Bison and cattle grazing influences on soil microbial N cycling and ecosystem N pools in annually burned tallgrass prairies

by

Nicholas Vega Anguiano

B.S., California State Polytechnic University, Humboldt, 2021

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Division of Biology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2024

Approved by:

Major Professor
Lydia Zeglin

Copyright

Abstract

Nitrogen (N) cycling is soil microbially mediated and essential for soil fertility. Grazing animals are integral to N cycling in grassland systems, especially historically grazed grasslands like the tallgrass prairie ecosystem which supported large populations of bison pre-colonization. Grazing animals impact the N cycle by recycling forage through consumption and deposition of more bioavailable N to the soil, promoting soil fertility. However, bison and cattle may have different impacts on N cycling due to differences in management, physiology, and behavior. Further comparative impacts of bison and cattle grazing on N cycling in tallgrass prairie have not been investigated. I predicted that N cycling microbial activities and plant and soil N pools would be similarly higher under both types of grazing relative to ungrazed conditions. To evaluate these predictions, I sampled upland mineral soils in annually burned bison-grazed, cattle-grazed, and ungrazed experimental tallgrass prairie watersheds at the Konza Prairie Biological Station near Manhattan, KS, USA, over the 2020-2022 summer growing seasons; and measured soil characteristics, N availability indices, microbial N cycling activity rate potentials, and N pools including total soil N, soil microbial biomass N, and aboveground primary productivity and forage N uptake within moveable exclosures with paired ungrazed plots.

Overall, soil N cycling rates in bison-grazed watersheds were higher than in ungrazed watersheds, and generally intermediate between the two in cattle-grazed watersheds. Under bison, there was higher soil pH, plant available N, lower extracellular enzyme activity estimated N limitation, and greater nitrification and denitrification potential than in ungrazed soils, though effects on denitrification were weaker than on N mineralization and nitrification. Forage N content, soil N concentration and N stock was also higher under bison grazing than in cattle grazed or ungrazed conditions. Interannual variability in microbial N cycling was high, and likely related to higher precipitation and soil moisture in 2020 than 2021 and 2022 but did not change grazing effects. However, dung N content did not differ between the two grazers, and forage production and N uptake into grass was significantly greater under cattle than bison grazing. In conclusion, while ungrazed soils had lower N-cycling rates and higher C:N ratios than either bison or cattle grazed soils, both soil fertility and forage quality was consistently greater under bison than cattle grazing. Results show that higher grass %N under bison grazing is related to more plant available N, accelerated soil N cycling rates, and a larger soil N stock, showing that stronger grazer-plant-soil fertility feedbacks exist in bison-grazed prairie than cattle-grazed prairie. Differences may be attributed either to animal physiology and behavior, and/or to greater grazing pressure from bison than cattle due to different management. This investigation establishes that relationships between animal and soil microbial processes mediate terrestrial N cycling in tallgrass prairie, and may be used to inform cattle and bison rangeland management decisions.

Table of Contents

List of Figures

List of Tables

Acknowledgements

None of this writing and scientific work could be done without the hard work of others. Thank you to my lab mates, friends, family and mentors who have all invested parts of themselves into me in some way, shape or form so that I could have the privilege of starting and finishing this endeavor. My time here has been a truly unique experience, one that I would not trade despite everything. Some of the lessons I've learned during my time here I will keep for the rest of my life, those bad and good. The sacrifices that these decent people have made in giving me their time and love will not be forgotten and will mean more to me than they'll ever know.

First thank you to my committee members, funding from the National Science Foundation, the Grassland Heritage Foundation and Rachel Snyder, to collaborative support from KBPS, KNZ LTER, Amanda Kuhl and team and the KSU Division of Biology. Second, here are some of the names these people: Rosalinda Vega, Christopher Anguiano, Julia, Victoria, Nana, tio Mark, tio Louie, Sophia, Cali, tio Lalo, tio Meno, tio Guero, tio Michael tia Mimi, tia Chelo, tia Qika, tia Lupe, tia Carmela, tia Angi, tio Ernest, tio David, tia Julieta, nina Pati, nina Silva, Tata, Nana Rosa, Tata Preto. Grandma, Grandpa, tio Moses, tio Junior, tio Tino, tia Charito, tia Rachel, tia Vero, tia Lori, tio Roy, tia Maria, Jessica, Isaiah, Valarie, Gigi, Christian and Laura and all my cousins. Thank you to Mario, Vera, Gilbert, Andre, Thomas, Lonyx, Susan and INRSEP. Thank you to Nick "Taco" Garrett, Nic Byrd, Josh "Slipknot" Pfeiffer, Brian, Rosa and CSC. Thanks to Andrew, Jeff, Patrick, Greg, Matthew, Janaye, Rissa, Jaide, Kiona, Caroline, Harley, Brett, Chester, Dale, Debra, Daisy, Thurman, Alex, LBB and JPH and Charlee and their families. Finally, thanks to all my ancestors for this blessing, thank you to the ancestors of this soil, so that we may all find peace and balance within ourselves so that we may be at peace with this land.

Chapter 1 - Introduction

In North America, populations of the keystone megaherbivore, the American buffalo (*Bison bison*), or bison are essential to the tallgrass prairie (Paine, 1995; Knapp *et al.*, 1999). Yet, on two occasions bison have hit two population bottlenecks: The first during the last ice age due to low forage availability (Davies *et al.*, 2019), and the second as a result of the *bellum romanum*[1] war (Dunbar-Ortiz, 2014) the early United States government employed to pacify Indian[2] nations in the early 19th century (Davies *et al.*, 2019). The slaughter of the bison directly parallels the decimation of Indian nations, and both reflect a physical, cultural, moral and social separation that allowed both sides to engage in war, with many results, including the loss of human life, indigenous ways of knowing, and the loss of bison, catalyzing the decline of grassland ecosystems (Deloria, Jr. and Wildcat, 2001; Grossman, 2014). Conventional agriculture, including cattle production, in the North American Great Plains increased coincident with the decline of bison populations during the American colonial period. Cattle were introduced to North America in what we now call Mexico by early Spanish expeditions (Bowling, 1942; Powell, 1975), members of which realized the potential for monetary profit enabled by eventual cattle proliferation across the continent.

To help sustain the critical food and material resources that bison provided, Indian nations practiced a lifestyle that supported a healthy tallgrass prairie ecosystem. For example,

---

[1] Defined as “War the way the Romans did it” as described in Julius Caesar’s War Commentaries or total war was used to break the will of conquered tribal peoples and was not limited to scorched earth tactics but all manner of cutting off of supply chains in an effort to slaughter all the warriors and subjugate survivors.

[2] Here I define Indian nations as those indigenous tribes, bands or groups of people living in North America prior to first contact from European expeditions led by Columbus, Cortez, and others.

fire was used as a tool to keep grasslands healthy and with select areas of woody vegetation to create a landscape mosaic for human use and for bison grazing (Stewart, 2002; Allred *et al.*, 2011; Ratajczak *et al.*, 2014; Lake and Christianson, 2019; Larson *et al.,* 2020; Wilcox *et al.*, 2021). As such, if Indian nations managed grasslands with fire that had been in place since time immemorial, it makes sense that without their style of land management, biological systems may have experienced perturbations (Deloria, Jr. and Wildcat, 2001). This rationale is rooted in Lockard's Exchange principle where "Every contact leaves a trace" (Fisher, Tilstone and Woytowicz, 2009). Further, using bison as a metaphor for Indian people, they have both survived and tribes continue to inhabit the land in which they were borne from. The effects of total war all those years ago is reflected as imbalances in the landscape. At present, remaining grasslands are at risk of loss due to woody encroachment as a result of fire suppression (Stewart, 2002; Lake and Christianson, 2019)

Other grassland ecosystem properties may have changed as a consequence of bison extirpation, such as the availability of nitrogen (N), a necessary component of soil fertility, since it is an essential element for life on earth to exist (Schlesinger and Bernhardt, 2020). Both soil microorganisms and large grazing animals are responsible for regulating N cycling, as the keystone bison who move N from forage (grass) biomass and deposit it back to the soil as dung and urine for faster microbial processing (Robertson and Groffman, 2014). Thus, keystone megaherbivores in historically grazed regions are also necessary to N cycling and thus soil fertility, as evidenced by research done in Yellowstone National Park (Frank *et al.*, 1994, 1995; Frank and Evans, 1997; Frank and Groffman, 1998; Tracy and Frank, 1998) and tallgrass prairie (Groffman, Rice and Tiedje, 1993; Knapp *et al.*, 1999), and in grasslands around the world

(Sitters and Olde Venterink, 2015; Roy and Bagchi, 2022; Abraham *et al.*, 2023; de Jonge *et al.*, 2023; Roy, Naidu and Bagchi, 2023).

Some investigations have implicated megaherbivores as having the ability to alter soil nutrient resource availability (Sitters and Olde Venterink, 2015), soil physical and chemical properties (Sharpley, 1991), and soil microbial biomass (Tracy and Frank, 1998) and microbial enzymatic activities (Groffman, Rice and Tiedje, 1993) compared to ungrazed conditions. Large grazer effects on soil N in the form of dung and urine depositions allows for soil N to be more bioavailable, whereby N is more available for plant uptake (Moore and Bower, 2001). In annually burned tallgrass prairie, soils have the tendency to retain N because the nutrient is in high demand (Dell, Williams and Rice, 2005), and N cycling microbial activities respond to shifts in changing soil N as a result of grazing pressure (Groffman, Rice and Tiedje, 1993). That said, little research exists comparing bison and cattle in reference to their impact on N cycling in tallgrass prairie. Thus, my thesis investigates multiple aspects of the N cycle under both types of grazing relative to ungrazed conditions, with the null hypothesis that cattle and bison have similar effects on the ecosystem.

References Cited

Abraham, A.J. *et al.* (2023) ‘Understanding anthropogenic impacts on zoogeochemistry is essential for ecological restoration’, *Restoration Ecology*, 31(3), p. e13778. Available at: https://doi.org/10.1111/rec.13778.

Allred, B.W. *et al.* (2011) ‘Ungulate preference for burned patches reveals strength of fire–grazing interaction’, *Ecology and Evolution*, 1(2), pp. 132–144. Available at: https://doi.org/10.1002/ece3.12.

Bowling, G.A. (1942) ‘The Introduction of Cattle into Colonial North America*’, *Journal of Dairy Science*, 25(2), pp. 129–154. Available at: https://doi.org/10.3168/jds.S0022-0302(42)95275-5.

Cotrufo, M.F. *et al.* (2015) ‘Formation of soil organic matter via biochemical and physical pathways of litter mass loss’, *Nature Geoscience*, 8(10), pp. 776–779. Available at: https://doi.org/10.1038/ngeo2520.

Davies, G. *et al.* (2019) 'Isotopic paleoecology of Northern Great Plains bison during the Holocene', *Scientific Reports*, 9(1), p. 16637. Available at: https://doi.org/10.1038/s41598-019-52873-4.

Deloria, Jr., V. and Wildcat, D. (2001) *Power and Place: Indian education in America*.

Dodds, W. k. *et al.* (1996) 'Nitrogen Transport from Tallgrass Prairie Watersheds', *Journal of Environmental Quality*, 25(5), pp. 973–981. Available at: https://doi.org/10.2134/jeq1996.00472425002500050007x

Dunbar-Ortiz, R. (2014) *An Indigenous Peoples' History of the United States*. Beacon Press.

Fisher, B.A.J., Tilstone, W.J. and Woytowicz, C. (2009) *Introduction to Criminalistics: The Foundation of Forensic Science*. Academic Press.

Frank, A.B. *et al.* (1995) 'Soil Carbon and Nitrogen of Northern Great Plains Grasslands as Influenced by Long-Term Grazing', *Journal of Range Management*, 48(5), p. 470. Available at: https://doi.org/10.2307/4002255.

Frank, D.A. *et al.* (1994) 'The biogeochemistry of a north-temperate grassland with native ungulates: Nitrogen dynamics in Yellowstone National Park', *Biogeochemistry*, 26(3), pp. 163–188. Available at: https://doi.org/10.1007/BF00002905.

Frank, D.A. and Evans, R.D. (1997) 'Effects of native grazers on grassland N cycling in Yellowstone National Park', *Ecology*, 78(7), pp. 2238–2248. Available at: https://doi.org/10.1890/0012-9658(1997)078[2238:EONGOG]2.0.CO;2.

Frank, D.A. and Groffman, P.M. (1998) 'Ungulate Vs. Landscape Control of Soil C and N Processes in Grasslands of Yellowstone National Park', *Ecology*, 79(7), pp. 2229–2241. Available at: https://doi.org/10.1890/0012-9658(1998)079[2229:UVLCOS]2.0.CO;2.

Groffman, P.M., Rice, C.W. and Tiedje, J.M. (1993) 'Denitrification in a Tallgrass Prairie Landscape', *Ecology*, 74(3), pp. 855–862. Available at: https://doi.org/10.2307/1940811.

Grossman, D. (2014) *On Killing: The Psychological Cost of Learning to Kill in War and Society*. Open Road Media.

de Jonge, I.K. *et al.* (2023) 'The metamicrobiome: key determinant of the homeostasis of nutrient recycling', *Trends in Ecology & Evolution*, 38(2), pp. 183–195. Available at: https://doi.org/10.1016/j.tree.2022.10.003.

Dell, C.J., Williams, M.A. and Rice, C.W. (2005) 'Partitioning of Nitrogen Over Five Growing Seasons in Tallgrass Prairie', *Ecology*, 86(5), pp. 1280–1287. Available at: https://doi.org/10.1890/03-0790.

Knapp, A.K. *et al.* (1999) 'The Keystone Role of Bison in North American Tallgrass Prairie: Bison increase habitat heterogeneity and alter a broad array of plant, community, and ecosystem processes', *BioScience*, 49(1), pp. 39–50. Available at: https://doi.org/10.1525/bisi.1999.49.1.39.

Lake, F.K. and Christianson, A.C. (2019) 'Indigenous Fire Stewardship', in S.L. Manzello (ed.) *Encyclopedia of Wildfires and Wildland-Urban Interface (WUI) Fires*. Cham: Springer International Publishing, pp. 1–9. Available at: https://doi.org/10.1007/978-3-319-51727-8_225-1.

Larson, D.L. *et al.* (2020) 'Management of remnant tallgrass prairie by grazing or fire: effects on plant communities and soil properties', *Ecosphere*, 11(8), p. e03213. Available at: https://doi.org/10.1002/ecs2.3213.

Moore, A.J. and Bower, N.W. (2001) 'Chemometric Analysis of Compositional Variation in Bison and Cow Patties: A Biogeochemistry Environmental Chemistry Experiment', *The Chemical Educator*, 6(2), pp. 86–90. Available at: https://doi.org/10.1007/s00897010459a.

Paine, R.T. (1995) 'A Conversation on Refining the Concept of Keystone Species', *Conservation Biology*, 9(4), pp. 962–964.

Powell, P.W. (1975) *Soldiers, Indians & Silver: North America's First Frontier War*. Center for Latin American Studies, Arizona State University.

Ratajczak, Z. *et al.* (2014) 'Fire dynamics distinguish grasslands, shrublands and woodlands as alternative attractors in the Central Great Plains of North America', *Journal of Ecology*, 102(6), pp. 1374–1385.

Robertson, G.P. and Groffman, P.M. (2014) 'Nitrogen Transformations', in E.A. Paul (ed.) *Soil Microbiology, Ecology and Biochemistry (Fourth Edition)*. San Diego: Academic Press, pp. 341–364. Available at: https://www.elsevier.com/books/soil-microbiology-ecology-and-biochemistry/paul/978-0-12-415955-6 (Accessed: 14 March 2021).

Roy, S. and Bagchi, S. (2022) 'Large Mammalian Herbivores and the Paradox of Soil Carbon in Grazing Ecosystems: Role of Microbial Decomposers and Their Enzymes', *Ecosystems*, 25(4), pp. 976–988. Available at: https://doi.org/10.1007/s10021-021-00696-8.

Roy, S., Naidu, D.G.T. and Bagchi, S. (2023) 'Functional substitutability of native herbivores by livestock for soil carbon stock is mediated by microbial decomposers', *Global Change Biology*, 29(8), pp. 2141–2155. Available at: https://doi.org/10.1111/gcb.16600.

Sitters, J. and Olde Venterink, H. (2015) 'The need for a novel integrative theory on feedbacks between herbivores, plants and soil nutrient cycling', *Plant and Soil*, 396(1), pp. 421–426. Available at: https://doi.org/10.1007/s11104-015-2679-y.

Schlesinger, W.H. and Bernhardt, E.S. (2020) *Biogeochemistry - 4th Edition*. 4th edn.

Sharpley, A.N. (1991) ‘Effect of soil pH on cation and anion solubility’, *Communications in Soil Science and Plant Analysis*, 22(9–10), pp. 827–841. Available at: https://doi.org/10.1080/00103629109368457.

Stewart, O.C. (2002) *Forgotten Fires: Native Americans and the Transient Wilderness*. University of Oklahoma Press.

Tracy, B.F. and Frank, D.A. (1998) ‘Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem: Yellowstone National Park’, *Oecologia*, 114, pp. 556–562.

Wilcox, B.P. *et al.* (2021) ‘Saving imperiled grassland biomes by recoupling fire and grazing: a case study from the Great Plains’, *Frontiers in Ecology and the Environment*, n/a(n/a). Available at: https://doi.org/10.1002/fee.2448.

Chapter 2 - Bison and cattle influences are stronger than interannual variation in soil nitrogen cycling in a tallgrass prairie ecosystem

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 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.

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, and maintaining 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, Williams and Rice, 2005). In addition, tallgrass prairies were historically grazed by large mammalian herbivores, which often enhance soil N cycling rates and soil fertility (Hobbs, 1996; Frank and Evans, 1997; Blair *et al.*, 1998; Knapp *et al.*, 1999). Bison grazing can increase soil N cycling rates in areas managed with annual fire to levels equivalent to areas experiencing infrequent fire (Groffman, Rice and Tiedje, 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 European colonization (Dunbar-Ortiz, 2014) decimated bison populations from approximately 30 million to less than 1000 individuals (Hornaday, 1913; Flores, 1991; Shaw, 1995; Lott, 2002). Native American tribes who depended on bison experienced societal and economic collapse that resulted in economic impacts in First Nation communities still seen till this day (Feir, Gillezeau and Jones, 2021). Domesticated cattle (*Bos taurus*) replaced bison, now outnumbering them by an estimated 500,000 to 100 million individuals (Samson, Knopf and Ostlie, 2004; Kohl *et al.*, 2013). The replacement of bison with cattle raises concerns of whether both animals occupy the same functional roles (Allred, Fuhlendorf and Hamilton, 2011; Kohl *et al.*, 2013). Some case studies around the globe have similar concerns and have also begun investigating the alteration of nutrient cycling with the replacement of their native megaherbivores with domesticated cattle in managed rangeland like tallgrass prairie (Enquist *et al.*, 2020; Abraham *et al.*, 2023; Roy, Naidu and Bagchi, 2023).

Cattle and bison graze similar grasses in tallgrass prairie (Allred, Fuhlendorf and Hamilton, 2011) and they both 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). On the other hand, bison have different physiological characteristics than cattle, which may influence soil N cycling impacts. 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, Hudson and Christophersen, 1978; Allred, Fuhlendorf and Hamilton, 2011; Larson *et al.*, 2013; McMillan *et al.*, 2021, 2022). 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 by bison and cattle depends on decisions to travel and eat forage and drink water (Plumb and Dodd, 1993; Augustine and Frank, 2001; Raynor, 2015; 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, Leslie and Shaw, 1998; Towne, Hartnett and Cochran, 2005; McMillan, Pfeiffer and Kaufman, 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.

In addition, 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 soil N transformations (Robertson and Groffman, 2014). In soils, interannual variability in precipitation leads to variability in soil water affecting the mobility of NH^{+}_{4} sorbed to soil colloidal surfaces and N cycling microbial activity, affecting N mineralization from SOM (Soil Organic Matter), impacting rates of nitrification and denitrification (Robertson and Groffman, 2014). Interannual variability in precipitation can affect N cycling globally like the Kalahari of southern Africa, desert Mongolian steppe, warm-climate grasslands (Bolivia, Brazil, Colombia, Guyana, Venezuela), and tallgrass prairie (Aranibar *et al.*, 2004; Dubeux *et al.*, 2007; Delon *et al.*, 2014; Guo *et al.*, 2022; Nippert *et al.*, 2022). As such, there is strong evidence that indicates interannual variability is a natural occurrence, affecting ecosystem and N cycling dynamics in tallgrass prairie and other grasslands around the world (Groffman, Rice and Tiedje, 1993; Broderick *et al.*, 2022; Chen *et al.*, 2022).

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. 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 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 one 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 2020-2022. These years spanned a range of above-average rainfall (2020) to well below average (2021) and slightly below average (2022) (Figure 2.5). 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.

Each transect covered six sampling points at 0, 0.1, 0.5, 1, 5, and 10 meters, 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 meters were 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 hours before measuring N cycling activity potential rates.

Soil physical characteristics and N-cycling rates

Soil water content of all samples was measured gravimetrically by drying soil at 105° C for 24 hours. Soil pH was measured in a 1:3 slurry of field-moist soil and DI H_2O, on samples collected in 2021 and 2022. Plant available N, the level of soil N mineralized during the growing season, was measured using ion exchange resin bags installed from June to September (Baer and Blair, 2008; Nieland, 2023) 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).

Nitrification potential (NP) activity 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 15000 rpm, and the supernatant was frozen at -20° C until spectrophotometrically read in 96 well plates in sample triplicates at 540 nm using a Filtermax F5 Multimode Microplate Reader (Molecular Devices, San Jose, CA, USA) (Hood-Nowotny *et al.*, 2010; Taylor *et al.*, 2010). Ammonia oxidation by soil microorganisms, and the resulting increase in NO_3^- -N over time, allows for calculation of the rate of maximum nitrification potential activity 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 one hour, 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 four-hour 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 the assimilation of N by soil microorganisms and the net mineralization of N into the plant available pool (Schimel and Weintraub, 2003; Sinsabaugh, Hill and Follstad Shah, 2009). The expression of these N-acquiring enzymes is generally regulated by product suppression, i.e., if soil N availability is high, fewer enzymes to produce more available N are synthesized by soil microorganisms (Allison and Vitousek, 2005; Sinsabaugh and Follstad Shah, 2012; Nieland *et* *al.*, 2021). 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 (*ln*(βG))/(*ln*(NAG + LAP)); this ratio is an index of N demand relative to C by soil microbes (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). 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 Honestly Significant Difference (HSD) tests for pairwise comparisons of within-group differences. Linear regression was used to assess regression models describing the relationship between soil characteristics, N cycling assays and EEAs. Coefficient of determination (R-squared) was used to assess linear relationship strength. For each predictor and response 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 best satisfying 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 2.1). 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 (Figure 2.1). 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 2.1, Figure 2.1).

Resin-sorbed N responses to grazing and year were also independent of one another (Table 2.1). 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 (Figure 2.2). 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 (Figure 2.2).

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 consistently higher in bison grazed soils than cattle grazed or ungrazed soils (Table 2.1, Figure 2.3). Soil denitrification potential (DNP) did not respond to grazing treatment, but was higher in 2020 than in 2021 or 2022 (Table 2.1, Figure 2.3). Notably, DNP rates were only 14% (on average) of the Denitrification enzyme activity (DEA) when detectable, and below detectable limits in 2022 and in cattle and ungrazed treatments in 2021. DEA responses to grazing varied interannually (Table 2.1). 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 (Figure 2.3).

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 2.1, Figure 2.6). 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 2.1, Figure 2.4, Figure 2.6). 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 (Figure 2.4).

Correlations

Many linear model correlations among soil characteristics and N cycling parameters were statistically significant, but none had an R^2 value higher than 0.15 (Table 2.2). 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 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 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 (Figure 2.1, 2.5). 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 (Figure 2.2). 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 (Figure 2.3). 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 (Figure 2.4, Figure 2.6). 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 (Figure 2.3), 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 affect 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, was likely related to the long period of time following a precipitation event preceding the sampling time: as soils dry, solute concentrations decline with shrinking water availability, and only 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, Ekenler and Senwo, 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 C and 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 (Figure 2.2, Figure 2.4). 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 (Figure 2.6). 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 (Figure 2.2). Notably, we also found that despite interannual variation, bison grazed soils maintained a higher (less acidic) pH than cattle grazed soils (Figure 2.1). 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 (Sharpley, 1991; Zeglin *et al.*, 2007; Sinsabaugh *et al.*, 2008; Nannipieri *et al.*, 2018). In this study, soil acidity was likely buffered by bison grazing activity, likely through urine and dung inputs coupled with soil can retard acidification; altering the generation and production of hydrogen ions leading to changes in available N and microbial enzyme production (Voroney and Heck, 2015; Hong *et al.*, 2021; Barber *et al.*, 2023).

Grazing and nitrification potential

Both NP and resin-sorbed NO_3^- -N were consistently higher in bison treatments (Figure 2.2, Figure 2.3), 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, Rice and Tiedje, 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 nitrate production is not necessarily strongly tied to ecosystem N 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 2.2). 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 may have higher abundance and/or a pH-specialized population of soil nitrifiers (Prosser and Nicol, 2012), resulting in 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 (Figure 2.3). 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 (Figure 2.2, Figure 2.3). 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, Martiny and Martiny, 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

While bison grazing substantially increased soil N-cycling rate potentials at this site in the North American Great Plains, 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, Rice and Tiedje, 1993). Comparatively, given the three decades of additional grazing pressure between these two studies, it is somewhat surprising that rates are so similar. However, the fact that variable precipitation mediates the magnitude of N-cycling must be taken into account, such that 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. 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 carrying capacity (Holechek, Pieper and Herbel, 2011).

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, Williams and Rice, 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, Nippert and Blair, 2020). As such, ecosystem N retention may be high in all treatments of this study because they are burned annually (Dell, Williams and Rice, 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; and also, that 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 because it is currently lacking (Pellant *et al.*, 2020). Long term data on N cycling activities are sparse and considering changing climate will provide useful information for future N cycling and budgeting in tallgrass prairie. Nonetheless, human societies past and present around the world have always depended on large grazing animals, especially the bison in North America and more recently cattle. Bison are tied to the existing cultural identity of tribal nations that have lived on grasslands for at least 10,000 years (Kornfeld, Frison and Larson, 2016). Thus, soil fertility by default is a mutual requirement for the large grazer to exist since they depend on forage, which depends on the management of bison and cattle to limit overgrazing.

Yet, the question remains how much did the loss of bison change N cycling on tallgrass prairie? The evidence in this study shows that bison can accelerate N cycling significantly, compared to cattle and ungrazed conditions in which N cycling is slower. Therefore, is it possible that the loss of bison contributed to a collapse of the pre-colonial N cycling regime. In our investigation the fact that bison are keystone species that promote grassland plant diversity (Ratajczak *et al.*, 2022) and faster N cycling vs cattle begs the question: How much did the loss of bison change N cycling on tallgrass prairie? The functional redundancy of cattle then comes into question and cannot be truly answered since currently, the animals are managed differently; but even the way bison were managed pre-colonization was different too. With year-round grazing mimicking how bison historically grazed tallgrass prairie, grazing intensity must be kept in mind. At present, we are left with altered components of what once was, with the opportunity to grow into something better. Bison have not rebounded population wise, still a small fraction of their pre-colonization abundance. However, livestock practices have improved with innovations in research and applications to land management, with more data, more knowledge may be used to make better decisions. Future work should focus on investigating N cycling pools to connect rates of N cycling to pools of N. Bison can impact N cycling differently vs cattle grazing, yet, there is less evidence about N storage in tallgrass prairie soils and plants: Understanding the ecosystem N budget is a critical next step.

References Cited

Abraham, A.J. *et al.* (2023) 'Understanding anthropogenic impacts on zoogeochemistry is essential for ecological restoration', *Restoration Ecology*, 31(3), p. e13778. Available at: https://doi.org/10.1111/rec.13778.

Allison, S.D. and Vitousek, P.M. (2005) 'Responses of extracellular enzymes to simple and complex nutrient inputs', *Soil Biology and Biochemistry*, 37(5), pp. 937–944. Available at: https://doi.org/10.1016/j.soilbio.2004.09.014.

Allred, B.W., Fuhlendorf, S.D. and Hamilton, R.G. (2011) 'The role of herbivores in Great Plains conservation: comparative ecology of bison and cattle', *Ecosphere*, 2(3), p. art26. Available at: https://doi.org/10.1890/ES10-00152.1.

Anderson, R.C. (2006) 'Evolution and origin of the Central Grassland of North America: climate, fire, and mammalian grazers1', *The Journal of the Torrey Botanical Society*, 133(4), pp. 626–647. Available at: https://doi.org/10.3159/1095-5674(2006)133[626:EAOOTC]2.0.CO;2.

Aranibar, J.N. *et al.* (2004) 'Nitrogen cycling in the soil–plant system along a precipitation gradient in the Kalahari sands', *Global Change Biology*, 10(3), pp. 359–373. Available at: https://doi.org/10.1111/j.1365-2486.2003.00698.x.

Augustine, D.J. and Frank, D.A. (2001) 'Effects of Migratory Grazers on Spatial Heterogeneity of Soil Nitrogen Properties in a Grassland Ecosystem', *Ecology*, 82(11), pp. 3149–3162. Available at: https://doi.org/10.1890/0012-9658(2001)082[3149:EOMGOS]2.0.CO;2.

Baer, S.G. and Blair, J.M. (2008) 'Grassland Establishment Under Varying Resource Availability: A Test of Positive and Negative Feedback', *Ecology*, 89(7), pp. 1859–1871. Available at: https://doi.org/10.1890/07-0417.1.

Barber, N.A. *et al.* (2023) 'Restoration age and reintroduced bison may shape soil bacterial communities in restored tallgrass prairies', *FEMS Microbiology Ecology*, 99(3), p. fiad007. Available at: https://doi.org/10.1093/femsec/fiad007.

Blair, J. (2023) 'CBH01 Konza Prairie bison herd information'. Environmental Data Initiative. Available at: https://doi.org/10.6073/PASTA/4912DE8FAF25E93D45551CB1891E7FAD.

Blair, J.M. (1997) 'Fire, N Availability, and Plant Response in Grasslands: A Test of the Transient Maxima Hypothesis', *Ecology*, 78(8), pp. 2359–2368. Available at: https://doi.org/10.1890/0012-9658(1997)078[2359:FNAAPR]2.0.CO;2.

Blair, J.M. *et al.* (1998) 'Terrestrial nutrient cycling in tallgrass prairie', *Grassland dynamics: long-term ecological research in tallgrass prairie. Oxford University Press, New York*, pp. 222–243.

Broderick, C.M. *et al.* (2022) 'Climate Legacy Effects Shape Tallgrass Prairie Nitrogen Cycling', *Journal of Geophysical Research: Biogeosciences*, 127(10), p. e2022JG006972. Available at: https://doi.org/10.1029/2022JG006972.

Burns, R.G. *et al.* (2013) 'Soil enzymes in a changing environment: Current knowledge and future directions', *Soil Biology and Biochemistry*, 58, pp. 216–234. Available at: https://doi.org/10.1016/j.soilbio.2012.11.009.

Chen, H. *et al.* (2022) 'Carbon and nitrogen cycling on the Qinghai–Tibetan Plateau', *Nature Reviews Earth & Environment*, 3(10), pp. 701–716. Available at: https://doi.org/10.1038/s43017-022-00344-2.

Christopherson, R.J., Hudson, R.J. and Christophersen, M.K. (1978) 'Seasonal energy expidentures and thermoregulatory responses of bison and cattle', *Canadian Journal of Animal Science* [Preprint]. Available at: https://doi.org/10.4141/cjas79-077.

Clegg, C.D. (2006) 'Impact of cattle grazing and inorganic fertiliser additions to managed grasslands on the microbial community composition of soils', *Applied Soil Ecology*, 31(1), pp. 73–82. Available at: https://doi.org/10.1016/j.apsoil.2005.04.003.

Connell, R.K., Nippert, J.B. and Blair, J.M. (2020) 'Three Decades of Divergent Land Use and Plant Community Change Alters Soil C and N Content in Tallgrass Prairie', *Journal of Geophysical Research: Biogeosciences*, 125(8), p. e2020JG005723. Available at: https://doi.org/10.1029/2020JG005723.

Coppedge, B.R., Leslie, D.M. and Shaw, J.H. (1998) 'Botanical composition of bison diets on tallgrass prairie in Oklahoma', *51* [Preprint]. Available at: https://doi.org/10.2307/4003321.

Coppedge, B.R. and Shaw, J.H. (1997) 'Effects of Horning and Rubbing Behavior by Bison (Bison bison) on Woody Vegetation in a Tallgrass Prairie Landscape', *The American Midland Naturalist*, 138(1), pp. 189–196. Available at: https://doi.org/10.2307/2426665.

Dell, C.J. and Rice, C.W. (2005) 'Short-Term Competition for Ammonium and Nitrate in Tallgrass Prairie', *Soil Science Society of America Journal*, 69(2), pp. 371–377. Available at: https://doi.org/10.2136/sssaj2005.0371.

Dell, C.J., Williams, M.A. and Rice, C.W. (2005) 'Partitioning of Nitrogen Over Five Growing Seasons in Tallgrass Prairie', *Ecology*, 86(5), pp. 1280–1287. Available at: https://doi.org/10.1890/03-0790.

Delon, C. *et al.* (2014) 'Interannual Variability of the Atmospheric Nitrogen Budget in West African Dry Savannas', in M.A. Sutton et al. (eds) *Nitrogen Deposition, Critical Loads and Biodiversity*. Dordrecht: Springer Netherlands, pp. 93–105. Available at: https://doi.org/10.1007/978-94-007-7939-6_11.

Detling, J.K. (1988) 'Grasslands and Savannas: Regulation of Energy Flow and Nutrient Cycling by Herbivores', in L.R. Pomeroy and J.J. Alberts (eds) *Concepts of Ecosystem Ecology*.

New York, NY: Springer (Ecological Studies), pp. 131–148. Available at: https://doi.org/10.1007/978-1-4612-3842-3_7.

Dodds, W. k. *et al.* (1996) ‘Nitrogen Transport from Tallgrass Prairie Watersheds’, *Journal of Environmental Quality*, 25(5), pp. 973–981. Available at: https://doi.org/10.2134/jeq1996.00472425002500050007x.

Dubeux, J.C.B. *et al.* (2007) ‘Nutrient Cycling in Warm-Climate Grasslands’, *Crop Science*, 47(3), pp. 915–928. Available at: https://doi.org/10.2135/cropsci2006.09.0581.

Dunbar-Ortiz, R. (2014) *An Indigenous Peoples’ History of the United States*. Beacon Press.

Eldridge, D.J. *et al.* (2017) ‘Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores’, *Ecology*, 98(7), pp. 1922–1931. Available at: https://doi.org/10.1002/ecy.1879.

Enquist, B.J. *et al.* (2020) ‘The megabiota are disproportionately important for biosphere functioning’, *Nature Communications*, 11(1), p. 699. Available at: https://doi.org/10.1038/s41467-020-14369-y.

Fay, P.A. *et al.* (2003) ‘Productivity responses to altered rainfall patterns in a C4-dominated grassland’, *Oecologia*, 137(2), pp. 245–251. Available at: https://doi.org/10.1007/s00442-003-1331-3.

Feir, D., Gillezeau, R. and Jones, M.E.C. (2021) ‘The Slaughter of the Bison and Reversal of Fortunes on the Great Plains’, p. 24.

Flores, D. (1991) ‘Bison Ecology and Bison Diplomacy: The Southern Plains from 1800 to 1850’, *The Journal of American History*, 78(2), p. 465. Available at: https://doi.org/10.2307/2079530.

Frank, D.A. and Evans, R.D. (1997) ‘Effects of native grazers on grassland N cycling in Yellowstone National Park’, *Ecology*, 78(7), pp. 2238–2248. Available at: https://doi.org/10.1890/0012-9658(1997)078[2238:EONGOG]2.0.CO;2.

Groffman, P.M. *et al.* (2009) ‘Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models’, *Biogeochemistry*, 93(1), pp. 49–77. Available at: https://doi.org/10.1007/s10533-008-9277-5.

Groffman, P.M., Rice, C.W. and Tiedje, J.M. (1993) ‘Denitrification in a Tallgrass Prairie Landscape’, *Ecology*, 74(3), pp. 855–862. Available at: https://doi.org/10.2307/1940811.

Guo, X. *et al.* (2022) ‘Direct and indirect effects of precipitation change and nutrients addition on desert steppe productivity in Inner Mongolia, northern China’, *Plant and Soil*, 471(1), pp. 527–540. Available at: https://doi.org/10.1007/s11104-021-05206-2.

Hawkins, Jaide.H. and Zeglin, L.H. (2022) ‘Microbial dispersal, including bison dung vectored dispersal, increases soil microbial diversity in a grassland ecosystem’, *Frontiers in*

Microbiology [Preprint]. Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.825193/abstract.

Hobbs, N.T. (1996) 'Modification of Ecosystems by Ungulates', *The Journal of Wildlife Management*, 60(4), pp. 695–713. Available at: https://doi.org/10.2307/3802368.

Holechek, J., Pieper, R. and Herbel, C. (2011) *Range management principles and practices*. 6th edn.

Hong, J. *et al.* (2021) 'Significant soil acidification caused by grazing exclusion across China's grassland areas', *Land Degradation & Development*, 32(2), pp. 535–545. Available at: https://doi.org/10.1002/ldr.3722.

Hood-Nowotny, R. *et al.* (2010) 'Alternative Methods for Measuring Inorganic, Organic, and Total Dissolved Nitrogen in Soil', *Soil Science Society of America Journal*, 74(3), pp. 1018–1027. Available at: https://doi.org/10.2136/sssaj2009.0389.

Hornaday, W.T. (William T. (1913) *Our vanishing wild life; its extermination and preservation*. New York, C. Scribner's sons. Available at: http://archive.org/details/ourvanishingwild00horn (Accessed: 18 April 2023).

IPCC (2022) 'Sythesis report of the IPCC, sixth assesment report (AR6)'. Available at: https://www.ipcc.ch/report/ar6/syr/ (Accessed: 21 May 2023).

Johnson, L.C. and Matchett, J.R. (2001) 'Fire and grazing regulate belowground processes in tallgrass prairie', 82(12).

Kemmitt, S.J. *et al.* (2006) 'pH regulation of carbon and nitrogen dynamics in two agricultural soils', *Soil Biology and Biochemistry*, 38(5), pp. 898–911. Available at: https://doi.org/10.1016/j.soilbio.2005.08.006.

Knapp, A.K. *et al.* (1999) 'The Keystone Role of Bison in North American Tallgrass Prairie: Bison increase habitat heterogeneity and alter a broad array of plant, community, and ecosystem processes', *BioScience*, 49(1), pp. 39–50. Available at: https://doi.org/10.1525/bisi.1999.49.1.39.

Kohl, M.T. *et al.* (2013) 'Bison Versus Cattle: Are They Ecologically Synonymous?', *Rangeland Ecology & Management*, 66(6), pp. 721–731. Available at: https://doi.org/10.2111/REM-D-12-00113.1.

Kornfeld, M., Frison, G.C. and Larson, M.L. (2016) *Prehistoric Hunter-Gatherers of the High Plains and Rockies: Third Edition*. Routledge.

Larson, D.M. *et al.* (2013) 'Blazing and grazing: influences of fire and bison on tallgrass prairie stream water quality', *Freshwater Science*, 32(3), pp. 779–791. Available at: https://doi.org/10.1899/12-118.1.

List, R. *et al.* (2007) ‘Historic Distribution and Challenges to Bison Recovery in the Northern Chihuahuan Desert’, *Conservation Biology*, 21(6), pp. 1487–1494. Available at: https://doi.org/10.1111/j.1523-1739.2007.00810.x.

Lott, D.F. (2002) *American Bison: A Natural History*. University of California Press.

McMillan, B.R., Pfeiffer, K.A. and Kaufman, D.W. (2011) ‘Vegetation Responses to an Animal-generated Disturbance (Bison Wallows) in Tallgrass Prairie’, *The American Midland Naturalist*, 165(1), pp. 60–73. Available at: https://doi.org/10.1674/0003-0031-165.1.60.

McMillan, N.A. *et al.* (2019) ‘Plant community responses to bison reintroduction on the Northern Great Plains, United States: a test of the keystone species concept’, *Restoration Ecology*, 27(2), pp. 379–388. Available at: https://doi.org/10.1111/rec.12856.

McMillan, N.A. *et al.* (2021) ‘Are bison movements dependent on season and time of day? Investigating movement across two complex grasslands’, *Ecosphere*, 12(1), p. e03317. Available at: https://doi.org/10.1002/ecs2.3317.

McMillan, N.A. *et al.* (2022) ‘Bison movements change with weather: Implications for their continued conservation in the Anthropocene’, *Ecology and Evolution*, 12(12), p. e9586. Available at: https://doi.org/10.1002/ece3.9586.

McNaughton, S.J. (1983) ‘Compensatory Plant Growth as a Response to Herbivory’, *Oikos*, 40(3), pp. 329–336. Available at: https://doi.org/10.2307/3544305.

Mehnaz, K.R. and Dijkstra, F.A. (2016) ‘Denitrification and associated N2O emissions are limited by phosphorus availability in a grassland soil’, *Geoderma*, 284, pp. 34–41. Available at: https://doi.org/10.1016/j.geoderma.2016.08.011.

Nannipieri, P. *et al.* (2018) ‘Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis’, *Biology and Fertility of Soils*, 54(1), pp. 11–19. Available at: https://doi.org/10.1007/s00374-017-1245-6.

Nelson, M.B., Martiny, A.C. and Martiny, J.B.H. (2016) ‘Global biogeography of microbial nitrogen-cycling traits in soil’, *Proceedings of the National Academy of Sciences*, 113(29), pp. 8033–8040. Available at: https://doi.org/10.1073/pnas.1601070113.

Nicol, G.W. *et al.* (2008) ‘The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria’, *Environmental microbiology*, 10(11), pp. 2966–2978.

Nieland, M.A. *et al.* (2021) ‘Differential Resilience of Soil Microbes and Ecosystem Functions Following Cessation of Long-Term Fertilization’, *Ecosystems*, 24(8), pp. 2042–2060. Available at: https://doi.org/10.1007/s10021-021-00633-9.

Nieland, M.A. (2023) ‘Ecosystem recovery from chronic fertilization: Biotic mechanisms underpinning soil nitrogen legacies in burned and unburned grasslands’.

Nippert, J. *et al.* (2022) ‘Climate Change in Grassland Ecosystems’, in Groskinsky, B., *Climate Actions*. 1st edn. Boca Raton: CRC Press, pp. 65–100. Available at: https://doi.org/10.1201/9781003048701-4.

Olson, K.C. (2023) ‘PBG06 Cattle grazing and cattle performance in the Patch-Burn Grazing experiment at Konza Prairie’. Environmental Data Initiative. Available at: https://doi.org/10.6073/PASTA/28C9AA49E942E3BD489A05E867F38ECC.

Pellant, M. *et al.* (2020) *Interpreting Indicators of Rangeland Health, Version 5: Bureau of Land Management Technical Reference 1734-6*. Bureau of Land Management. Available at: https://pubs.er.usgs.gov/publication/70215720 (Accessed: 12 December 2022).

Plumb, G.E. and Dodd, J.L. (1993) ‘Foraging Ecology of Bison and Cattle on a Mixed Prairie: Implications for Natural Area Management’, *Ecological Applications*, 3(4), pp. 631–643. Available at: https://doi.org/10.2307/1942096.

Prosser, J.I. and Nicol, G.W. (2012) ‘Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation’, *Trends in Microbiology*, 20(11), pp. 523–531. Available at: https://doi.org/10.1016/j.tim.2012.08.001.

Ratajczak, Z. *et al.* (2022) ‘Reintroducing bison results in long-running and resilient increases in grassland diversity’, *Proceedings of the National Academy of Sciences*, 119(36), p. e2210433119. Available at: https://doi.org/10.1073/pnas.2210433119.

Raynor, E.J. (2015) ‘Ecological hierarchy of foraging in a large herbivore: the plains bison perspective in tallgrass prairie’. Available at: https://krex.k-state.edu/dspace/handle/2097/20348 (Accessed: 1 December 2020).

Raynor, E.J. *et al.* (2021) ‘Cattle Grazing Distribution Patterns Related to Topography Across Diverse Rangeland Ecosystems of North America’, *Rangeland Ecology & Management*, 75, pp. 91–103. Available at: https://doi.org/10.1016/j.rama.2020.12.002.

Robertson, G.P. and Groffman, P.M. (2014) ‘Nitrogen Transformations’, in E.A. Paul (ed.) *Soil Microbiology, Ecology and Biochemistry (Fourth Edition)*. San Diego: Academic Press, pp. 341–364. Available at: https://www.elsevier.com/books/soil-microbiology-ecology-and-biochemistry/paul/978-0-12-415955-6 (Accessed: 14 March 2021).

Roy, S., Naidu, D.G.T. and Bagchi, S. (2023) ‘Functional substitutability of native herbivores by livestock for soil carbon stock is mediated by microbial decomposers’, *Global Change Biology*, 29(8), pp. 2141–2155. Available at: https://doi.org/10.1111/gcb.16600.

R Core Team (2022). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Samson, F.B., Knopf, F.L. and Ostlie, W.R. (2004) ‘Great Plains ecosystems: past, present, and future’, *Wildlife Society Bulletin*, 32(1), pp. 6–15. Available at: https://doi.org/10.2193/0091-7648(2004)32[6:GPEPPA]2.0.CO;2.

Sahrawat, K.L., 2008. Factors affecting nitrification in soils. *Communications in Soil Science and Plant Analysis*, *39*(9-10), pp.1436-1446.

Schimel, J.P. (2018) ‘Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes’, *Annual Review of Ecology, Evolution, and Systematics*, 49(1), pp. 409–432. Available at: https://doi.org/10.1146/annurev-ecolsys-110617-062614.

Schimel, J.P. and Weintraub, M.N. (2003) ‘The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model’, *Soil Biology and Biochemistry*, 35(4), pp. 549–563. Available at: https://doi.org/10.1016/S0038-0717(03)00015-4.

Schlesinger, W.H. and Bernhardt, E.S. (2020) *Biogeochemistry: An Analysis of Global Change*. 4th Edition. Academic Press.

Schlesinger, W.H. and Hartley, A.E. (1992) ‘A Global Budget for Atmospheric NH3’, *Biogeochemistry*, 15(3), pp. 191–211.

Sharpley, A.N. (1991) ‘Effect of soil pH on cation and anion solubility’, *Communications in Soil Science and Plant Analysis*, 22(9–10), pp. 827–841. Available at: https://doi.org/10.1080/00103629109368457.

Shaw, H. (1995) ‘How ManyBison Originally Populated Western Rangelands?’

Sinsabaugh, R. and Follstad Shah, J. (2012) ‘Ecoenzymatic Stoichiometry and Ecological Theory’, *Annual Review of Ecology Evolution and Systematics*, 43, pp. 313–343. Available at: https://doi.org/10.1146/annurev-ecolsys-071112-124414.

Sinsabaugh, R., Hill, B. and Follstad Shah, J. (2009) ‘Sinsabaugh RL, Hill BH, Follstad Shah JJ.. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462: 795-798’, *Nature*, 462, pp. 795–8. Available at: https://doi.org/10.1038/nature08632.

Sinsabaugh, R.L. *et al.* (2008) ‘Stoichiometry of soil enzyme activity at global scale: Stoichiometry of soil enzyme activity’, *Ecology Letters*, 11(11), pp. 1252–1264. Available at: https://doi.org/10.1111/j.1461-0248.2008.01245.x.

Soong, J.L. and Cotrufo, M.F. (2015) ‘Annual burning of a tallgrass prairie inhibits C and N cycling in soil, increasing recalcitrant pyrogenic organic matter storage while reducing N availability’, *Global Change Biology*, 21(6), pp. 2321–2333. Available at: https://doi.org/10.1111/gcb.12832.

Srikanthasamy, T. *et al.* (2018) ‘Contrasting effects of grasses and trees on microbial N-cycling in an African humid savanna’, *Soil Biology and Biochemistry*, 117, pp. 153–163. Available at: https://doi.org/10.1016/j.soilbio.2017.11.016.

Tabatabai, M.A., Ekenler, M. and Senwo, Z.N. (2010) ‘Significance of Enzyme Activities in Soil Nitrogen Mineralization’, *Communications in Soil Science and Plant Analysis*, 41(5), pp. 595–605. Available at: https://doi.org/10.1080/00103620903531177.

Tang, S. *et al.* (2019) ‘Heavy grazing reduces grassland soil greenhouse gas fluxes: A global meta-analysis’, *Science of The Total Environment*, 654, pp. 1218–1224. Available at: https://doi.org/10.1016/j.scitotenv.2018.11.082.

Taylor, A.E. *et al.* (2010) ‘Evidence for Different Contributions of Archaea and Bacteria to the Ammonia-Oxidizing Potential of Diverse Oregon Soils’, *Applied and Environmental Microbiology* [Preprint]. Available at: https://doi.org/10.1128/AEM.01324-10.

Towne, E.G., Hartnett, D.C. and Cochran, R.C. (2005) ‘Vegetation Trends in Tallgrass Prairie from Bison and Cattle Grazing’, *Ecological Applications*, 15(5), pp. 1550–1559. Available at: https://doi.org/10.1890/04-1958.

Voroney, R.P. and Heck, R.J. (2015) ‘Chapter 2 - The Soil Habitat’, in E.A. Paul (ed.) *Soil Microbiology, Ecology and Biochemistry (Fourth Edition)*. Boston: Academic Press, pp. 15–39. Available at: https://doi.org/10.1016/B978-0-12-415955-6.00002-5.

Wallenstein, M.D. *et al.* (2006) ‘Environmental Controls on Denitrifying Communities and Denitrification Rates: Insights from Molecular Methods’, *Ecological Applications*, 16(6), pp. 2143–2152. Available at: https://doi.org/10.1890/1051-0761(2006)016[2143:ECODCA]2.0.CO;2.

Wang, Z. *et al.* (2019) ‘Impact of long-term grazing exclusion on soil microbial community composition and nutrient availability’, *Biology and Fertility of Soils*, 55(2), pp. 121–134. Available at: https://doi.org/10.1007/s00374-018-01336-5.

Yingjin, S. *et al.* (2022) ‘Short-term grazing rather than mowing stimulates N2O production potential through enhancing the bacterial pathway in semiarid grasslands’, *Journal of Soils and Sediments*, 22(1), pp. 32–42. Available at: https://doi.org/10.1007/s11368-021-03051-5.

Zeglin, L.H. *et al.* (2007) ‘Microbial responses to nitrogen addition in three contrasting grassland ecosystems’, *Oecologia*, 154(2), pp. 349–359. Available at: https://doi.org/10.1007/s00442-007-0836-6

Tables and Figures

Table 2.1. 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. Bolded values indicate P < 0.05. *Sqrt* denotes square root transformation and *ln* denotes natural log transformation.

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**

Table 2.2. Correlation test results (r statistic and P values) among soil GWC and pH, N-cycling rates, and the microbial N limitation index. Bolded values indicate $P < 0.05$. *Sqrt* denotes square root transformation and *ln* denotes natural log transformation.

r, P *Soil core resolution*	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

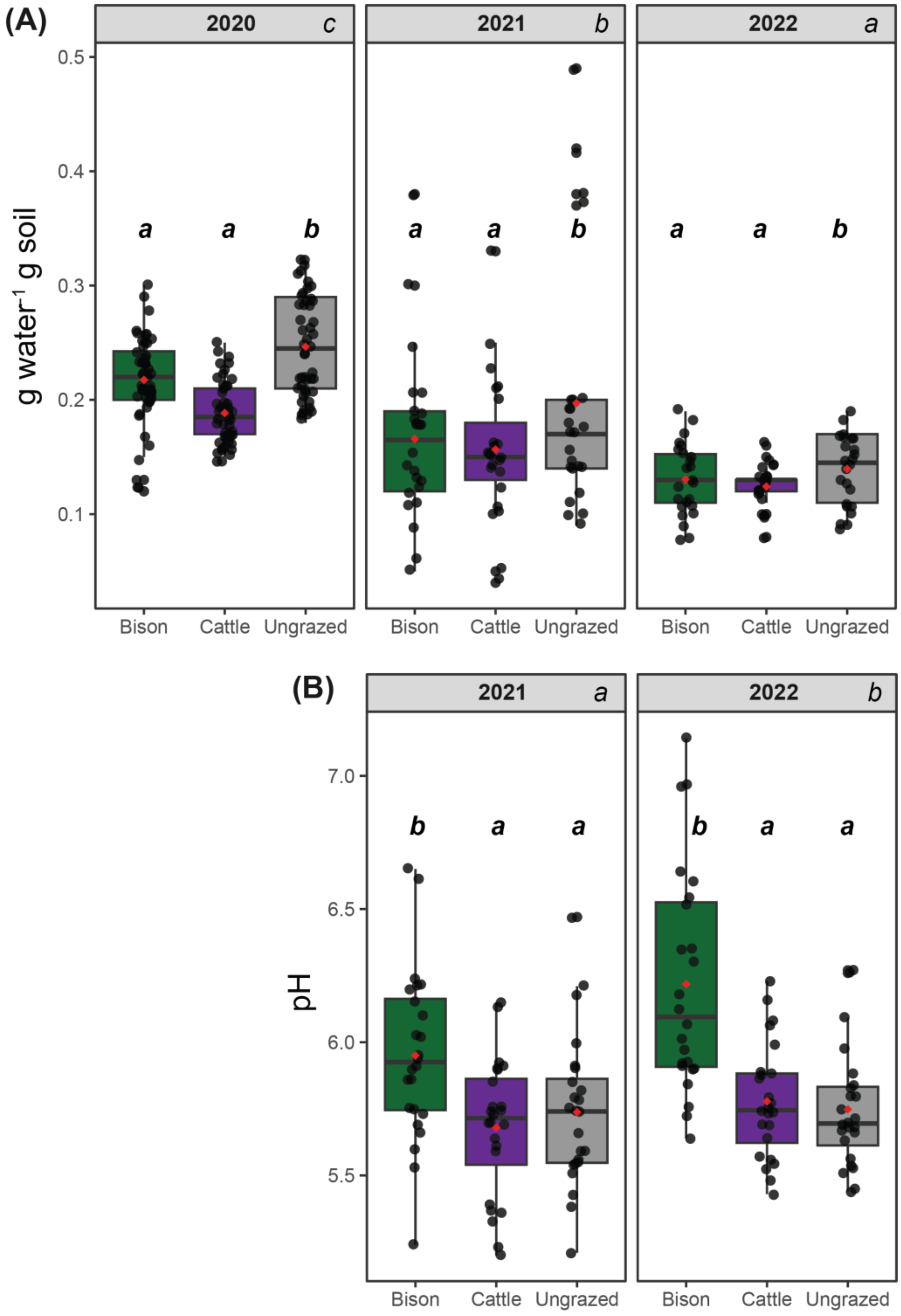


Figure 2.1. A) Soil gravimetric water content over a three-year period in grazed and ungrazed soils and (B) soil pH over a two-year period. Tukey's HSD post-hoc results are shown with different letters indicating years (top) or grazing treatments (center) that differed from each other at $P < 0.05$.

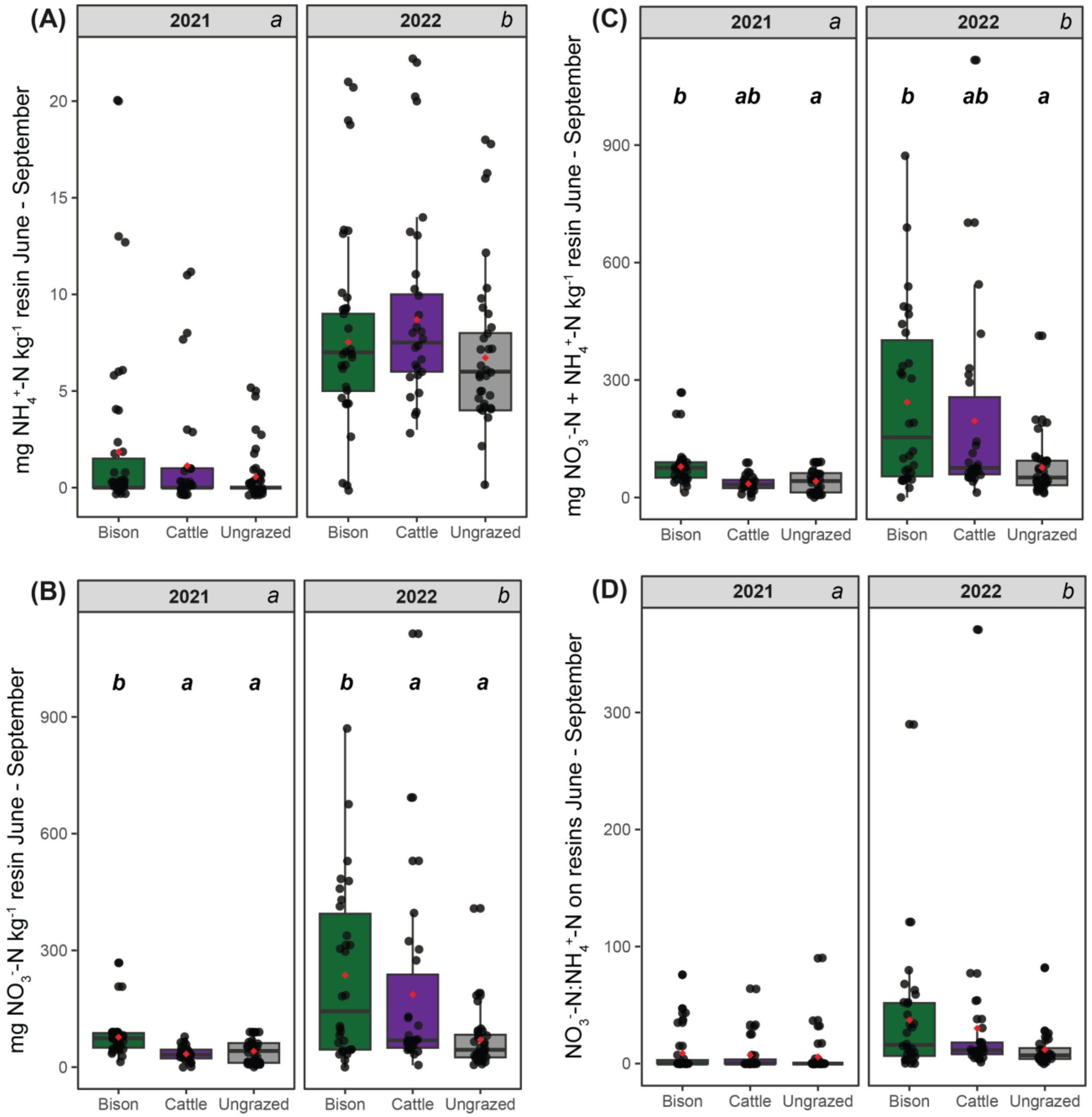


Figure 2.2. (A) NH_4^+-N, (B) NO_3^--N, (C) NO_3^--N + NH_4^+-N, and (D) NO_3^--N : NH_4^+-N sorbed to resin bags through the summer growing season in two study years. Tukey's HSD post-hoc results are shown with different letters indicating years (top) or grazing treatments (center) that differed from each other at $P < 0.05$.

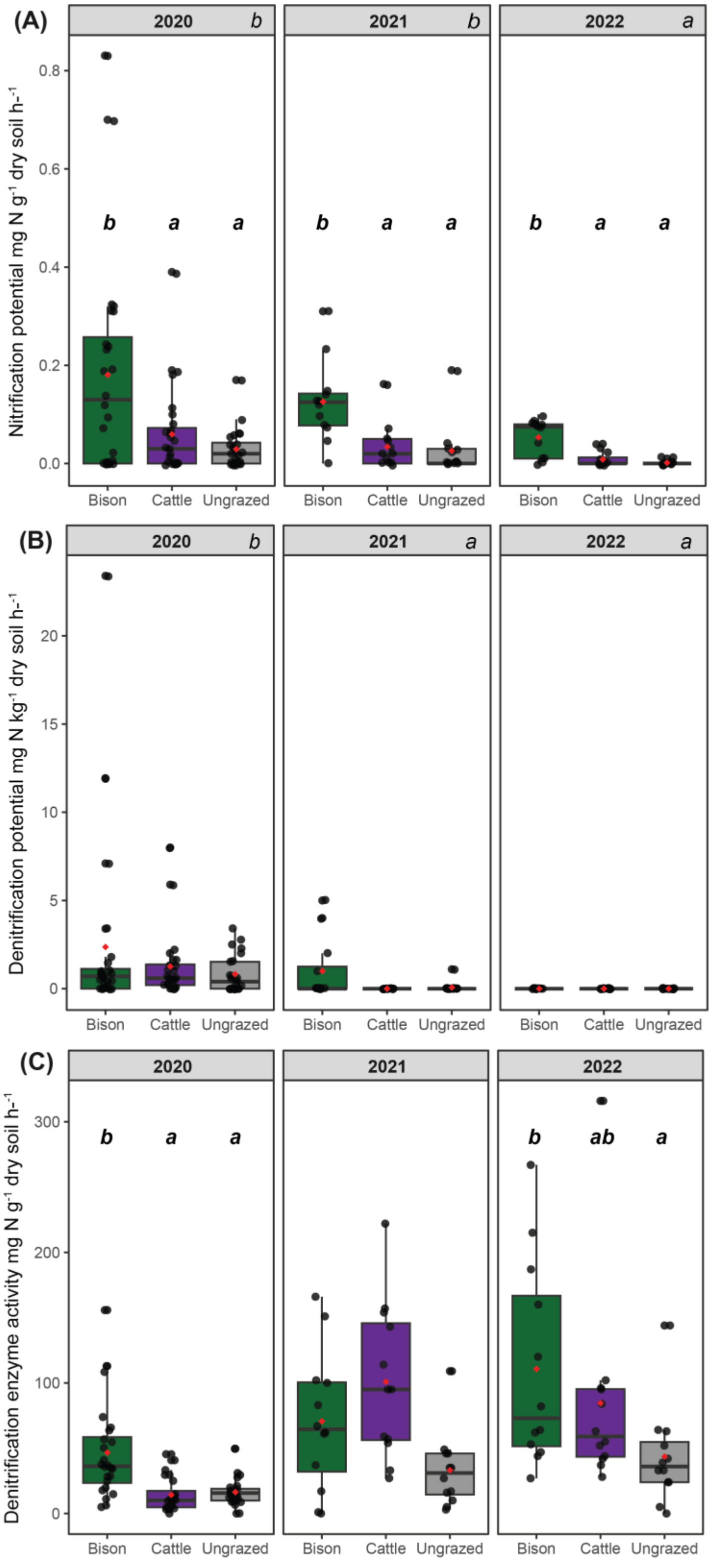


Figure 2.3. (A) NP, (B) DNP, and (c) DEA in soils from different grazing treatments over three years. Tukey's HSD post-hoc results are shown with different letters indicating years (top) or grazing treatments (center) that differed from each other at P < 0.05.

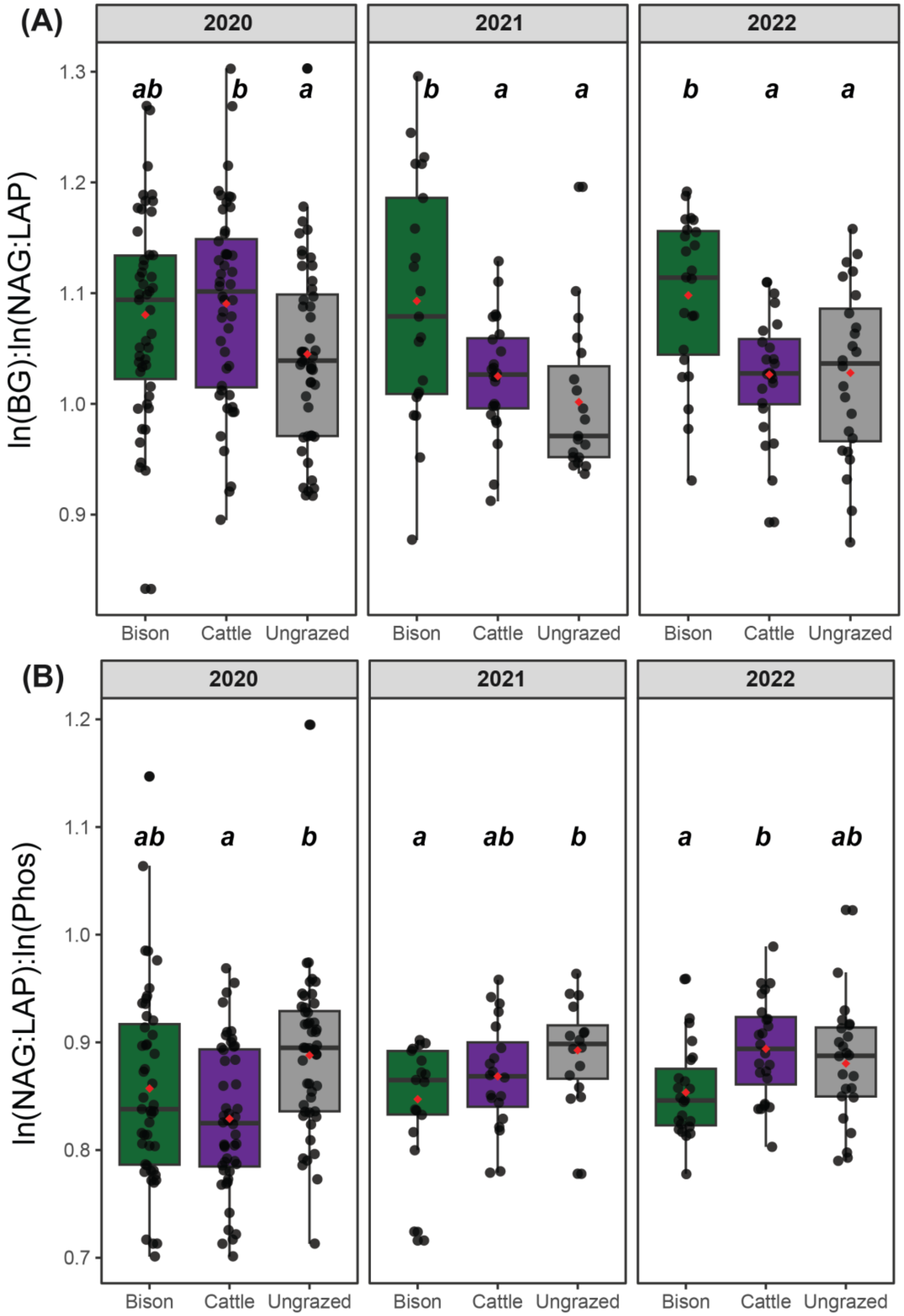


Figure 2.4. (A) ln(BG):ln(NAG:LAP) and (B) ln(NAG:LAP):ln(Phos), indicators of relative soil microbial demand for C and N, and N and P, respectively, in soils from different grazing treatments over three years. Tukey's HSD post-hoc results are shown with different letters indicating grazing treatments that differed from each other at $P < 0.05$ level each year.

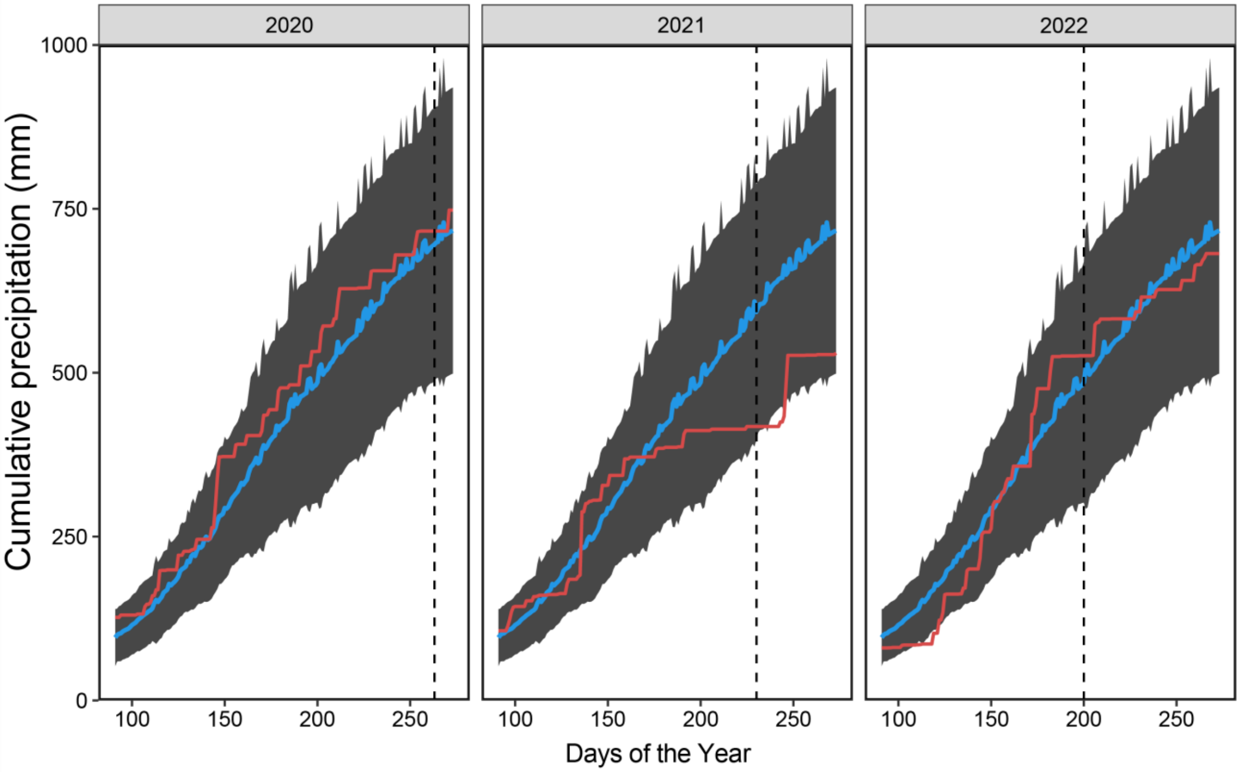


Figure 2.5. Cumulative precipitation on KPBS in the 2020, 2021, and 2023 growing seasons. Historic average precipitation is denoted by a blue line overlaying the gray one standard deviation boundaries. Average precipitation is denoted by the red line for each respective year and dashed vertical lines indicate soil sampling dates for the year.

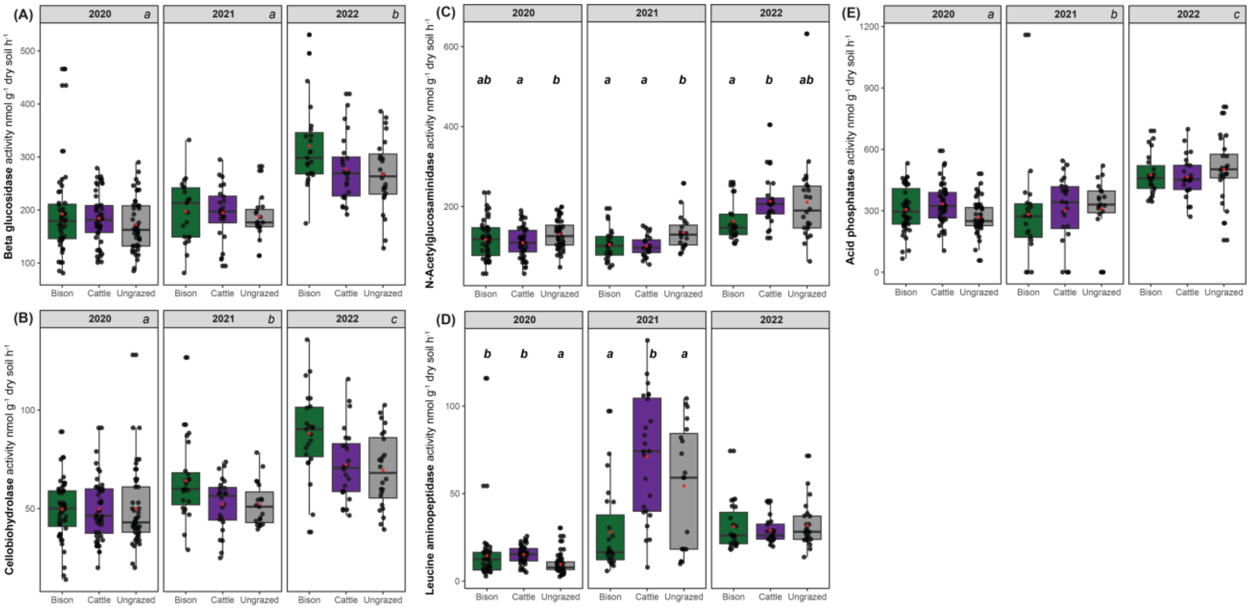


Figure 2.6. (A) Beta glucosidase, (B) cellobiohydrolase, (C) N-acetyl glucosaminidase, (D) leucine aminopeptidase, and (E) acid phosphatase enzyme activity rates (nmol substrate released g^{-1} dry soil h^{-1}) through the summer growing season in three sampling years. Tukey's HSD post-hoc results are shown with different letters indicating years or grazing treatment units within year that differed at the $P < 0.05$ level.

Chapter 3 - Plant soil feedbacks on soil fertility in bison and cattle grazed tallgrass prairie

Abstract

Bison and cattle can recycle nitrogen (N) by consuming N rich forage and depositing urine and dung for transformation into bioavailable N for incorporation into soil, microbial and plant N pools. Pre-colonization, the keystone bison numbered in the millions in north American grasslands, including the tallgrass prairie ecosystem. At present, cattle have largely replaced bison and we do not know how both grazers comparatively impact N pools and fluxes in tallgrass prairie. Thus, we investigated whether bison and cattle had similar impacts on N pools and fluxes in annually burned tallgrass prairie relative to ungrazed conditions and complied a partial N budget over a two-year period at the Konza Prairie Biological Station. We examined soil N, microbial biomass N, dung N and forage N concentration and uptake, and paired these measurements with nitrification potential, denitrification potential and other estimated fluxes from the peer reviewed literature. We found that bison and cattle impacted soil N pools differently with soil total N stocks and forage N concentrations being higher in the bison grazed treatment than either cattle grazed or ungrazed treatments. N uptake into forage was higher in the cattle grazed treatment than either bison grazed or ungrazed treatments. Soil microbial biomass N was highest in ungrazed treatments while dung N concentrations did not differ between bison or cattle. This evidence indicates that bison grazed treatments had higher N recycling potential relative to cattle grazed treatments from decomposition of N rich litter or dung, with a concomitant potential for greater N loss to the atmosphere via combustion and denitrification, while the potential for N export due to animal movement away from the study area is higher

under cattle than bison grazing. Overall this evidence provide evidence for different N cycling in bison vs cattle grazed tallgrass prairie.

Introduction

Nitrogen (N) is an essential nutrient supporting primary producers and their consumers in all ecosystems, including forage grasses that are eaten by large grazing animals in tallgrass prairies like the American plains buffalo (*Bison bison*) and domestic cattle (*Bos taurus*). Globally, grazed rangelands cover 50% of the earth's terrestrial surface (Flintan *et al.*, 2021), and support an abundance and diversity of large grazing animals. However, many grasslands do not exist to the extent that they once did, as in the case of North American tallgrass prairie, of which less than 1% remains (Samson and Knopf, 1994). In parallel with reduction of the tallgrass prairie ecosystem due to anthropogenic land use change following settler colonization, there was a mass eradication of the keystone grazer bison and managed first nation lands (Hornaday, 1913; Lott, 2002; Dunbar-Ortiz, 2014). For tens of thousands of years the keystone bison contributed to N cycling in soils across North America (Meagher, 1986; Knapp *et al.*, 1999; Lott, 2002; Zedeño, Ballenger and Murray, 2014; Davies *et al.*, 2019), and it is possible that bison eradication in the early 19th century disrupted microbial N recycling and storage in soils (Fierer *et al.*, 2013; Hawkins and Zeglin, 2022; Abraham *et al.*, 2023).

N is critical for grazers because it is indicative of the protein content or quality of the forage that bison and cattle consume, and it is also the hallmark of soil fertility in tallgrass prairie ecosystems (Robertson and Groffman, 2014). Forage growth relies on soil N stocks, which receive inputs from decomposing plant litter, grazer urine and dung deposition, and N fixation by soil microorganisms, algal soil crusts, and rhizobia in legume roots (Eisele *et al.*, 1989; Blair *et al.*, 1998; Valdés-Correcher *et al.*, 2019). Additionally, soil N retention and limitation in tallgrass prairie soils is high because of frequent controlled burning, a common land management practice and landscape driver (Dodds *et al.*, 1996; Dell, Williams and Rice, 2005; Allred *et al.*, 2011). Periodic fire, caused by lightning strikes or intentionally or accidently set by the indigenous peoples and now land managers, maintains the tallgrass prairie mosaic and is a mechanism for N loss through combustion of plant litter (Anderson, 2006; Nowacki, MacCleery and Lake, 2012; Lake and Christianson, 2019). In annually burned grasslands, however, grazing may reduce the amount of N that is lost by fire and instead increase the amount stored within large grazer biomass (de Mazancourt, Loreau and Abbadie, 1998, 1999).

Additionally, bison and cattle can increase soil N availability by consuming N containing forage and depositing bioavailable N in dung that is more easily decomposed by insects and soil microorganisms than plant material (Moore and Bower, 2001; Sitters *et al.*, 2017, 2020). Soil microbes can incorporate N from soil organic matter or dung, and mineralize nitrogenous compounds like ammonium (NH_4^+) and nitrate (NO_3^-), which are the primary N compounds that are built into microbial biomass and assimilated by plants (Blair *et al.*, 1998; Tracy and Frank, 1998; Robertson and Groffman, 2014; Singh and Gupta, 2018). Grasses can compensate for being grazed upon by growing faster, using more soil N (de Mazancourt, Loreau and Abbadie, 1998, 1999; Schoenecker, Zeigenfuss and Augustine, 2022) to support higher aboveground net primary productivity (ANPP) (Frank *et al.*, 1994; Hobbs, 1996; McNaughton, Milchunas and Frank, 1996; Singer and Schoenecker, 2003). In a parallel study, we showed significantly elevated soil N availability and N cycling rates under bison grazing relative to cattle grazing or in ungrazed prairie (Vega Anguiano, 2023), but we did not assess the extent to which the whole ecosystem N cycle was affected by grazing, or whether bison and cattle can impact the N cycle similarly (Elser and Urabe, 1999; Atkinson *et al.*, 2017).

Therefore, accounting for current N stocks and calculating a budget is useful to contextualize the past and future ecological context of large ungulate grazers on the tallgrass prairie. N budgets are very useful to quantify grazer relationships with grassland N cycles, as was done thoroughly in Yellowstone National Park (Frank *et al.*, 1994, 2000; Tracy and Frank, 1998; Singer and Schoenecker, 2003). In this investigation, we completed a partial budget of N in grazed and ungrazed tallgrass prairie, and predicted that there would be more N in forage (grass) in bison and cattle grazed tallgrass prairie than ungrazed prairie, but that bison and cattle N budgets would be similar to one another. To assess this prediction, we sampled upland soils, collected bison and cattle dung, and clipped forage in movable exclosure paired plots in annually burned watershed-scale experimental grazing treatments at the Konza Prairie Biological Station (KPBS), during the growing seasons of 2021 and 2022. From these samples, we measured total C and N in soils, soil microbial biomass, grass forage, and bison and cattle dung; and we estimated ANPP in the ungrazed and grazed areas (the latter from moveable exclosures). In combination with previously reported soil N-cycling rate measurements (Vega Anguiano, 2023), we created a partial N budget for both grazed and ungrazed prairie; we also used additional N-cycling parameters from the peer reviewed literature to estimate a more robust N budget for grazed tallgrass prairie.

Methods

Study location and site description

The Konza Prairie Biological Station (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 the Konza Prairie Long-Term

Ecological Research (KNZ LTER) site in 1980. The dominant characteristic plants of this area include *Sorgastrum 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. KPBS has maintained watershed scale treatments of variable fire return intervals since the 1970s. Bison were reintroduced to a subset of experimental watersheds in the late 1980s to early 1990s, are stocked at 0.4 ha/AUM, and are present year-round (Blair, 2023; Taylor, 2023), while cattle were introduced to another subset of watersheds in the 1990s, are present on KBPS annually from approximately April to October, and are stocked at 0.7 ha/AUM (Olson, 2023)

KPBS has supported a target herd size of 245 bison in a naturally reproducing mixed herd, and a herd size of 215 cattle in cow-calf pairs (Olson, 2023), each in separate open pastures of just over ~860 ha or ~2100 acres where the animals have free range across historical 1, 2, 4, and 20-year fire return intervals for bison treatments and of 1 and 3-year fire return intervals for cattle treatments. As such, the main differences between bison and cattle management at KPBS are months of the year present, and the minimal fire management in two subunits totaling 167 ha or 412 acres, of the bison-grazed pasture: This comparison is sufficient to investigate grazer effects on N cycling as a consequence of management, particularly relative to ungrazed prairie (Hoy, 2009; Beam, 2010).

Soil collection

Soil samples were collected during the summer growing seasons of 2020 and 2021 on the Benfield-Florence complex soil map unit (Clayey-skeletal, smectitic, mesic Udic Argiustolls and Fine, mixed, superactive, mesic Udertic Argiustolls), which covers ~288 ha of uplands on KPBS (NRCS Soil Survey Staff, 2022), and is the most consistently distributed soil mapping unit in all three grazing treatments (Figure 3.1). A stratified random sampling design was employed, by sampling along four transects per watershed grazing treatment, in a total of six watersheds: two annually burned bison grazed watersheds (N1A, N1B), two annually burned cattle grazed watersheds (C1A, C1B) and two annually burned ungrazed watersheds (1D, SpB). These transects were placed parallel to plant community sampling transects used by the KNZ LTER. Along each 10 m transect, we collected six mineral soil samples using a 2-cm diameter Oakfield corer (Oakfield, WI) to a depth of up to 15 cm. Each core was aseptically sieved using a No. 4 (4 mm) sieve to protect soil aggregates, plant roots were removed, and ~50 g of each soil sample was frozen at -20°C for storage before running soil microbial biomass and total C and N assays.

Total soil C and N

To prepare samples for total C and N analysis, dried soil samples were ground and passed through a Wiley Mill using a 40 mm-mesh screen. Approximately 0.15 g of each sample type was used to determine total C and N with a LECO TruSpec CN combustion analyzer (Laboratory Equipment Company, Michigan, USA) at the Kansas State University Soil Testing Laboratory.

Soil microbial biomass C and N

Microbial biomass C (MBC) was estimated as the exchangeable dissolved organic C (DOC) liberated after a 24-h chloroform fumigation (Vance, Brookes and Jenkinson, 1987) of the field-moist soil. DOC and inorganic N were extracted from fumigated and unfumigated soils by shaking in a 0.05 M K_2SO_4 solution (approximately 10 g soil in 40 mL extractant) for 1 h, and quantified via combustion/chromatography analysis with a Shimadzu TOC analyzer (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). Extractable DOC of the unfumigated soils is a stand-alone variable, and the excess DOC in fumigated soils was assumed to reflect MBC pool sizes. From the same K_2SO_4 extracts as DOC, ammonium-N concentration was quantified using a modified indophenol method and nitrate-N concentration was quantified using the VCl3/Greiss method, using microplate spectrophotometry (Hood-Nowotny *et al.*, 2010). All values are reported as µg C or N g^{-1} dry soil.

Soil Bulk Density

Soil bulk density estimates were downloaded in 2021 from SoilGrids (Poggio et al., 2021) a digital soil mapping product curated by the International Soil Reference and Information Center (ISRIC, Wageningen, The Netherlands) and imported into ArcPro (ESRI, Redlands, CA, USA) where geolocated soil core points 0m, 5m and 10m were paired with bulk density measurements and extracted using the Sample tool within the image analysis raster toolbox. Soil bulk density was used to convert soil N cycling rates and C and N concentrations to area-specific rates and stocks. Soil N and C content were calculated on a gram per unit area basis as follows:

$$\frac{Total\ C\ \%}{100} * Dry\ soil\ wieght\ (g) * Bulk\ density\ \left(\frac{g}{m^3}\right) * \frac{10000\ cm}{1\ m} = C\ or\ N\ g/m2$$

Dung C and N

To estimate the egested N and C pools, we collected fresh bison and cattle dung in June, July, and August 2022 in bison and cattle grazed watersheds only. Each month, 24 dung samples per grazer type (bison and cattle) were collected, for a total of 144 dung samples. Fresh dung was collected within a three-day window after deposition (Moore and Bower, 2001), by observing herds ahead of sampling: If there were at least 24 adult animals greater than 2 years old present, we marked the location for dung collection and waited until the animals left the area and then returned to collect fresh dung samples. At each location, 20 g of fresh bison and cattle dung was collected using sterile technique into 15 ml sterile conical tubes on ice for transport back to the lab. Then, 2 g of fresh dung was dried for 48 hours at 70 °C (Valdés-Correcher *et al.*, 2019), before total C and N analysis using the same approach described above for soil samples.

Forage collection and ANPP calculation

Forage samples were collected in 2021 and 2022 during the summer growing season, using a moveable enclosure approach (Knapp et al.; 2012). Moveable exclosures and unprotected plots were established parallel to soil sampling transects in annually burned bison and cattle grazed watersheds (Figure 3.9). The modified paired plot design consisted of three 1 m^2 moveable exclosures made of 2 m high cattle panel and placed along a 50 m transect on upland tallgrass prairie at 0, 16 and 38 m. Paired with each moveable exclosure was an unprotected plot, open to grazing, 4m in length and 2m in width and set laterally on the 50m transect at 8, 25, and 46 m. Each summer month (June, July, and August), moveable exclosures and unprotected plots were clipped within a 0.1 m^2 (50 x 20 cm) quadrat, then moved 4 m away from the previous location to allow fresh grass growth and collection in the next month (Knapp *et al.*, 2012). To compare grazed forage to ungrazed forage, we used samples collected by the KNZ LTER program for sample archive and ANPP estimation (Blair and Nippert, 2023). These samples are collected in early October to represent peak biomass at the end of the growing season, so, in 2022, we added a final grazed forage sampling point in October for comparison of plant tissue chemistry. All clipped plant material (forbs and grass) was dried for 48 hours in a drying oven set to 60° C to measure a dry weight, then a subsample of grass tissue only (forage) was used for total C and N analysis using the same approach described above for soil and dung samples.

To estimate aboveground net primary productivity (ANPP) in ungrazed treatments, we scaled dry weight of end-of-season clipped biomass to a m^{-2} basis. To estimate ANPP in grazed treatments, we summed the unprotected dry weight in August with the differences between protected dry weights and the unprotected dry weights of the clipped forage from June and July and August. We calculated N uptake into forage by calculating N in grazed grass based on %N in tissues each month, then summing these values in the same manner as grazed ANPP. In the case that unprotected dry grass weights was less than the protected dry grass weights in a paired location, which occurred frequently due to heterogeneity in plant distribution (34.5% of paired plot replicates), we called that amount a zero instead of a negative number, since negative ANPP is not biologically realistic.

Data analysis

All statistical analysis was done using the R programing language in the R studio interface (R Core Team, 2022). To test for the direct effects of bison vs cattle grazing monthly (individual monthly measurements) and annually (compilation of end of growing season values for respective response) on soil, dung, and forage C and N content, and ANPP, we used two-way analysis of variance (ANOVA) models for each response variable, after using diagnostic Q-Q plots and histograms to assess assumptions of statistical normality and log-transforming any variables as appropriate (soil total C, N, and C:N concentrations and stocks; grass C:N; all ANPP data). Statistical results with a P-value of < 0.05 are reported in the text.

N budget estimation

Building on the measurements of C and N stocks in soil and forage, we also estimated the movement of N through pools, based on the microbial nitrification potential (NP) and denitrification potential (DNP) activity rates from Chapter 2 (Vega Anguiano; 2023). Some N fluxes were not quantified in this thesis, and we used values from Blair et al. (1998) to fill in those parts of the estimated N budget.

Results

Soil C and N concentrations and stocks

Soil microbial biomass C and N pools were similar under bison and cattle grazing, but were higher in ungrazed treatments than both grazed treatments (MBC: F, P = 3.3, 0.044; MBN: F, P = 16.5, <0.001) (Figure 3.2). Microbial biomass C:N in cattle grazed soil was significantly higher than in both bison and ungrazed soils (F, P = 14.2, < 0.001) (Figure 3.2). Soil %C and %N were both lower in cattle grazed treatments than both bison grazed and ungrazed treatments, and soil %N was highest in bison grazed treatments (%C: F, P = 12.2, < 0.001; %N: F, P = 40.1, < 0.001) (Figure 3.2). Soil total C:N ratio was greatest in the ungrazed treatment, lowest in the bison grazed treatment, and intermediate in the cattle grazed treatment (F, P = 23.3, < 0.001) (Figure 3.2).

Soil bulk density ranged between 1.24 and 1.29 g cm^{-3}, and was higher on average in cattle grazed units than either bison or ungrazed treatments (F, P = 6.9, 0.003) (Figure 3.6). As a consequence, treatment effects on soil C and N stocks (g m^{-2}) differed slightly from effects on C and N concentrations. In bison grazed watersheds, soil C and N content per unit area was greater than both cattle grazed and ungrazed watersheds (g C m^{-2}: F, P = 8.2, 0.001; g N m^{-2}: F, P = 36.0, < 0.001) (Figure 3.5, Figure 3.6). Microbial biomass N stocks were higher in ungrazed soils than in bison or cattle grazed soils (F, P = 15.1, < 0.001) (Figure 5).

Dung C and N concentrations

Cattle dung had consistently higher water content, and tended to have higher %C, than bison dung; but differences between bison and cattle dung %N and C:N were inconsistent, with cattle dung %N higher than bison dung in June only, and bison dung C:N lower than cattle dung in August only (Table 1, Figure 3.7).

Forage C and N concentrations, ANPP, and N stocks

Forage C and N content responded to grazing and changed through the growing seasons in both 2021 and 2022 (Table 1). Forage %C varied weakly among treatments or months, being lower in August than June or July in 2021, and higher in bison grazed treatment in August 2022 (Figure 3.8). Forage %N decreased though each growing season, with the decline in 2021 beginning in August, and the decline in 2022 beginning in July; in parallel, forage %N was consistently higher in the bison grazed treatment relative to the cattle grazed treatment (Table 1, Figure 3.3). In October 2022, forage %N was highest in the bison grazed treatment, lowest in the cattle grazed treatment, and intermediate between the two in the ungrazed treatment (Figure 3.3). Forage C:N increased though each growing season, and forage C:N was consistently lower in the bison grazed treatment relative to the cattle grazed treatment (Table 1, Figure 3.3). In 2022, forage C:N increased most sharply between August and October, and was intermediate in the ungrazed treatment relative to the bison and cattle grazed treatments (Figure 3.3).

Total ANPP was higher in the bison and cattle grazed treatments than the ungrazed treatment (F, P = 29.6, < 0.001); however, forage ANPP (grass only) was higher in the cattle grazed than both bison grazed and ungrazed treatments (F, P = 8.7, 0.002), while forb was ANPP highest in the bison grazed treatment, intermediate in the cattle grazed treatment, and lowest in the ungrazed treatment (F, P = 72.6, < 0.001). Because there was more grass, N uptake into forage was higher in cattle grazed than bison grazed treatments in both 2021 and 2022 (respectively: F, P = 28.4, < 0.001; 24.0, < 0.001).

Discussion

This study’s goal was to learn how much N is present in soil, dung, and forage pools and is potentially lost via N fluxes in annually burned tallgrass prairie in bison and cattle grazed soils compared to ungrazed soils. We predicted that N cycling would be similar in bison and cattle grazed treatments, but higher than in ungrazed treatments. By measuring soil and forage N, we found that bison and cattle impacted soil N pools differently. While soil total N stocks and forage N concentrations were higher in the bison grazed treatment than either cattle grazed or ungrazed treatments, N uptake into forage was higher in the cattle grazed treatment than either bison grazed or ungrazed treatments. Also, soil microbial biomass N was highest in ungrazed treatments, while dung N concentrations did not differ between bison or cattle. Implications of these results include that in bison grazed treatments, there is higher potential relative to cattle grazed treatments for recycling of N via decomposition of higher nitrogen litter or dung, but also higher potential for N loss to the atmosphere via combustion and denitrification; whereas potential for export of N due to animal movement away from the study area is higher under cattle than bison grazing.

Soil microbial N stocks and cycling rates

Soil MBC and MBN in bison and cattle grazed treatments were similar, but lower than the ungrazed treatments by 8-11% and 20-28%, respectively (Figure 3.2). Microbial biomass is known to vary over both space and time, and is affected by soil bulk density and water content, which are related to habitat space and nutrient supply for soil microorganisms (Garcia and Rice, 1994; Tracy and Frank, 1998), and by plant litter biomass and belowground root biomass, which both promote C inputs into soil (Johnson and Matchett, 2001; Singh and Gupta, 2018). Soil water content was slightly higher in ungrazed treatments during the study period (Vega Anguiano et al., 2023); additionally, while we did not measure root biomass in this study, it is expected to be higher in ungrazed treatments, which in turn increases C rich root exudates, stimulating microbial biomass growth (Tracy and Frank, 1998; Johnson and Matchett, 2001; Nippert *et al.*, 2011). Notably, root production is stimulated by N limitation, as plants send more energy to roots to forage for scarcer resources, and N limitation is greater in ungrazed than grazed treatments at this site (Johnson and Matchett, 2001; Nippert *et al*., 2011 Vega Anguiano, 2023). Like all ecosystem C or N stocks, pool size may not predict turnover rate; in fact, lower microbial biomass levels often co-occur with higher growth and death rates of soil microorganisms, and this faster turnover supports higher soil N availability (Schmidt *et al.*, 2007). In this study, bison grazed soils also supported higher N availability and microbial N cycling rates, consistent with a higher degree of soil fertility relative to ungrazed treatments, while cattle grazed soils were intermediate between the two (Vega Anguiano et al., 2023).

Forage quality consistently higher in bison grazed prairie

As perennial grass grows each season, the tissue concentration of N and other nutrients is diluted by the increasing amount of carbon fixed over time; then, at the end of the growing season, some tissue N is retranslocated to roots and rhizomes and stored over-winter. This phenomenon is clearly shown in the decrease in forage %N, and increase in C:N, through the 2021 and 2022 growing seasons (Figure 3.8). Forage N concentration in bison grazed treatments was 21-38% higher than cattle grazed treatments through the growing season, and 81% higher after the end of the season. In October, N concentration remained 65% higher in bison grazed forage than in ungrazed grass, and 10% higher in ungrazed forage than in cattle grazed treatments, suggesting most N retranslocation in cattle grazed grasses, and least in bison grazed grasses. While this may not seem N efficient for the plant, it is well established that plant litter with lower C:N ratio decomposes faster and supports higher soil N availability (Parton *et al.*, 2007) thus higher litter quality is a positive feedback that supports higher soil fertility. While some of this material will be volatilized in the spring during prescribed fire, a significant proportion of litter N (~35%) can return to the soil over one winter of decomposition (Cotrufo *et al.*, 2015).

Similar dung N content in bison and cattle

In our study, despite different grass forage N content, bison and cattle dung had similar %N, but differed in dung %C and moisture content, which suggests differences in diet or N use efficiency by the animals. Nutrient contents of cattle dung vary by study (Williams and Haynes, 1995; Gupta, Aneja and Rana, 2016; Rayne and Aula, 2020; Joseph *et al.*, 2022), but there is little published information on bison dung (Moore and Bower, 2001). Interestingly, the inoculation of prairie grass fed bison rumen microbiota into the cattle rumen was recently shown to increase animal N uptake (Ribeiro *et al.*, 2017), suggesting that cattle and bison can have similar N metabolic efficiency, which is related to how much N is left over in fresh dung (Moore and Bower, 2001). Animal diet composition also affects N uptake, and in other studies, cattle tend to consume more forbs than bison which may contribute to higher %C (Coppedge, Leslie and Shaw, 1998; Moore and Bower, 2001); of course, we know that grass forage had higher C:N in cattle units, so some of this excess C may have been egested in dung. Dung moisture content is affected by thermoregulation and hydration needs, so differences in dung moisture are partially related to diet since some water is also stored in forage plants (Moore and Bower, 2001; Towne, Hartnett and Cochran, 2005), and also to bison's more efficient thermoregulation and lower drinking water demand than cattle (Christopherson, Hudson and Christophersen, 1978; McMillan *et al.*, 2021).

As with plant litter, dung decomposition also represents an important return of N to the soil in grazed grasslands (Knapp *et al.*, 1999). Because of the similar N content, for dung to support another stronger positive feedback on soil N availability under bison relative to cattle grazing, it would need to be more abundant by total mass on the ground; however, because we did not measure areal density of dung, we do not know whether or not this is the case. We do know that bison are on the study site year round while cattle only graze for six months out of the year, which would also support greater dung deposition intensity, so this consumer driven nutrient return of labile organic N pool remains a viable mechanism for supporting greater soil fertility under bison grazing (Sitters and Olde Venterink, 2015; Sitters *et al.*, 2017).

ANPP and grazed forage N

Interestingly, grass forage ANPP in cattle grazed treatments was 25-33% greater than in bison grazed watersheds, and also greater than in ungrazed watersheds; this also meant that more N per unit area was assimilated into grasses under cattle grazing, despite the lower forage quality there (Figure 3.4). Further, grazed forage N was similar under cattle and bison grazing: It seems that cattle ate more grass than bison did because there was more present, and this balanced the total amount of N moving into the animals from higher quality forage in the bison grazed treatment (Figure 3.5). However, total ANPP in bison grazed treatments was highest due to the larger amount of forb production (Figure 3.4). At our study site, bison grazed treatments have a higher diversity and proportion of forbs than grasses (Ratajczak *et al.*, 2022), such that the differences in grass ANPP may be attributed to the fact that cattle treatments have proportionally more grass cover and lower diversity and cover of forbs. This diversity effect could be related to long-term differences in grazing preferences between cattle and bison, or in overall grazing pressure and grass removal (Hickman *et al.*, 2004). Like forage N, the N in uneaten forb litter may be decomposed and returned to the soil, or lost to volatilization during prescribed fire: However, we did not measure forb N content, so cannot estimate the relative importance of forb N within the full N cycle.

Higher soil N in bison grazed soils

Soil N concentrations and stocks on the Benfield-Florence complex upland soils that we sampled were on average 26-27% higher on bison grazed than ungrazed or cattle grazed treatments, respectively, and also had lowest soil C:N (Figure 3.2, Figure 3.5). A different study at the same research station, using data from deeper lowland soils, found that bison grazing did not affect total soil N concentrations, but it did decrease soil C:N (Connell, Nippert and Blair, 2020). While contrasting soil development histories most likely explains the difference between studies, it is important to keep in mind that soil organic C and N levels are also heterogeneous at small spatial scales within a soil type, so data reported in any study do not completely represent landscape scale variation in soil properties. The Benfield-Florence soils that we measured are particularly rocky, complex, and heterogeneous. Still, if our soil measurements are representative, because this soil complex covers a large area, extrapolation on a soil map unit basis suggests a significant change in soil N status as a result of grazing management. It is also important to note that while our study shows clear evidence for plant-soil feedbacks promoting soil fertility under bison grazing, many of the parameters that measure this phenomenon were intermediate under cattle grazing (Vega Anguiano, 2023), including soil C:N, which was lower in the cattle grazed than the ungrazed treatment (Figure 3.2); so, we cannot rule out the possibility that cattle grazing might influence soil and forage quality more strongly under more intensive management.

Budgeting N and extrapolation

To put our data into context, we created a current partial N budget using back of the envelope calculations to extrapolate mean N pools and fluxes in equivalent units (g m^2) (Murillo-Gonzalez and Burkholder, 2022) (Figure 3.5). For some pieces of the budget, we turned to the literature to find relevant estimates, including: N lost by fire, estimated at 1-4 g m^{-2} y^{-1} (Blair *et al.*, 1998); and N fixed by free-living or plant-associated bacteria, estimated at 0.1-0.5 g m^{-2} y^{-1} (Eisele *et al.*, 1989; Blair *et al.*, 1998). Our estimates for soil total N, MBN, and denitrification potential are within range of the most recent N budget conducted for this tallgrass prairie site (Blair *et al.*, 1998); but previous studies reported lower nitrification than our estimates (Groffman, Rice and Tiedje, 1993) and very low potential soil nitrate leaching (Blair *et al.*, 1998). The nitrification assay that we used measures the maximum potential of nitrate production under ideal conditions, and it is unlikely that gross nitrification ever reaches this rate *in situ*: Instead, the higher potential activity that we measured represents a larger population of soil nitrifying microorganisms in bison treatments, which can support the higher soil fertility and forage quality that we also observed.

Despite the accumulation of evidence for positive feedbacks between bison grazing, soil N cycling rates, and organic N return to the soil, it was still a surprise that this added up to enough N to augment total soil N pools in the bison grazed treatment, and not in the cattle or ungrazed treatments. Unmeasured factors that might help balance this budget include higher N return to soil from forb litter, dung, or urine in the bison grazed treatment, higher N input to the whole cycle, i.e., more N fixation, in the bison grazed treatment, and/or higher N loss from the whole cycle in the cattle grazed treatment. With higher forb cover in bison-grazed prairies (Ratajczak et al. 2022), there may also be higher abundance of N-fixing legumes; however, legume abundance does not predict N-fixation activity, and rates of actual N-fixation by plant symbionts have not been measured at the study site. As previously noted, bison here are managed as year-round residents, while cattle graze only during the growing season: This entails both a higher amount of forage N intake than we quantified here, as well as a larger amount of N egestion, under bison grazing than cattle grazing. Further, cattle may export N from the study area both by leaving over-winter, and by moving more frequently through the lowland and riparian portions of the landscape that we did not sample in this study. In other ecosystems, including grazed and logged forested systems, the export of C and N from the system can result from land management (Kreutzweiser, Hazlett and Gunn, 2008; Piñeiro *et al.*, 2010). Future research should better constrain ecosystem N inputs and losses in both ungrazed and grazed prairie.

Conclusion

In sum, N cycling in bison grazed treatments was faster, and the soils also contain larger N pools, so N was more conserved overall, leading to higher forage quality as a result. This was not the case in cattle grazed treatments, which could be related to lower grazing intensity (either by different management or through cattle choice to spend less time on upland soils), and/or differences in diet selection by cattle and consequent shifts in vegetation cover. In a recent global metanalysis, heavier grazing decreased plant, microbial, and soil C:N, while light to moderate grazing had weaker effects (He *et al.*, 2020). Critically, grasslands globally, including tallgrass prairie in the North American Great Plains, evolved with large ungulate grazers like bison, and the “grazing lawn” phenomenon, in which higher intensity animal use supports higher forage quality in a positive feedbacks, is a widespread feature of grazed grasslands (McNaughton, 1984; Hempson *et al.*, 2015). Whether or not cattle can sustain grazing lawns, and what level of grazing intensity entails “overgrazing”, are ongoing topics of debate (Venter, Hawkins and Cramer, 2019; Hempson *et al.*, 2022; Joseph and Seymour, 2022). In addition to grazing intensity, we suggest that tallgrass prairie grazing management should also consider the possibility of N movement within pastures as well as export from pastures in the long term.

Further, while the dilution of nutrients in plant material for consumption in uncultivated ecosystems is of increasing concern (Welti *et al.*, 2020; Mason *et al.*, 2022) it is not clear whether grazed grasslands have experienced the same level of N decline over recent decades. If grazing treatments here are reframed as land uses, rangelands are common in the North American Great Plains, and our estimates may be extrapolated to indicate N levels under similar land management at larger scales. Finally, this work indicates that grazing is a vital process to grassland soil fertility, which also suggests that the extirpation of bison in the early 19th century disrupted N pools and fluxes across the continent. At that time there were tens of millions of bison in North America, and with their reduction of habitat and population down to the thousands (Hornaday, 1887) there was likely a disruption of past levels of N cycling leading to an altered landscape today. It will be difficult to estimate how much N went unrecycled following bison slaughter, or how much N was stored in tallgrass prairie soil before bison loss, but our work makes it clear that the presence of bison on the tallgrass prairie can lead to a significantly different N cycle.

References Cited

Abraham, A.J. *et al.* (2023) ‘Understanding anthropogenic impacts on zoogeochemistry is essential for ecological restoration’, *Restoration Ecology*, 31(3), p. e13778. Available at: https://doi.org/10.1111/rec.13778.

Allred, B.W. *et al.* (2011) ‘Ungulate preference for burned patches reveals strength of fire–grazing interaction’, *Ecology and Evolution*, 1(2), pp. 132–144. Available at: https://doi.org/10.1002/ece3.12.

Anderson, R.C. (2006) ‘Evolution and origin of the Central Grassland of North America: climate, fire, and mammalian grazers1’, *The Journal of the Torrey Botanical Society*, 133(4), pp. 626–647. Available at: https://doi.org/10.3159/1095-5674(2006)133[626:EAOOTC]2.0.CO;2.

Atkinson, C.L. *et al.* (2017) ‘Consumer-driven nutrient dynamics in freshwater ecosystems: from individuals to ecosystems’, *Biological Reviews*, 92(4), pp. 2003–2023. Available at: https://doi.org/10.1111/brv.12318.

Beam, M. (2010) ‘Beef Production in the Flint Hills’. Available at: https://newprairiepress.org/cgi/viewcontent.cgi?article=1010&context=sfh.

Blair, J. (2023) ‘CBH01 Konza Prairie bison herd information’. Environmental Data Initiative. Available at: https://doi.org/10.6073/PASTA/4912DE8FAF25E93D45551CB1891E7FAD.

Blair, J. and Nippert, J. (2023) ‘PAB01 Aboveground net primary productivity of tallgrass prairie based on accumulated plant biomass on core LTER watersheds (001d, 004b, 020b)’. Environmental Data Initiative. Available at: https://doi.org/10.6073/PASTA/BFD08C670671CE975AF4B17CDA2D5179.

Blair, J.M. *et al.* (1998) ‘Terrestrial nutrient cycling in tallgrass prairie’, *Grassland dynamics: long-term ecological research in tallgrass prairie. Oxford University Press, New York*, pp. 222–243.

Bouché, P., Lejeune, P. and Vermeulen, C. (2012) ‘How to count elephants in West African savannahs? Synthesis and comparison of main gamecount methods’, *Biotechnol. Agron. Soc. Environ.*, p. 15.

Christopherson, R.J., Hudson, R.J. and Christophersen, M.K. (1978) ‘Seasonal energy expidentures and thermoregulatory responses of bison and cattle’, *Canadian Journal of Animal Science* [Preprint]. Available at: https://doi.org/10.4141/cjas79-077.

Connell, R.K., Nippert, J.B. and Blair, J.M. (2020) ‘Three Decades of Divergent Land Use and Plant Community Change Alters Soil C and N Content in Tallgrass Prairie’, *Journal of Geophysical Research: Biogeosciences*, 125(8), p. e2020JG005723. Available at: https://doi.org/10.1029/2020JG005723.

Coppedge, B.R., Leslie, D.M. and Shaw, J.H. (1998) 'Botanical composition of bison diets on tallgrass prairie in Oklahoma', *51* [Preprint]. Available at: https://doi.org/10.2307/4003321.

Cotrufo, M.F. *et al.* (2015) 'Formation of soil organic matter via biochemical and physical pathways of litter mass loss', *Nature Geoscience*, 8(10), pp. 776–779. Available at: https://doi.org/10.1038/ngeo2520.

Davies, G. *et al.* (2019) 'Isotopic paleoecology of Northern Great Plains bison during the Holocene', *Scientific Reports*, 9(1), p. 16637. Available at: https://doi.org/10.1038/s41598-019-52873-4.

Dell, C.J., Williams, M.A. and Rice, C.W. (2005) 'Partitioning of Nitrogen Over Five Growing Seasons in Tallgrass Prairie', *Ecology*, 86(5), pp. 1280–1287. Available at: https://doi.org/10.1890/03-0790.

Dodds, W. k. *et al.* (1996) 'Nitrogen Transport from Tallgrass Prairie Watersheds', *Journal of Environmental Quality*, 25(5), pp. 973–981. Available at: https://doi.org/10.2134/jeq1996.00472425002500050007x.

Dunbar-Ortiz, R. (2014) *An Indigenous Peoples' History of the United States*. Beacon Press.

Eisele, K.A. *et al.* (1989) 'Effects of available P and N:P ratios on non-symbiotic dinitrogen fixation in tallgrass prairie soils', *Oecologia*, 79(4), pp. 471–474. Available at: https://doi.org/10.1007/BF00378663.

Elser, J.J. and Urabe, J. (1999) 'The Stoichiometry of Consumer-Driven Nutrient Recycling: Theory, Observations, and Consequences', *Ecology*, 80(3), pp. 735–751. Available at: https://doi.org/10.1890/0012-9658(1999)080[0735:TSOCDN]2.0.CO;2.

Fierer, N. *et al.* (2013) 'Reconstructing the Microbial Diversity and Function of Pre-Agricultural Tallgrass Prairie Soils in the United States', *Science*, 342(6158), pp. 621–624. Available at: https://doi.org/10.1126/science.1243768.

Flintan, F. *et al.* (2021) 'Rangelands Atlas'. Global. Available at: https://www.globallandscapesforum.org/publication/rangelands-atlas/ (Accessed: 7 November 2023).

Frank, D.A. *et al.* (1994) 'The biogeochemistry of a north-temperate grassland with native ungulates: Nitrogen dynamics in Yellowstone National Park', *Biogeochemistry*, 26(3), pp. 163–188. Available at: https://doi.org/10.1007/BF00002905.

Frank, D.A. *et al.* (2000) 'Ungulate stimulation of nitrogen cycling and retention in Yellowstone Park grasslands', *Oecologia*, 123(1), pp. 116–121. Available at: https://doi.org/10.1007/s004420050996.

Garcia, F.O. and Rice, C.W. (1994) 'Microbial biomass dynamics in tallgrass prairie', *Soil Science Society of America Journal*, 58(3), pp. 816–823.

Groffman, P.M., Rice, C.W. and Tiedje, J.M. (1993) ‘Denitrification in a Tallgrass Prairie Landscape’, *Ecology*, 74(3), pp. 855–862. Available at: https://doi.org/10.2307/1940811.

Gupta, K.K., Aneja, K.R. and Rana, D. (2016) ‘Current status of cow dung as a bioresource for sustainable development’, *Bioresources and Bioprocessing*, 3(1), p. 28. Available at: https://doi.org/10.1186/s40643-016-0105-9.

Hawkins, Jaide.H. and Zeglin, L.H. (2022) ‘Microbial dispersal, including bison dung vectored dispersal, increases soil microbial diversity in a grassland ecosystem’, *Frontiers in Microbiology* [Preprint]. Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.825193/abstract.

He, M. *et al.* (2020) ‘Grazing intensity significantly changes the C : N : P stoichiometry in grassland ecosystems’, *Global Ecology and Biogeography*, 29(2), pp. 355–369. Available at: https://doi.org/10.1111/geb.13028.

Hempson, G.P. *et al.* (2015) ‘Ecology of grazing lawns in Africa’, *Biological Reviews*, 90(3), pp. 979–994. Available at: https://doi.org/10.1111/brv.12145.

Hempson, G.P. *et al.* (2022) ‘Grazing lawns and overgrazing in frequently grazed grass communities’, *Ecology and Evolution*, 12(9), p. e9268. Available at: https://doi.org/10.1002/ece3.9268.

Hickman, K.R. *et al.* (2004) ‘Grazing management effects on plant species diversity in tallgrass prairie’, *Journal of Range management*, 57(1), pp. 58–65.

Hobbs, N.T. (1996) ‘Modification of Ecosystems by Ungulates’, *The Journal of Wildlife Management*, 60(4), pp. 695–713. Available at: https://doi.org/10.2307/3802368.

Hood-Nowotny, R. *et al.* (2010) ‘Alternative Methods for Measuring Inorganic, Organic, and Total Dissolved Nitrogen in Soil’, *Soil Science Society of America Journal*, 74(3), pp. 1018–1027. Available at: https://doi.org/10.2136/sssaj2009.0389.

Hornaday, W.T. (1887) ‘The extermination of the American bison, with a sketch of its discovery and life history’, *Report of the United States National Museum for the year ending June 30, 1887 (Pt. 2 of the Annual Report of the Board of Regents of the Smithsonian Institution for the year ending June 30, 1887.)* [Preprint]. Available at: https://repository.si.edu/bitstream/handle/10088/29938/Hornaday_1887_366-549.pdf (Accessed: 7 November 2023).

Hornaday, W.T. (William T. (1913) *Our vanishing wild life; its extermination and preservation*. New York, C. Scribner’s sons. Available at: http://archive.org/details/ourvanishingwild00horn (Accessed: 18 April 2023).

Hoy, J. (2009) ‘Cattle in the Flint Hills’. Available at: https://newprairiepress.org/sfh/2009/flinthills/6.

Johnson, L.C. and Matchett, J.R. (2001) ‘Fire and grazing regulate belowground processes in tallgrass prairie’, 82(12).

Joseph, G.S. and Seymour, C.L. (2022) ‘Are Madagascar’s obligate grazing-lawns ancient and evolved with endemic herbivores, or recently selected by introduced cattle?’, *Biology Letters*, 18(9), p. 20220212. Available at: https://doi.org/10.1098/rsbl.2022.0212.

Joseph, R. *et al.* (2022) ‘Comparative analysis on bioactive compounds presents in dung material of Bos taurus and Bos indicus’, *Materials Today: Proceedings*, 48, pp. 181–185. Available at: https://doi.org/10.1016/j.matpr.2020.06.457.

Knapp, A.K. *et al.* (1999) ‘The Keystone Role of Bison in North American Tallgrass Prairie: Bison increase habitat heterogeneity and alter a broad array of plant, community, and ecosystem processes’, *BioScience*, 49(1), pp. 39–50. Available at: https://doi.org/10.1525/bisi.1999.49.1.39.

Knapp, A.K. *et al.* (2012) ‘A test of two mechanisms proposed to optimize grassland aboveground primary productivity in response to grazing’, *Journal of Plant Ecology*, 5(4), pp. 357–365. Available at: https://doi.org/10.1093/jpe/rts020.

Kreutzweiser, D.P., Hazlett, P.W. and Gunn, J.M. (2008) ‘Logging impacts on the biogeochemistry of boreal forest soils and nutrient export to aquatic systems: A review’, *Environmental Reviews*, 16(NA), pp. 157–179. Available at: https://doi.org/10.1139/A08-006.

Lake, F.K. and Christianson, A.C. (2019) ‘Indigenous Fire Stewardship’, in S.L. Manzello (ed.) *Encyclopedia of Wildfires and Wildland-Urban Interface (WUI) Fires*. Cham: Springer International Publishing, pp. 1–9. Available at: https://doi.org/10.1007/978-3-319-51727-8_225-1.

Lott, D.F. (2002) *American Bison: A Natural History*. University of California Press.

Mason, R.E. *et al.* (2022) ‘Evidence, causes, and consequences of declining nitrogen availability in terrestrial ecosystems’, *Science*, 376(6590), p. eabh3767. Available at: https://doi.org/10.1126/science.abh3767.

de Mazancourt, C., Loreau, M. and Abbadie, L. (1998) ‘Grazing optimization and nutrient cycling: When do herbivores enhance plant production?’, *Ecology*, 79(7), pp. 2242–2252. Available at: https://doi.org/10.1890/0012-9658(1998)079[2242:GOANCW]2.0.CO;2.

de Mazancourt, C., Loreau, M. and Abbadie, L. (1999) ‘Grazing Optimization and Nutrient Cycling: Potential Impact of Large Herbivores in a Savanna System’, *Ecological Applications*, 9(3), pp. 784–797. Available at: https://doi.org/10.1890/1051-0761(1999)009[0784:GOANCP]2.0.CO;2.

McMillan, N.A. *et al.* (2021) ‘Are bison movements dependent on season and time of day? Investigating movement across two complex grasslands’, *Ecosphere*, 12(1), p. e03317. Available at: https://doi.org/10.1002/ecs2.3317.

McNaughton, S.J. (1984) ‘Grazing Lawns: Animals in Herds, Plant Form, and Coevolution’, *The American Naturalist*, 124(6), pp. 863–886. Available at: https://doi.org/10.1086/284321.

McNaughton, S.J., Milchunas, D.G. and Frank, D.A. (1996) ‘How can net Primary Productivity be Measured in Grazing Ecosystems?’, *Ecology*, 77(3), pp. 974–977. Available at: https://doi.org/10.2307/2265518.

Meagher, M. (1986) ‘Bison bison’, *Mammalian Species*, (266), pp. 1–8.

Moore, A.J. and Bower, N.W. (2001) ‘Chemometric Analysis of Compositional Variation in Bison and Cow Patties: A Biogeochemistry?Environmental Chemistry Experiment’, *The Chemical Educator*, 6(2), pp. 86–90. Available at: https://doi.org/10.1007/s00897010459a.

Murillo-Gonzalez, G. and Burkholder, E.W. (2022) ‘What decisions do experts make when doing back-of-the-envelope calculations?’, *Physical Review Physics Education Research*, 18(1), p. 010125. Available at: https://doi.org/10.1103/PhysRevPhysEducRes.18.010125.

Nippert, J.B., Wieme, R.A., Ocheltree, T.W. and Craine, J.M., 2012. Root characteristics of C 4 grasses limit reliance on deep soil water in tallgrass prairie.in *Plant and Soil*, 355, pp.385-394.

Nowacki, G.J., MacCleery, D.W. and Lake, F.K. (2012) ‘Native Americans, Ecosystem Development, and Historical Range of Variation’, in *Historical Environmental Variation in Conservation and Natural Resource Management*. John Wiley & Sons, Ltd, pp. 76–91. Available at: https://doi.org/10.1002/9781118329726.ch6.

NRCS Soil Survey Staff (2022) ‘Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture’. Available at: http://websoilsurvey.sc.egov.usda.gov/ (Accessed: 14 June 2022).

Olson, K.C. (2023) ‘PBG06 Cattle grazing and cattle performance in the Patch-Burn Grazing experiment at Konza Prairie’. Environmental Data Initiative. Available at: https://doi.org/10.6073/PASTA/28C9AA49E942E3BD489A05E867F38ECC.

Parton, W. *et al.* (2007) ‘Global-Scale Similarities in Nitrogen Release Patterns During Long-Term Decomposition’, *Science*, 315(5810), pp. 361–364. Available at: https://doi.org/10.1126/science.1134853.

Piñeiro, G. *et al.* (2010) ‘Pathways of Grazing Effects on Soil Organic Carbon and Nitrogen’, *Rangeland Ecology & Management*, 63(1), pp. 109–119. Available at: https://doi.org/10.2111/08-255.1.

Ratajczak, Z. *et al.* (2022) ‘Reintroducing bison results in long-running and resilient increases in grassland diversity’, *Proceedings of the National Academy of Sciences*, 119(36), p. e2210433119. Available at: https://doi.org/10.1073/pnas.2210433119.

Rayne, N. and Aula, L. (2020) ‘Livestock manure and the impacts on soil health: A review’, *Soil Systems*, 4(4), p. 64.

R Core Team (2022). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ribeiro, G.O. *et al.* (2017) ‘Repeated inoculation of cattle rumen with bison rumen contents alters the rumen microbiome and improves nitrogen digestibility in cattle’, *Scientific Reports*, 7(1), p. 1276. Available at: https://doi.org/10.1038/s41598-017-01269-3.

Robertson, G.P. and Groffman, P.M. (2014) ‘Nitrogen Transformations’, in E.A. Paul (ed.) *Soil Microbiology, Ecology and Biochemistry (Fourth Edition)*. San Diego: Academic Press, pp. 341–364. Available at: https://www.elsevier.com/books/soil-microbiology-ecology-and-biochemistry/paul/978-0-12-415955-6 (Accessed: 14 March 2021).

Samson, F. and Knopf, F. (1994) ‘Prairie Conservation in North America’, *BioScience*, 44(6), pp. 418–421. Available at: https://doi.org/10.2307/1312365.

Schmidt, S.K. *et al.* (2007) ‘Biogeochemical Consequences of Rapid Microbial Turnover and Seasonal Succession in Soil’, *Ecology*, 88(6), pp. 1379–1385. Available at: https://doi.org/10.1890/06-0164.

Schoenecker, K.A., Zeigenfuss, L.C. and Augustine, D.J. (2022) ‘Can grazing by elk and bison stimulate herbaceous plant productivity in semiarid ecosystems?’, *Ecosphere*, 13(4), p. e4025. Available at: https://doi.org/10.1002/ecs2.4025.

Singer, F.J. and Schoenecker, K.A. (2003) ‘Do ungulates accelerate or decelerate nitrogen cycling?’, *Forest Ecology and Management*, 181(1), pp. 189–204. Available at: https://doi.org/10.1016/S0378-1127(03)00133-6.

Singh, J.S. and Gupta, V.K. (2018) ‘Soil microbial biomass: A key soil driver in management of ecosystem functioning’, *Science of The Total Environment*, 634, pp. 497–500. Available at: https://doi.org/10.1016/j.scitotenv.2018.03.373.

Sitters, J. *et al.* (2017) ‘The Stoichiometry of Nutrient Release by Terrestrial Herbivores and Its Ecosystem Consequences’, *Frontiers in Earth Science*, 5. Available at: https://www.frontiersin.org/article/10.3389/feart.2017.00032 (Accessed: 22 February 2022).

Sitters, J. *et al.* (2020) ‘Nutrient availability controls the impact of mammalian herbivores on soil carbon and nitrogen pools in grasslands’, *Global Change Biology*, 26(4), pp. 2060–2071. Available at: https://doi.org/10.1111/gcb.15023.

Sitters, J. and Olde Venterink, H. (2015) ‘The need for a novel integrative theory on feedbacks between herbivores, plants and soil nutrient cycling’, *Plant and Soil*, 396(1), pp. 421–426. Available at: https://doi.org/10.1007/s11104-015-2679-y.

Taylor, J. (2023) ‘Personal Communication regarding Konza Prairie Biological Station bison and cattle stocking rates and density’s’.

Towne, E.G., Hartnett, D.C. and Cochran, R.C. (2005) ‘Vegetation Trends in Tallgrass Prairie from Bison and Cattle Grazing’, *Ecological Applications*, 15(5), pp. 1550–1559. Available at: https://doi.org/10.1890/04-1958.

Tracy, B.F. and Frank, D.A. (1998) ‘Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem: Yellowstone National Park’, *Oecologia*, 114, pp. 556–562.

Valdés-Correcher, E. *et al.* (2019) ‘Herbivore dung quality affects plant community diversity’, *Scientific Reports*, 9(1), p. 5675. Available at: https://doi.org/10.1038/s41598-019-42249-z.

Vance, E.D., Brookes, P.C. and Jenkinson, D.S. (1987) ‘Microbial biomass measurements in forest soils: The use of the chloroform fumigation-incubation method in strongly acid soils’, *Soil Biology and Biochemistry*, 19(6), pp. 697–702. Available at: https://doi.org/10.1016/0038-0717(87)90051-4.

Vega Anguiano, N. (2023) *Bison and cattle grazing influences on soil microbial N cycling and ecosystem N pools in annually burned tallgrass prairies*. Kansas State University.

Venter, Z.S., Hawkins, H.-J. and Cramer, M.D. (2019) ‘Cattle don’t care: Animal behaviour is similar regardless of grazing management in grasslands’, *Agriculture, Ecosystems & Environment*, 272, pp. 175–187. Available at: https://doi.org/10.1016/j.agee.2018.11.023.

Welti, E.A.R. *et al.* (2020) ‘Nutrient dilution and climate cycles underlie declines in a dominant insect herbivore’, *Proceedings of the National Academy of Sciences*, 117(13), pp. 7271–7275. Available at: https://doi.org/10.1073/pnas.1920012117.

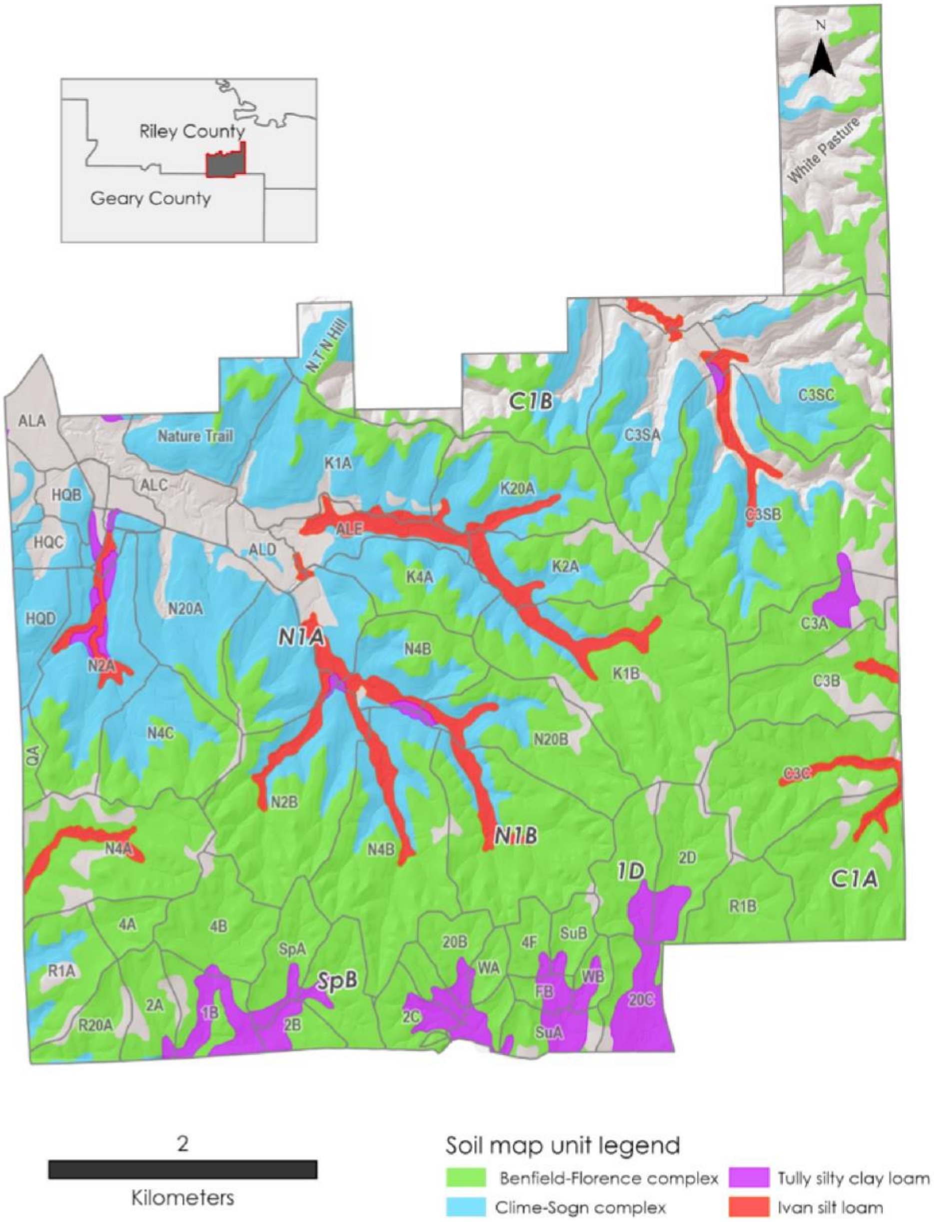
Williams, P.H. and Haynes, R.J. (1995) ‘Effect of sheep, deer and cattle dung on herbage production and soil nutrient content’, *Grass and Forage Science*, 50(3), pp. 263–271. Available at: https://doi.org/10.1111/j.1365-2494.1995.tb02322.x.

Zedeño, M.N., Ballenger, J.A.M. and Murray, J.R. (2014) ‘Landscape Engineering and Organizational Complexity among Late Prehistoric Bison Hunters of the Northwestern Plains’, *Current Anthropology*, 55(1), pp. 23–58. Available at: https://doi.org/10.1086/674535.

Tables and Figures

Table 3.1. Two-way ANOVA results (F statistic and P values) showing the direct and interactive effects of sampling month and grazing treatment on C and N from protected and grazed forage in 2021 and 2022, and dung in 2022. Bolded values indicate P < 0.05.

Year	Response variable	Grazing treatment F, P	Month F, P	Grazing*Month F, P
2021	forage total %C	0.095, 0.76	**27.7, < 0.001**	0.08, 0.92
	forage total %N	**107.9, < 0.001**	**42.7, < 0.001**	**9.4, 0.001**
	forage C:N	**99.7, < 0.001**	**40.5, < 0.001**	**6.0, 0.003**
2022	forage total %C	**31.6, < 0.001**	**22.0, < 0.001**	**7.7, < 0.001**
	forage total %N	**68.1, < 0.001**	**102.9, < 0.001**	1.7, 0.17
	forage C:N	**80.4, < 0.001**	**132.7, < 0.001**	**8.3, < 0.001**
	dung water content	**12.9, < 0.001**	**33.0, < 0.001**	0.40, 0.67
	dung total C%	**69.2, < 0.001**	**86.3, < 0.001**	**20.5, < 0.001**
	dung total N%	1.6, 0.21	**26.5, < 0.001**	**9.4, < 0.001**
	dung C:N	**3.6, 0.06**	**59.5, < 0.001**	**5.2, 0.006**



Prepared by Nicholas Vega Anguiano | Sources: NRCS soil survey and KPBS GIS data portal | NAD84 UTM Zone 14

Figure 3.1. Map of Konza Prairie Biological Station (KBPS) in Riley and Geary Counties, KS, USA, with overlay of main soil units (colors, legend), and watershed scale fire and grazing treatments (label codes including "N" denote units with native grazers, i.e., bison; "C" denotes cattle grazed units; numbers indicate historical fire return interval in years).

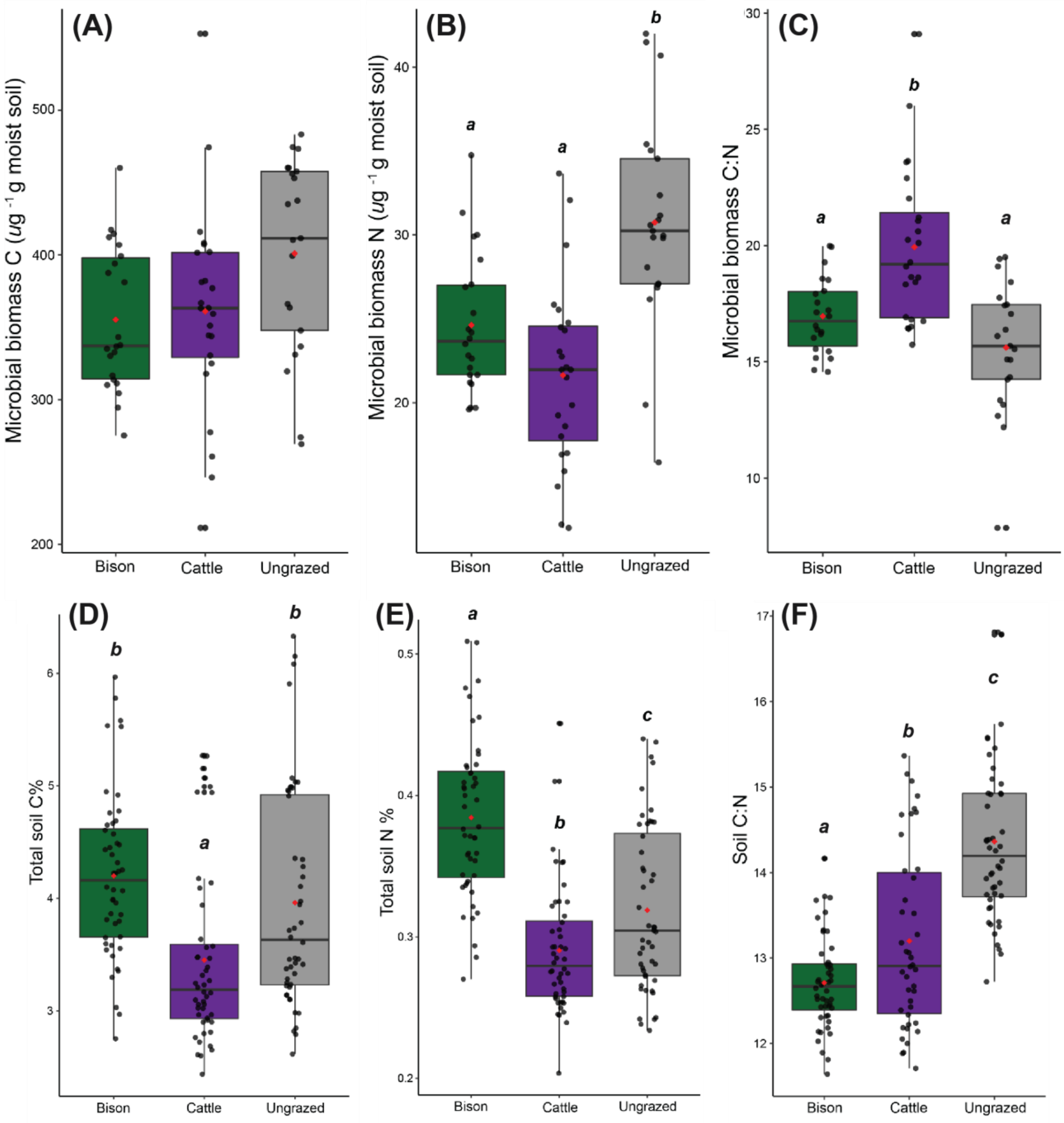


Figure 3.2. Soil microbial biomass (MB) C (A), N (B), and C:N (C) collected in 2021, and soil total C (D), N (E) and C:N (F) collected in 2020 from grazed and ungrazed watershed treatments. The height of the error bars represent mean ± standard error (SE), and means are show in red within each treatment. Letters indicate Tukey's post-hoc differences at $P < 0.05$ level.

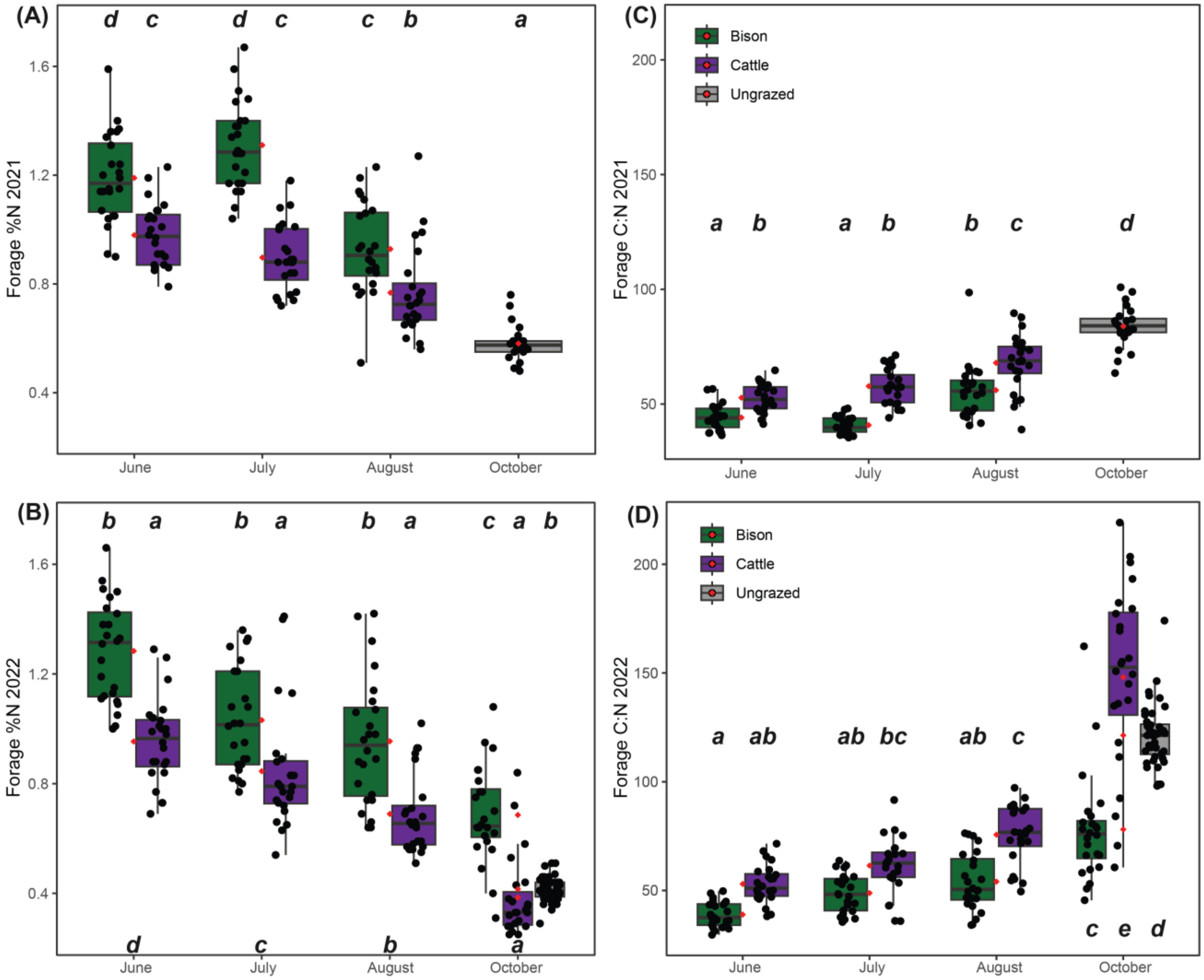


Figure 3.3. Forage (grass) total N% over the summer growing season 2021 (A) and 2022 (B), and C:N in 2021 (C) and 2022 (D) in bison, cattle, and ungrazed treatments. The height of the error bars represent mean ± standard error (SE), and means are show in red within each treatment. Letters indicate Tukey's post-hoc differences at $P < 0.05$ level.

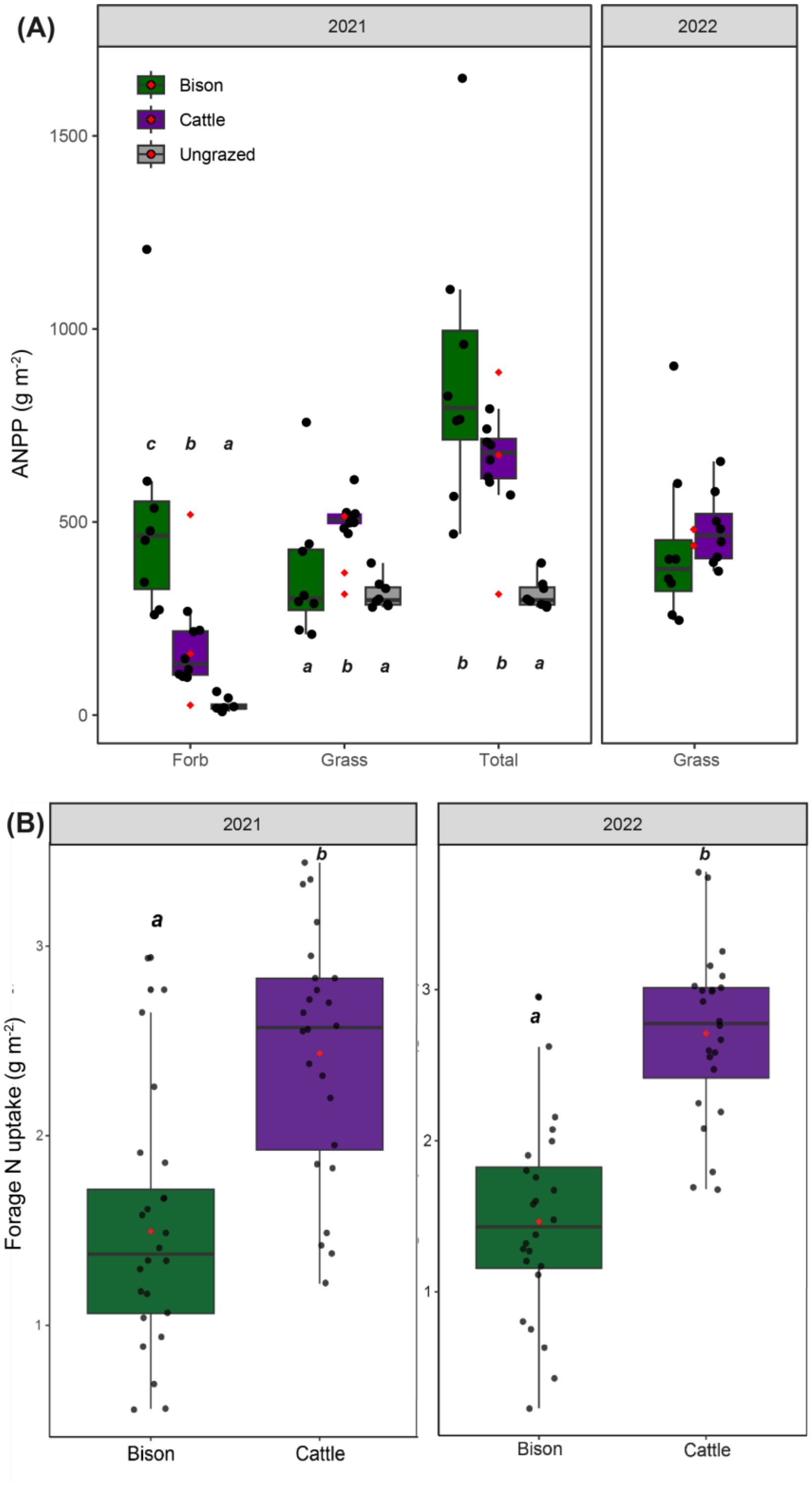


Figure 3.4. Aboveground net primary productivity (ANPP) of forb, grass, and total (forb + grass) biomass in 2021, and grass biomass in 2022, from bison, cattle and ungrazed treatments (A); and forage (grass only) N uptake in 2021 and 2022 (B). The height of the error bars represent mean ± standard error (SE), and means are show in red within each treatment. Letters indicate Tukey's post-hoc differences at P < 0.05 level.

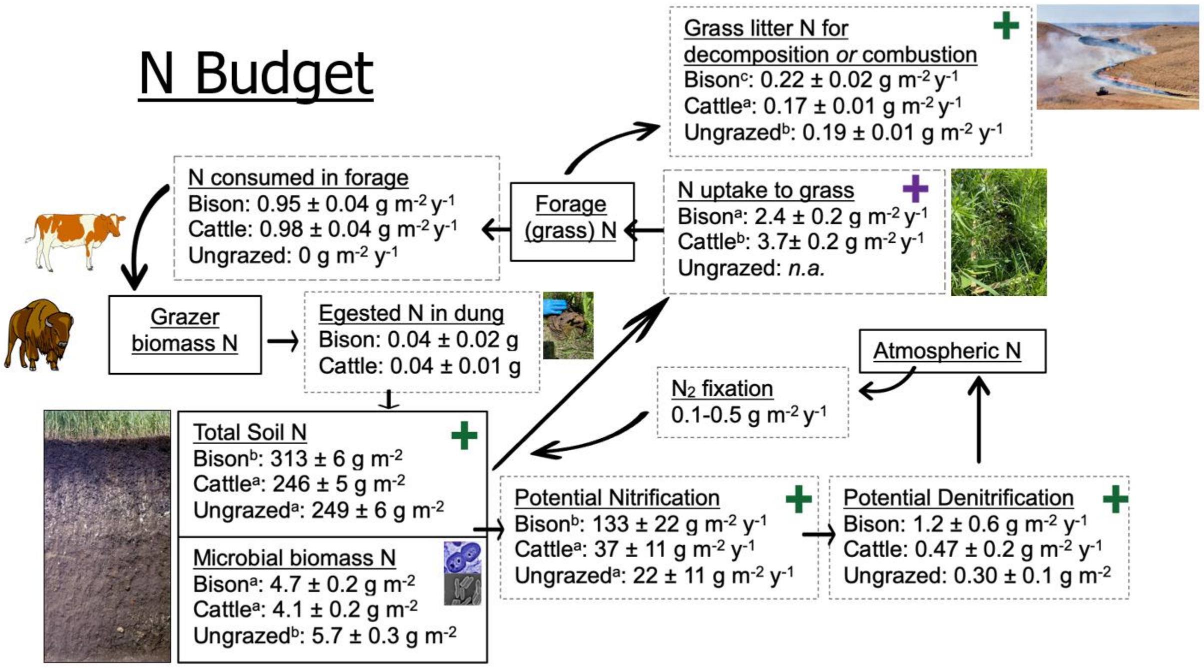


Figure 3.5. A projected N budget using values derived from our investigation, and from references cited in the text, with mean ± SE. Solid boxes represent pools, dashed boxes represent fluxes within grazed treatments. Green plus symbols represent greater fluxes and pools in bison treatments and purple plus symbols indicate greater fluxes and pools in cattle treatments.

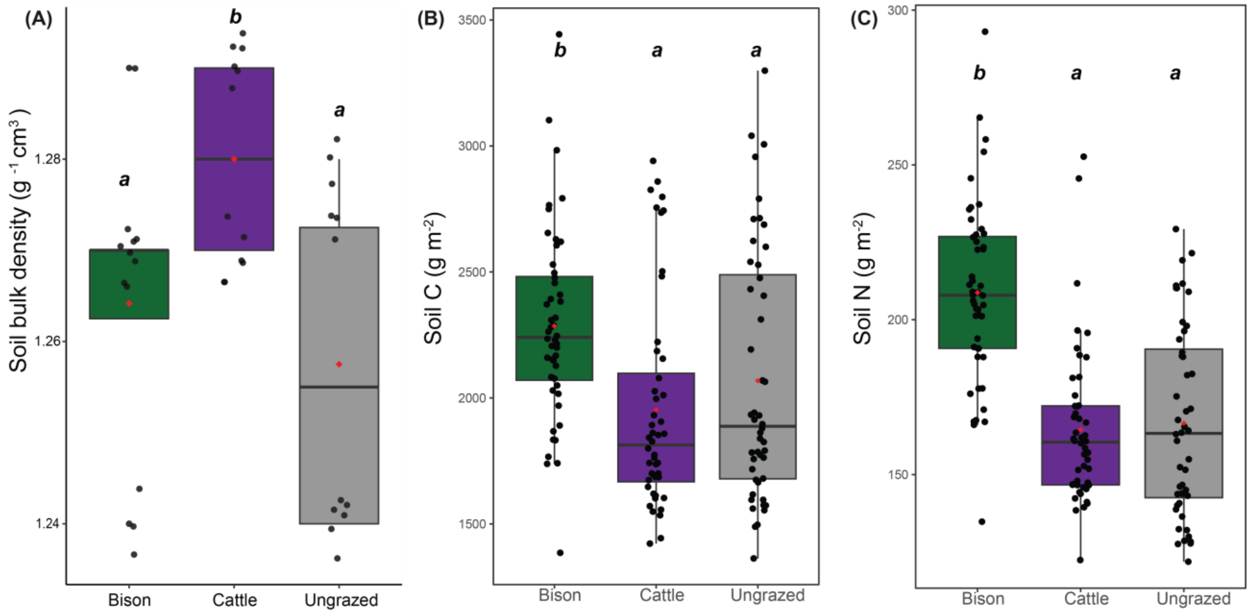


Figure 3.6. Soil bulk density (A) was acquired from SoilGrids and used to convert total soil C and N percentages into soil C stocks (B) and N stocks (C) for grazed and ungrazed treatment sampling points. The height of the error bars represent mean ± standard error (SE), and means are show in red within each treatment. Letters indicate Tukey's post-hoc differences at $P < 0.05$ level.

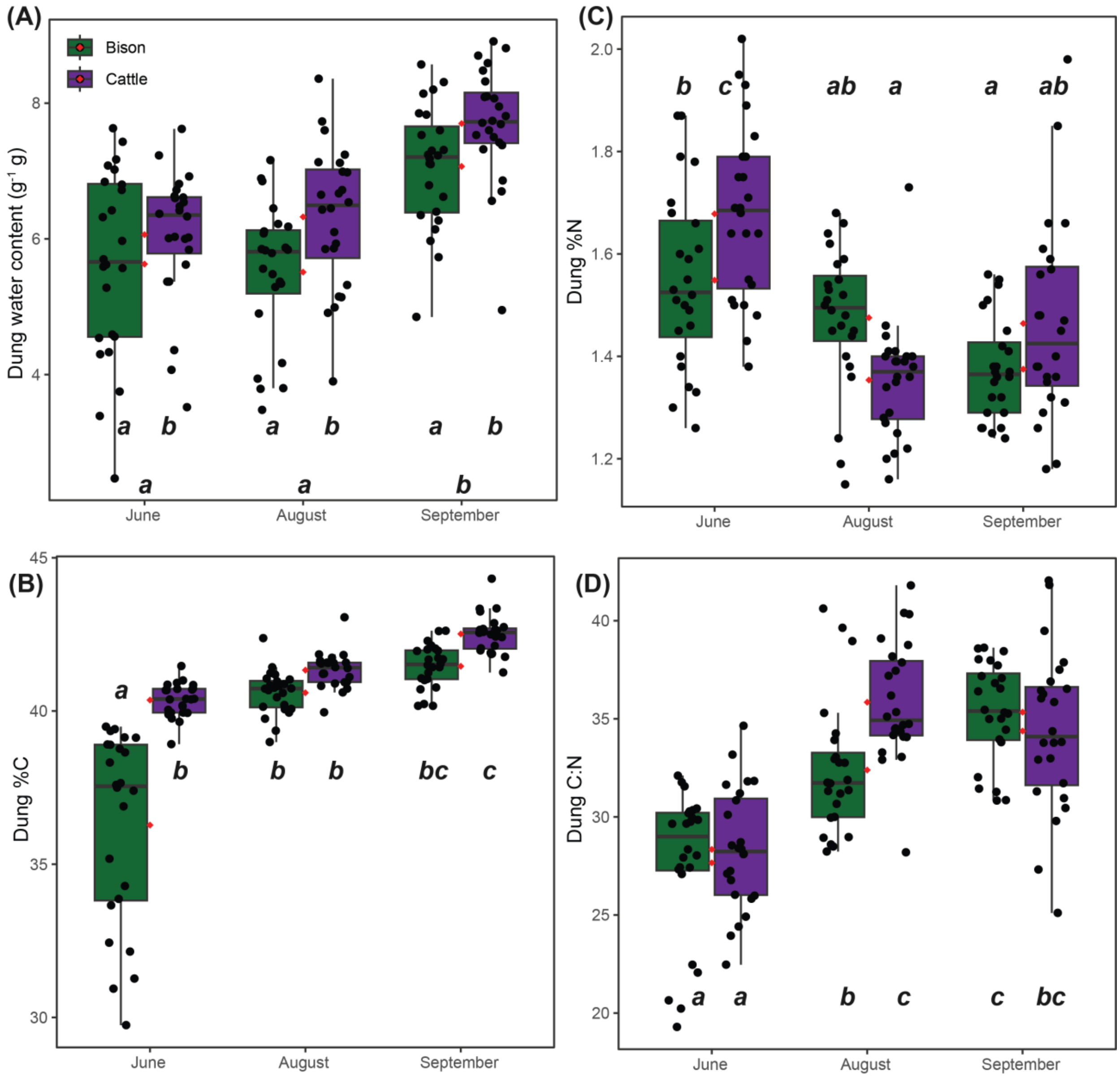


Figure 3.7. Boxplots of dung moisture content (A), total %C (B), total %N (C), and C:N ratio (D) in samples collected monthly over the growing season 2022. The height of the error bars represent mean ± standard error (SE), and means are show in red within each treatment. Letters indicate Tukey's post-hoc differences at P < 0.05 level.

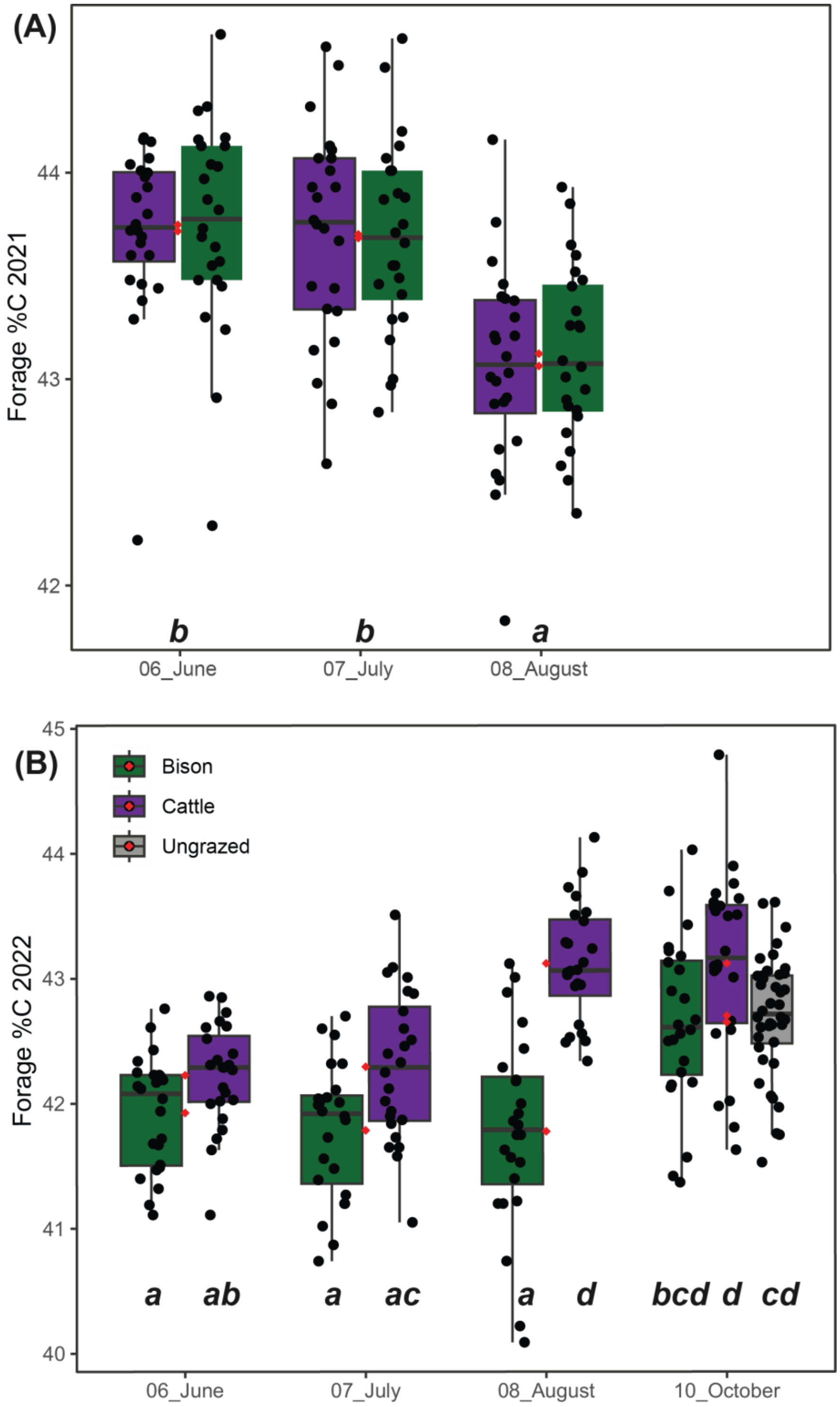
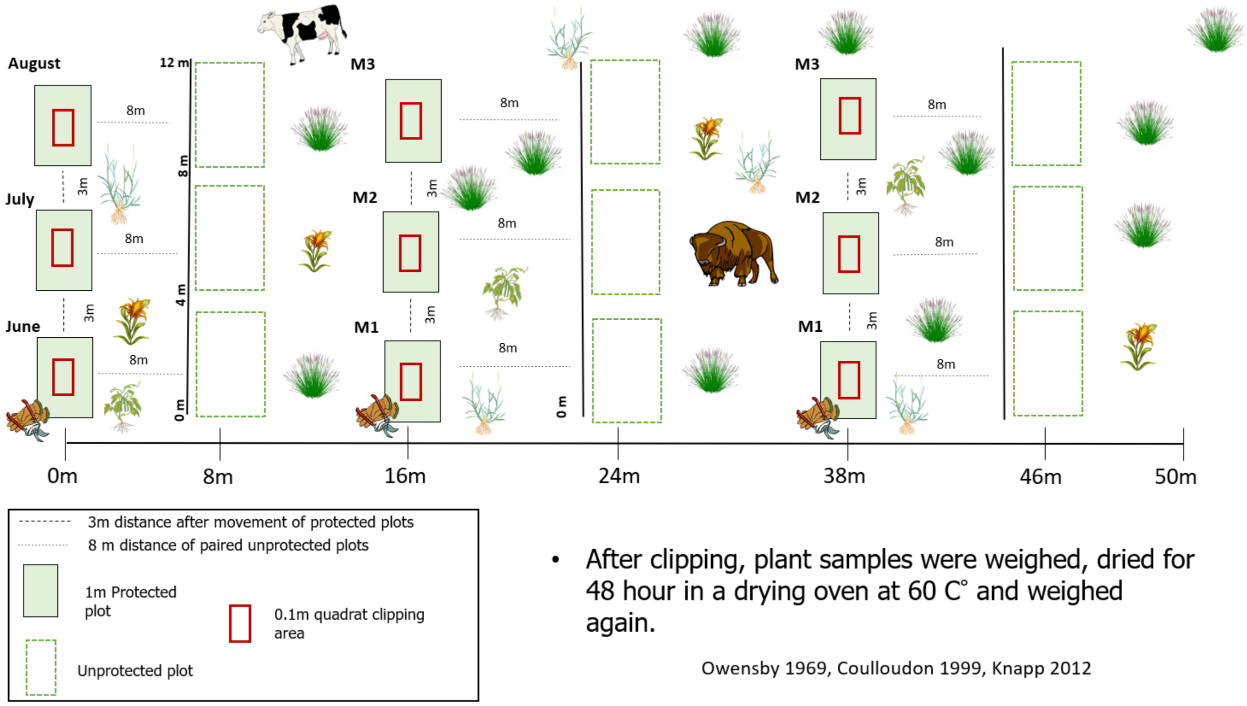


Figure 3.8. Forage %C over the growing season in 2021 (A) and 2022 (B). In 2021, ungrazed units were clipped in different months than in 2022. The height of the error bars represent mean ± standard error (SE), and means are show in red within each treatment. Letters indicate Tukey's post-hoc differences at $P < 0.05$ level.



- After clipping, plant samples were weighed, dried for 48 hour in a drying oven at 60 C° and weighed again.

Owensby 1969, Coulloudon 1999, Knapp 2012

Figure 3.9. Schematic of the paired plot method employed in this investigation, depicting protected plots and unprotected plots on a 50m transect length near each respective long-term plant transect within selected watershed treatments. Paired plots were moved and clippings were taken each month from June through August.

Chapter 4 - Conclusion

Nitrogen (N) is necessary for life on earth to exist, and is elementally limiting in many ecosystems (Schlesinger and Bernhardt, 2020). Thus, N in soils is also important for primary producing plants to grow and feed primary consumers, and is an essential component of soil fertility (Robertson and Groffman, 2014). Soil N and its chemical species are regulated by soil microbial organisms and their ability to cycle N in and out of terrestrial ecosystems like the tallgrass prairie. Large grazers like the keystone bison and cattle livestock are primary consumers, and also recycle N when they eat prairie grasses, as they deposit more bioavailable N in urine and dung forms for microbial and plant use after decomposition (Moore and Bower, 2001). Bison once ranged across North America and were responsible for N recycling as their populations numbered in at least the tens of millions (Hornaday, 1913; Feir, Gillezeau and Jones, 2021). Indian Nations pre-colonization used controlled burning as a technology to aid in the creation tallgrass prairie for bison to eat grass forage, also making hunting grounds where meat could be attained to feed entire villages and useful plants could be gathered (Kimmerer and Lake, 2001; Stewart, 2002). At present, land managers have implemented the drivers of grazing and fire as a prescription to maintain and restore tallgrass prairie ecosystems (Fuhlendorf and Engle, 2001, 2004) because a fraction of the original extent of tallgrass prairie remains (Samson, Knopf and Ostlie, 2004) .

Because cattle have replaced native bison on the landscape as the dominant herbivore, many question whether the two animals are functionally redundant (Kohl *et al.*, 2013). Within the context of N cycling and its prominence in soil fertility, N cycling in bison vs cattle grazed soil remains understudied in grazed tallgrass prairie. Even though bison and cattle both recycle N, they have yet to be compared within the context of their soil N cycling microbial responses.

My research fills this gap and shows how bison can support a significantly different and faster N cycle in their soils, despite differences in summer precipitation, leading to feedbacks of greater forage quality and soil N stock when compared to cattle grazed soils.

In my second research chapter, I investigated if bison and cattle had different microbial N cycling activities i.e., the rates of microbial nitrification and denitrification, over a three-year period. We also measured soil available N (an index of soil N availability) over the growing season and N limitation of the soils according to the ratios of microbial extracellular decomposition enzymes relative to N acquiring enzymes. We found that there was greater soil N availability in bison treatments relative to ungrazed treatments with cattle not differing from bison in this regard over time. Yet, nitrification potential displayed a consistently higher rate than cattle and ungrazed treatments regardless of seasonal variation in summer precipitation, a strong control on N cycling but not enough to overpower grazing effects.

We measured denitrification in two ways: The maximum rate, and a more realistic rate under ambient conditions of substrate availability. Maximum potential denitrification was highest in grazed soils, where bison and cattle did not differ from each other over the three-year period, while the more realistic estimate of denitrification was only highest in one out the three years where there was a weak and almost undetectable microbial response in the second two years of the study, likely because the first year was the wettest. N limitation was lowest in bison grazed soils in two out of the three years of the study (the two driest), which we found was related to variability in precipitation. Also, we measured a consistent difference in soil pH, which allows for the more energetically favorable non-protonated form, ammonia, to be more available for nitrification in bison treatments than cattle and ungrazed treatments. Together, these results suggest that bison grazed soils were less N limited, which promoted higher soil microbial nitrification rates, which allowed for faster N cycling that was indeed different in bison grazed soils vs cattle grazed soils.

My third research chapter revolved around measuring N pools in soils, microbial biomass, dung and grass forage and in estimated N fluxes (using nitrification and denitrification from Chapter 2), forage N assimilated from soils and lost from annual burning and potential forage N consumed by bison and cattle. We also measured above ground primary productivity (how much the grass forage grew in a year) over a two-year period and how much N was in that grass. All these measurements were taken to figure out if N pools and fluxes were different in bison vs cattle grazed tallgrass prairie. Taking it a step further, a partial N budget was created with all these measurements in mind to account for pools and fluxes between the bison and cattle respectively.

We learned that surprisingly, soil N stocks were larger in bison grazed treatments than in cattle and ungrazed treatments, and that forage quality or the N concentration (%N) in bison grazed forage was also higher than in cattle treatments. Also, through the growing season, %N decreased, characteristic of grass having the highest %N at the beginning of the growing season, and with N retranslocation for overwintering at the end of the season. However, calculations of N that was assimilated into forage grass tissues indicated that forage took up greater N per unit area in the cattle grazed than bison grazed treatment. Taken with the fact there is proportionally more forbs in bison grazed units (less grass total) and less forbs in cattle units (more grass total) (Ratajczak et al.; 2022), it makes sense that N in grass forage cattle treatments would be higher. However, microbial biomass N and dung N did not differ between cattle and bison treatments. Soil N could be greater in bison treatments for a number of reasons, but is most likely in part due to a greater N content in grass litter that gets decomposed by soil microbes since they prefer a lower carbon to nitrogen ratio food substrate, which can end up back into the soil N pool since these soils can retain N effectively after microbial death or be made available to plants again via microbial N mineralization.

Furthermore, with higher nitrification and maximum denitrification potentials and N loss from fire, bison grazed treatments would have the higher potential for loss back to the atmosphere or via soil leaching.With consideration to land management, specifically animal movement away from the site as N within the animal body, cattle may have a higher N removal capacity than bison. Together these results suggest that even though more N can be lost from bison grazed tallgrass prairie, this has a dual effect of also having more N recycling and thus greater soil fertility as observed in the larger N pool in bison grazed soils creating a plant soil feedback of higher quality forage. The existence of grazing lawns within bison treatments thus cannot not be overstated, as bison create these areas of high soil fertility which then produce higher quality forage. Yet, all things considered, rangeland management decisions are the boundaries set within which bison and cattle may or may not overgraze the landscape and lead to poor soil fertility, loss of N and degradation of the already endangered tallgrass prairie.

Overall this thesis demonstrates that N cycling can be fundamentally different down to the microbial level between two large grazing animals; one the last native surviving keystone megaherbivore who has been in North America since before the last ice age (195,000 -135,000 years ago) (Davies *et al.*, 2019); the other a nonnative herbivore of which the majority of American rangelands support. My thesis provides strong evidence that bison can have greater soil N cycling and thus greater soil N fertility than cattle, also supporting the overall long-term health of the tallgrass prairie, indicating that in the context of the N cycle these two animals are not functionally redundant.

References Cited

Davies, G. *et al.* (2019) ‘Isotopic paleoecology of Northern Great Plains bison during the Holocene’, *Scientific Reports*, 9(1), p. 16637. Available at: https://doi.org/10.1038/s41598-019-52873-4.

Feir, D., Gillezeau, R. and Jones, M.E.C. (2021) ‘The Slaughter of the Bison and Reversal of Fortunes on the Great Plains’, p. 24.

Fuhlendorf, S.D. and Engle, D.M. (2001) ‘Restoring heterogeneity on rangelands: ecosystem management based on evolutionary grazing patterns: we propose a paradigm that enhances heterogeneity instead of homogeneity to promote biological diversity and wildlife habitat on rangelands grazed by livestock’, *BioScience*, 51(8), pp. 625–632.

Fuhlendorf, S.D. and Engle, D.M. (2004) ‘Application of the fire–grazing interaction to restore a shifting mosaic on tallgrass prairie’, *Journal of Applied Ecology*, 41(4), pp. 604–614. Available at: https://doi.org/10.1111/j.0021-8901.2004.00937.x.

Hornaday, W.T. (William T. (1913) *Our vanishing wild life; its extermination and preservation*. New York, C. Scribner’s sons. Available at: http://archive.org/details/ourvanishingwild00horn .

Kimmerer, R.W. and Lake, F.K. (2001) ‘The role of indigenous burning in land management’, *Journal of Forestry*, 99(11), pp. 36–41.

Kohl, M.T. *et al.* (2013) ‘Bison Versus Cattle: Are They Ecologically Synonymous?’, *Rangeland Ecology & Management*, 66(6), pp. 721–731. Available at: https://doi.org/10.2111/REM-D-12-00113.1.

Moore, A.J. and Bower, N.W. (2001) ‘Chemometric Analysis of Compositional Variation in Bison and Cow Patties: A Biogeochemistry?Environmental Chemistry Experiment’, *The Chemical Educator*, 6(2), pp. 86–90. Available at: https://doi.org/10.1007/s00897010459a.

Robertson, G.P. and Groffman, P.M. (2014) ‘Nitrogen Transformations’, in E.A. Paul (ed.) *Soil Microbiology, Ecology and Biochemistry (Fourth Edition)*. San Diego: Academic Press, pp. 341–364. Available at: https://www.elsevier.com/books/soil-microbiology-ecology-and-biochemistry/paul/978-0-12-415955-6 (Accessed: 14 March 2021).

Samson, F.B., Knopf, F.L. and Ostlie, W.R. (2004) ‘Great Plains ecosystems: past, present, and future’, *Wildlife Society Bulletin*, 32(1), pp. 6–15. Available at: https://doi.org/10.2193/0091-7648(2004)32[6:GPEPPA]2.0.CO;2.

Schlesinger, W.H. and Bernhardt, E.S. (2020) *Biogeochemistry - 4th Edition*. 4th ed.

Stewart, O.C. (2002) *Forgotten Fires: Native Americans and the Transient Wilderness*. University of Oklahoma Press.