Grassland soil microbial community composition and distribution response to grazing by *Bison bison*

by

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Abstract

In Great Plains prairie ecosystems, bison were historically a keystone species, and still exert significant influence on the composition of aboveground communities through their grazing activity. Given that soil microorganisms also support essential grassland ecosystem services, there is a need for greater understanding of how bison grazing activity affects soil microbial communities. Although the mechanisms controlling soil microbial community assembly at different spatial scales are known to be the same as for all larger organisms – environmental filtering, drift, dispersal, and mutation – the context in which each mechanism becomes important is not well understood. I predicted that bison would weaken the soil microbial community distance-dissimilarity relationship, one of the most common spatial patterns, making microbial communities more similar across space. More specifically, I predicted this pattern would be a result of bison physically distributing microbial cells or altering the environment to increase competitive dominance of certain taxa, and that bison dung would be a main contributor to these mechanisms.

To address these predictions, I carried out an observational project evaluating regional soil microbial composition and distribution and an experimental project investigating dispersal mechanisms. For the observational project, surface soils were collected from bison grazed and ungrazed areas at nine grassland sites across the Great Plains for analysis of microbial community composition (as bacterial and archaeal 16S rRNA gene sequence libraries), along with information on soil chemistry and plant community cover. The experimental project involved manipulating the openness of the soil microbial community to passive dispersal or the addition of bison dung to simulate active dispersal, in combination with the presence or absence of bison and spring burning at a focal tallgrass prairie site (Konza Prairie Biological Station). To

assess soil microbial dispersal rates under these contrasting conditions, change in soil microbial community composition was measured over time.

Results indicate that bison grazing does weaken the soil microbial distance-dissimilarity relationship when evaluated at a regional level, but at a local site level the strength and direction of this relationship relative to ungrazed areas is mediated by plant community structure and soil factors. Still, variation in the strength of the grazing effect on distance-dissimilarity relationships could be driven by both relative ease of microbial dispersal and environmental filtering at the small-scale sample level. Experimental results show that passive dispersal occurred throughout the duration of the project, but dispersal limitation of microbial taxa does not vary with grazing or fire management. Furthermore, bison dung can directly disperse microbes and influence community assembly over time. Overall, both projects support the importance of bison grazing in structuring and mediating soil microbial community dynamics across Great Plains grasslands, and provide impetus for future research and conservation of soil microbial communities, especially in relation to belowground ecosystem services.

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the most unique landscape scale bison grazing experiments running since the 1980s. It was such a privilege to visit each of the grassland sites and see the beauty of the plains exemplified in unique and varied ways; I truly wish everyone could have a similar experience. I also acknowledge that the land each site is located on was cared for and called home by Indigenous peoples before European settlement, and that these lands still hold historical, spiritual, and personal significance for Native Nations and people across the Great Plains. I would also like to thank my funding sources: National Science Foundation, Konza Long-Term Ecological Research Program, KPBS, and the Grassland Heritage Foundation for making my research possible.

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Chapter 1 - Introduction

Ecological communities assemble through, and are maintained by, a variety of similar mechanisms that define which populations coexist, regardless from which branch on the tree of life the organism has evolved. The interplay of mechanisms results in the profound diversity that is prevalent across our planet. A central goal in ecology is to understand how this diversity is maintained and why organisms are found in specific environments or at specific times. Distributions and spatial structure have been studied extensively for plants and animals, allowing for the understanding of broad geographical patterns of species or community assemblages (Rosenzweig 1995), and metacommunity dynamics (Leibold et al. 2004) but this knowledge is still being built for microbial systems. The recognition of biogeographical patterns and constrained spatial distribution for microbial taxa is a relatively new frontier (Martiny et al. 2006, Van der Gast 2015).

In grasslands, fire and grazing are important for maintaining biological diversity and ecosystem function (Frank et al. 1998; Bond et al. 2005). The interaction of these drivers creates a patchwork structure of high spatial and temporal heterogeneity in plant diversity across grasslands (Collins et al. 1998; Collins and Smith, 2006). In particular, large herbivore grazing has been found to increase plant heterogeneity and biodiversity by creating open patches across prairies that result in a mosaic of light and resource availability, allowing non-dominant plants to establish or increase in abundance (Collins et al. 1998, Bakker et al. 2003; Borer et al. 2014; Koerner et al. 2018). The strong plant responses to grazing and fire in grasslands begs the question of how these factors affect belowground communities, but unfortunately less research exists on belowground microbial responses, even though many ecosystem services are mediated by soil microbial communities (Schimel et al. 2007 and Delgado-Baquerizo et al. 2016).

The same fundamental processes that determine macro-organismal community assembly also are hypothesized to act on microbial communities (Hanson et al. 2012; Nemergut et al. 2013), but the relative importance of each process will depend on the environment and the target organisms. In grassland ecosystems, grazing and fire are strong determinants of environmental state (Briggs et al. 2005) and therefore are likely influencing the biogeography of soil microbes by altering the relative importance of different assembly mechanisms. One of the most ubiquitous biogeographical patterns across trophic levels is distance-dissimilarity (Soininen et al. 2007), or the decrease in community similarity with geographic distance, which is driven by the balance between local environmental filtering and dispersal (Leibold et al. 2004, Martiny et al. 2006). Environmental filtering is the result of abiotic constrains and competitive exclusion by populations that are best suited to live in unique local environmental conditions; for example, in environments with extreme pH, this is the strongest factor and results in communities that are similar at closer distances (Tripathi et al. 2018). Dispersal, the movement of new populations into a local community, can become the dominating mechanism when the rate of dispersal is high and continuous, leading to homogenization of communities across space. However, even at high dispersal rates, local environmental conditions can favor specific taxa, emphasizing that communities are assembled from multiple processes acting simultaneously (Lindström and Östman 2011).

With mounting evidence that microbial taxa display constrained geographical patterns of diversity, possibly reflecting either dispersal limitation or strong local filtering (Bell 2010; Lindström and Langenheder 2012; Albright and Martiny 2018), simply describing the pattern is not enough. To move forward, it is necessary to provide a mechanistic understanding of why these patterns occur and which factors act as modulators (Hanson et al. 2012). Thus, across

grasslands, we wanted to know how bison grazing affects soil microbial biogeography at both local and regional scales. Furthermore, to get a better understanding of the mechanistic link between human management choices on grazing and fire and soil microbial biogeography, we wondered how experimental alteration of dispersal mechanisms in differently grazed and burned areas at a single grassland site would affect local microbial diversity and composition. We hypothesized that regional soil microbial diversity would be constrained by climate and soil factors, and that grazing activity would decrease the distance-dissimilarity relationship within sites by decreasing dispersal limitation of microbes and consistently alter the soil environment that would cause increased similarity among sites. Secondly, we examined whether passive dispersal (promoted by a more open plant canopy) or active dispersal (in bison dung) could be a mechanism for decreasing microbial dispersal limitation, and whether fire and grazing management would modulate the strength of dispersal driven assembly.

References Cited

Albright, M. B. N., and J. B. H. Martiny. 2018. Dispersal alters bacterial diversity and composition in a natural community. The ISME Journal 12:296–299.

Bakker, C., J. M. Blair, and A. K. Knapp. 2003. Does resource availability, resource heterogeneity or species turnover mediate changes in plant species richness in grazed grasslands? Oecologia 137:385–391.

Bell, T. 2010. Experimental tests of the bacterial distance–decay relationship. The ISME Journal 4:1357–1365.

Bond, W. J., F. I. Woodward, and G. F. Midgley. 2004. The global distribution of ecosystems in a world without fire. New Phytologist 165:525–538.

Borer, E. T., E. W. Seabloom, D. S. Gruner, W. S. Harpole, H. Hillebrand, E. M. Lind, P. B. Adler, J. Alberti, T. M. Anderson, J. D. Bakker, L. Biederman, D. Blumenthal, C. S. Brown, L. A. Brudvig, Y. M. Buckley, M. Cadotte, C. Chu, E. E. Cleland, M. J. Crawley, P. Daleo, E. I. Damschen, K. F. Davies, N. M. DeCrappeo, G. Du, J. Firn, Y. Hautier, R. W. Heckman, A. Hector, J. HilleRisLambers, O. Iribarne, J. A. Klein, J. M. H. Knops, K. J. La Pierre, A. D. B. Leakey, W. Li, A. S. MacDougall, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, B. Mortensen, L. R. O'Halloran, J. L. Orrock, J. Pascual, S. M. Prober, D. A. Pyke, A. C. Risch, M. Schuetz, M. D. Smith, C. J. Stevens, L. L. Sullivan, R. J. Williams, P. D. Wragg, J. P. Wright, and L. H. Yang. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508:517–520.

Briggs, J. M., A. K. Knapp, J. M. Blair, J. L. Heisler, G. A. Hoch, M. S. Lett, and J. K. McCarron. 2005. An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. BioScience 55:243.

Collins, S. L., A. K. Knapp, J. M. Briggs, J. M. Blair, and E. M. Steinauer. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. Science 280:745–747.

Collins, S. L., and M. D. Smith. 2006. Scale-dependent interaction of fire and grazing on community heterogeneity in tallgrass prairie. Ecology 87:2058–2067.

Delgado-Baquerizo, M., F. T. Maestre, P. B. Reich, T. C. Jeffries, J. J. Gaitan, D. Encinar, M. Berdugo, C. D. Campbell, and B. K. Singh. 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nature Communications 7:10541.

Frank, D. A., S. J. McNaughton, and B. F. Tracy. 1998. The ecology of the earth's grazing ecosystems. BioScience 48:513–521.

Hanson, C. A., J. A. Fuhrman, M. C. Horner-Devine, and J. B. H. Martiny. 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. Nature Reviews Microbiology 10:497–506.

Koerner, S.E., Smith, M.D., Burkepile, D.E. et al. 2018. Change in dominance determines herbivore effects on plant biodiversity. Nature Ecology and Evolution 2, 1925–1932.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters 7:601–613.

Lindström, E. S., and Ö. Östman. 2011. The importance of dispersal for bacterial community composition and functioning. PLoS ONE 6:e25883.

Lindström, E. S., and S. Langenheder. 2012. Local and regional factors influencing bacterial community assembly: Bacterial community assembly. Environmental Microbiology Reports 4:1–9.

Martiny, J. B. H., B. J. M. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske, P. J. Morin, S. Naeem, L. Øvreås, A.-L. Reysenbach, V. H. Smith, and J. T. Staley. 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4:102–112.

Nemergut, D. R., S. K. Schmidt, T. Fukami, S. P. O'Neill, T. M. Bilinski, L. F. Stanish, J. E. Knelman, J. L. Darcy, R. C. Lynch, P. Wickey, and S. Ferrenberg. 2013. Patterns and processes of microbial community assembly. Microbiology and Molecular Biology Reviews 77:342–356.

Rosenzweig, M. L. 1995. Species diversity in space and time. Cambridge University Press, England

Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386–1394.

Soininen, J., R. McDonald, and H. Hillebrand. 2007. The distance decay of similarity in ecological communities. Ecography 30:3–12.

Tripathi, B. M., J. C. Stegen, M. Kim, K. Dong, J. M. Adams, and Y. K. Lee. 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. The ISME Journal.

van der Gast, C. J. 2015. Microbial biogeography: the end of the ubiquitous dispersal hypothesis?: Microbial biogeography. Environmental Microbiology 17:544–546.

Chapter 2 - Soil microbial communities respond to bison grazing at local and regional scales across Great Plains grasslands

Abstract

It is well recognized that soil microbial communities become less similar at increasing geographic distances, but the environmental and biotic factors that control the strength of distance-dissimilarity patterns, and how they change with spatial scale, are less understood. In Great Plains grasslands, bison grazing is an important driver of aboveground community dynamics, but their influence over soil microbial community assembly has not been well characterized. In this study, I sampled soil from bison grazed and ungrazed areas at nine Great Plains grassland sites across a broad geographic and climatic gradient to evaluate how bison activity affects soil microbial community spatial distribution and composition at varying scales. We expected communities to be more homogenous across space where bison are present at all sites, but that the strength of this grazing effect would depend on local environmental variables. At the regional scale, bison did significantly decrease soil microbial community distance-dissimilarity compared to ungrazed areas, but the strength and direction varied for each site which was related to openness of the plant canopy, the number of bison, and water availability. The results indicate that bison activity may consistently alter soil microbial assembly across the Great Plains prairie region.

Introduction

North American prairies can produce an exceptional amount of above- and below-ground primary production and harbor unique biodiversity. However, in the past century, 70% or more of North American grasslands have been lost or degraded due to fragmentation, abandonment,

and conversion for food production (Samson et al. 2004, Briggs et al. 2005). This loss can have profound negative consequences on the ecosystem services that prairies provide, such as a clean water supply, erosion control, and pollination (Foley et al. 2005, Bengtsson et al. 2019). Understanding the historical drivers of prairie diversity and function can enhance conservation and management of grasslands to ensure sustainable ecosystem provisions.

Grasslands can support high biodiversity because of frequent disturbance, such as fire and grazing (Frank et al. 1998; Bond et al. 2005). The interplay of these drivers creates a patchwork structure that increases spatial and temporal heterogeneity in plant diversity across grasslands (Collins et al. 1998; Collins and Smith, 2006). In particular, large herbivore grazing has been found to increase plant heterogeneity and biodiversity by creating open patches across prairies that result in a mosaic of light and resource availability, allowing non-dominant plants to establish (Collins et al. 1998, Bakker et al. 2003; Borer et al. 2014; Koerner et al. 2018). The American bison (*Bison bison*) is thought to have played a keystone role in the historical maintenance of prairie vegetation (Knapp et al. 1999), and although largely replaced by cattle grazing, their reintroduction and activity provide an important perspective on how large native grazers affect prairie structure and function (Allred et al. 2011, Truett et al. 2001). Aboveground plant response to grazing in grasslands is well-studied, but less research exists on belowground microbial responses even though most ecosystem services are mediated by soil microbial communities (Schimel et al. 2007 and Delgado-Baquerizo et al. 2016).

Although soil microbial (bacterial and archaeal) composition varies considerably across grasslands (Leff et al. 2015) and over time (Shade et al. 2013), certain microbial taxa may be indicators of grassland states. For example, the bacterial clade Verrucomicrobia DA101 has been identified as a dominant group in native tallgrass prairie and is predicted to have been prevalent

across the historical range of this ecosystem (Fierer et al. 2013). Also, when nutrient inputs to grasslands increase, the soil bacterial groups of Actinobacteria, Alphaproteobacteria, and Gammaproteobacteria tend to increase in abundance while Acidobacteria, Planctomyces, and Deltaproteobacteria tend to decrease, and this is consistent at grasslands sites across the globe (Leff et al. 2015). However, these studies and others are missing an important piece of historical context − that large herbivore grazers were present throughout the history of grasslands (Stebbins 1981). The importance of grazers in controlling aboveground community dynamics, coupled with more than 25% of the earth's surface currently managed for grazing (Asner et al. 2004), necessitates the urgency in understanding grassland soil microbial communities that exist in the presence of grazing ungulates.

The response of soil microbial community dynamics and activity to ungulate grazing may vary due to several factors. In a meta-analysis of grassland responses to herbivore grazing across Australia, Eldridge et al. (2016) found that an average response ratio index of soil function did not always respond to grazing, except under the highest grazing pressure where it markedly declined. Grazing intensity seems to also be an important determinant of belowground response globally; for example, heavy grazing reduced bacterial and fungal community biomass at multiple grassland sites (Zhao et al. 2017). More generally, it is it well known that the link between above ground grazers and belowground nutrient cycling is mediated by abiotic factors, such as precipitation and soil chemistry (Bardgett and Wardle 2003, Augustine and McNaughton 2006). Furthermore, after grazing is ceased and rangeland abandoned, the structure and function of microbial communities change, providing further evidence for the importance of large herbivore grazing in mediating belowground community dynamics (Aldezabal et al. 2015 and Oggioni et al. 2020).

These variable factors make the mechanistic relationships between above ground grazers and soil microbial community composition nuanced from site to site. In semi-arid grasslands of eastern Australia, different types of herbivore grazers can alter apparent competitive exclusion of certain bacterial taxa by reducing soil carbon and increasing plant richness, which increases the abundance of dominant bacteria, thereby altering overall community diversity (Eldridge et al. 2017). Large herbivore dung, which deposits biologically-available nutrients, can also shift soil microbial communities by enhancing the activity and growth of certain microbial functional groups (Patra et al. 2005, Esch et al. 2013). In both dry and mesic Great Plains prairies, bison grazing decreases root growth, increases N cycling, and changes root chemistry (Johnson and Matchett, 2001, Frank et al. 2017). Thus, grazing-induced changes to soil resources can lead to changes in soil microbial communities, (Mitchell et al. 2010) but there are few studies that have explicitly explored this across Great Plains prairies at varying scales.

Bison also play a pivotal role in the spatial distribution of plant communities (Collins and Smith 2006, Bakker et al. 2003), and we have evidence from a prior study conducted at Konza Prairie Biological Station (KBPS, Manhattan, KS, USA) that their grazing activity influences the spatial distribution of soil microbial communities as well (Zeglin et al., unpublished), such that the distance-dissimilarity relationship of soil microbial (bacterial and archaeal) communities is weaker in grazed areas. Distance-dissimilarity, or the decrease in community similarity as geographical distance increases, is a widespread pattern in all ecological communities, and can be attributed to both environmental filtering and dispersal limitation of microbial taxa (Nekola and White 1999, Soininen et al. 2007, Hanson et al. 2012). Thus, the presence of large ungulate grazers could be related either to direct dispersal of microbial cells by animal movement or

opening of the plant canopy indirectly allowing for aerial microbial dispersal to more distant soil locations, with either resulting in a weaker distance-dissimilarity slope.

With the understanding that both local and regional environmental characteristics are important drivers of microbial biogeography, we sought to examine whether bison grazing shifts soil microbial composition similarly among locations that vary in soil type, climate and management at multiple sites across the Great Plains prairie region. Broadly, we hypothesized that regional soil microbial community composition would be best predicted by climate and soil type, and that bison grazing would change the community composition and weaken the distance-dissimilarity relationship for soil microbes similarly at all sites. We also considered that the magnitude of impact (effect size) of bison grazing at each site would be modulated by local factors related to plant, soil, and land management attributes.

To address this question and hypothesis, we sampled soil in a spatially explicit design at nine grassland sites with varying climate, soils, and management backgrounds. We predicted that A) climate variables and soil type would the most important predictors of regional composition and diversity (de Vries et al. 2012, Stevens et al. 2020), B) bison grazing would weaken the distance-dissimilarity relationship as compared to ungrazed soil, but that the magnitude of this difference would be dependent on local factors related to management decisions (Pérez-Valera et al. 2017, Zhao et al. 2017), plant structure (Bardgett et al. 1999, Leff et al. 2018), and soil chemistry (Albright et al. 2019), and C) there would be specific microbial taxa that differentially responded to the presence of bison grazing across all sites.

Methods

Study locations, experimental design, sample collection

Samples were collected during the summer of 2019 from nine bison-grazed prairies across the Great Plains, with five sites located in Kansas, one site in Oklahoma, two sites in Nebraska, and one site in Minnesota (Figure 2.1). The sites represent tallgrass, mixed, and short grass prairie and are located on either Mollisols or Entisols. Precipitation and temperature varies widely across sites with a mean annual precipitation (MAP) ranging from 486 mm (FLR) to 1192 mm (TGP), and mean annual temperature (MAT) ranging from 7°C (CCR) to 15°C (TGP) (Table 2.1). Plant composition varies among the three prairie types, with short grass prairie dominated by *Bouteloua dactyloides* and *Bouteloua gracilis,* mixed-grass prairie dominated by *Pascopyrum smithii*, and tall grass prairie dominated *by Andropogon gerardii, Sorghastrum nutans, Panicum virgatum,* and *Schizachyrium scoparium*.

A stratified random sampling design was used to establish 3 replicate linear transects in both bison grazed and non-grazed areas at each site, for a total of 6 transects. The exact distance between transects varied depending on the site area but was always on the order of 100s of meters. At KNZ, one additional transect for each grazing type was added to match historical collection methods, for a total of 8. Transect locations were selected to maximize similarity between the grazed and ungrazed areas based on soil type, burn history, and other land use history factors. However, at some sites, this was a challenge due to management as wildlife areas or preserves, not as experimental stations, resulting in a limited extent of ungrazed land. In this case the location histories were recorded and accompanied by field notes. Each transect was 10 m long and included six sampling points, from which mineral soil was collected using aseptic technique by washing the soil auger in ethanol between each sample and wearing gloves. The

cores were 2 cm in diameter and 10 cm in depth, and were collected along log-distance intervals at 0, 0.1, 0.5, 1, 5, and 10 m with geographic locations recorded at 0, 1, 5, and 10 m using a Garmin GPSMAP 64x (Garmin, Olathe, KS, USA). Each core was placed into an individual WhirlPak bag and stored on ice for transport to the lab, where all were aseptically sieved using 4 mm mesh, plant roots removed, and frozen at -20°C until further analysis. In addition, aboveground percent cover for graminoids, forbs, woody plants, bare ground, and litter was recorded using a modified Daubenmire cover scale (Bailey and Poultan, 1968) with a 0.5 m x 0.5 m quadrat at 0, 1, 5, and 10 m, with the center of the quadrat in line with the location of the corresponding soil core.

Soil chemistry and other variables

For each of the 336 soil samples, gravimetric water content (GWC) was measured as mass lost from soil after drying ~5 g of fresh soil at 105°C for 48 hours. Soil organic matter (SOM) was measured by loss-on-ignition (LOI) using ~5 g of soil. Total carbon and nitrogen was measured using a LECO TruSpec CN combustion analyzer (LECO Corporation, St. Joseph, MI, USA) with ~0.35 g of dried and ground soil and reported on a weight percent basis. Soil pH was measured in 1:1 suspension of field moist soil:DI with a pH electrode. Mean annual precipitation (MAP), mean annual temperature (MAT), and mean monthly temperature data were obtained from the National Oceanic and Atmospheric Administration (NOAA) 30-year Climate Normals Search Tool using the station closest to each sampling site (Arguez et al. 2010). Aridity index (AI) was calculated as the proportion of mean annual precipitation relative to potential evapotranspiration (P/PET), which is a simple but widely used measure of dryness. PET was calculated using the Thornthwaite method with SPEI R package (Beguería and Vicente-Serrano, 2017).

DNA extraction and polymerase chain reaction (PCR)

Total genomic DNA (gDNA) was extracted from approximately 0.5 g of homogenized soil per sample using the Qiagen DNeasy PowerSoil kit (Qiagen Sciences, Germantown, MD, USA) following manufacturer's instructions but with the following modifications: PowerBead Tubes were disrupted by bead beating for 20 s using MP biomedicals sample disruptor set at 4 m/s velocity, supernatant was transferred using the recommended minimum volume, and for the final DNA elution step 50 μL of solution C6 was added and incubated for 5 min at room temperature before spinning down and repeated using the flow-through. gDNA was stored at -20°C until further analysis. From the gDNA extracts, the 16S rRNA gene was targeted for Illumina sequencing using universal bacterial and archaeal primers (515F/926R) following established protocols (Caporaso et al. 2012, Parada et al. 2016) with one modification: PCR was run for 25 cycles instead of 35. Three technical replicates were run for each barcoded sample and each reaction was confirmed with 1 % agarose gel electrophoresis. Upon successful PCR, technical replicates were pooled, cleaned using Qiagen Exo-SapIT (Qiagen Sciences, Germantown, MD, USA), and amplicon pools quantified using the Quant-iT PicoGreen assay kit (Life Technologies, Grand Island, NY, USA). Amplicon amounts were then normalized to 75 ng per barcoded sample, combined into one library and cleaned using a QIAquick Gel Extraction Kit (Qiagen, Germantown, MD, USA). The library was sequenced on a 2 x 250 paired-end read Ilumina MiSeq run (Caporaso et al. 2012; Zeglin et al. 2016), with a 15% PhiX spike, at the Kansas State University Integrated Genomics Facility.

Bioinformatics

Raw Illumina sequence data was processed using the QIIME2 software package (Boylen et al., 2019). Sequences were demultiplexed, joined and quality filtered using the q-score

filtering method set to a PHRED score threshold of 20. Proceeding with only the forward sequences, they were trimmed to 250 base pairs and denoised using deblur. The remaining sequences were clustered to 97% sequence similarity and assigned to operational taxonomic units (OTUs) using the open-source workflow. After removing chimeric sequences using vsearch, OTUs were aligned to the GreenGenes v 13.18 16S rRNA gene reference database and taxonomy assigned using a Naïve-Bayes classifier trained at 97% similarity. Singletons, doubletons, and non-prokaryotic OTUs (i.e. chloroplasts and mitochondria) and control and blank samples were removed using filter functions before further analysis. The remaining pre-processing, statistical analysis and visualizations were performed in R version 3.6.2 (R Core Team, 2017). The sequence library was further pre-processed using phyloseq version 3.10 (McMurdie and Holmes, 2013) by creating a phyloseq object and removing samples that did not have at least 4000 reads and removing OTUs that did not have at least 5 reads per sample, resulting in a dataset with 299 samples and 3,192,101 total sequences with 7437 unique OTUs. From this, two separate datasets were created: a rarefied dataset with all samples trimmed to 4000 sequences by random sampling resulting in 298 samples and 1,192,000 total sequences with 7388 unique OTUs, and a normalized data set by proportional transformation of each sample using total sequence counts resulting in 299 samples and 2,990,000 total sequences with 7437 unique taxa.

Diversity analysis

All alpha and beta diversity metrics were calculated using phyloseq (McMurdie and Holmes, 2013), and all statistical testing was done with base R version 3.6.2 (R Core Team, 2017) and the R package vegan (Oksanen et al. 2019), with a default P-value of 0.05 for all tests, unless otherwise stated. The alpha diversity metrics of chao1 and evenness were calculated for

each sample using the rarefied dataset. A Bray-Curtis dissimilarity matrix was calculated from the normalized dataset to evaluate beta diversity across all samples and community differences were visualized with non-metric multidimensional scaling (NMDS). A permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the effect on community composition of site, grazing history, and their interaction using the adonis function in package vegan (Oksanen et al. 2019) with 999 permutations. Correlation between site environmental variables and whole dataset community differences was assessed by fitting vectors to the Bray-Curtis NMDS ordination space using the envfit function. Redundancy analysis (RDA) was used to further understand how linear combinations of environmental variables might explain community structure at the regional level (Legendre & Legendre, 2012). Variables were categorized as edaphic/climate, management, or spatial, and 3 separate RDA models were constructed with the best variables in each category obtained by forward selection based on AIC and adjusted R^2. The community abundance data was Hellinger transformed prior to RDA analysis. Spatial variables were derived from distance-based Moran's Eigenvector Maps (db-MEM) using the eigenvectors as explanatory variables. This approach (formerly referred to as principle coordinate of neighbour matrices (PCNM)) allows for explicit incorporation of spatial scale into models and better captures spatial structure than alternative methods, such as trend surface analysis or mantel tests (Dray et al., 2006). This approach was carried out using the dbmem function in the adespatial package (Dray et al. 2020). Lastly, using the vegan varpart function, variance partitioning was performed to parse the amount of variation explained by each of the three covariate categories individually and jointly. The results were visualized using the eulerr package (Larsson, 2020).

Biogeographical analysis

The geographic coordinates for unrecorded sampling locations at 0.1 and 0.5 m were calculated using the transect bearing and distance from the 0 m sampling location, and all coordinates were used to construct a Euclidian distance matrix using the command geodesic in the package geosphere (Hijmans, 2019). A whole dataset distance-dissimilarity curve was made by plotting the pairwise Bray-Curtis dissimilarity values against corresponding log transformed pairwise Euclidian distances, and separate linear regression parameters for grazed and ungrazed soils were estimated using the lm function. Significance of these linear regression models was tested with ANOVA, and analysis of covariance (ANCOVA) was used to test whether the slopes of the line for grazed and ungrazed treatments were significantly different. The two distance matrices were then filtered to produce nine separate matrices for each site and the distance-dissimilarity lines estimated and tested using the same workflow.

Statistics on site and grazing effects

To test the effect of site, grazing history, and the interaction between the two, on soil microbial alpha diversity, aboveground plant structure, and edaphic variables, we used two-way analysis of variance (ANOVA) models. In addition to running ANOVA on each plant functional class cover (gram, forb, woody, litter, and bare), we tested the correlations between percent cover for each functional group and the management related variables of bison number, years of bison, and years since last fire (YSLF) using Pearson Product-Moment Correlation. For all ANOVAs, the post-hoc Tukey's test was used to assess which group means differed from one another.

Grazing effect size

We used two different measures to quantify the grazing effect size within each site. We first estimated the difference in microbial community composition between the grazed and ungrazed soils, as the difference between treatment centroids in Euclidian ordination space using the multivariate_difference function in the codyn package (Hallett et al. 2020). This output value ranges from 0-1 and is the Euclidean distance where 0 indicates no grazing treatment effect on community composition. Second, to estimate how grazing impacted the magnitude and direction of the distance-dissimilarity relationship at each site, we calculated the difference between regression slopes relative to our predicted response. That is, we subtracted the grazed slope from ungrazed slope, because we expected that a history of bison grazing would be associated with a weaker distance-dissimilarity relationship, thus positive slope difference values match our prediction. These two metrics of grazing effect were then used as response variables in two separate linear models to understand which site-specific variables contributed most to the grazing effect size. To quantify the grazing effect on the potential explanatory variables that were measured on each sample, we subtracted the site-average ungrazed value from the site-average grazed value (i.e., pH, total C and N, C:N, plant cover). Although the goal of this analysis was exploration, not prediction, we wanted to avoid overfitting the statistical models given there were only 9 observations (9 sites). Therefore, forward selection was performed on a subset of 8 preselected variables based on correlation coefficients, reducing collinearity, and established importance (Legendre & Legendre, 2012).

Grazing indicator taxa

To identify specific bacterial or archaeal OTUs that characterized grazed soils across all sites, we tested for differentially abundant OTUs between grazed and ungrazed samples using

negative binomial generalized linear models in the package DESeq2 (Love et al., 2014). We used the cleaned raw OTU counts dataset to create a DESeqDataSet object, during which the grazed covariate (grazed vs ungrazed) was designated as the study design factor and the initial steps of the calculation performed (McMurdie and Holmes, 2013). The DESeq2 analysis was then run calling the DSEeq function with default testing framework. The resulting differentially abundant taxa were filtered to only retain OTUs with a false-detection-rate adjusted P-value of 0.01 or less. The design factor was specified such that negative log fold changes correspond to lower OTU abundance in grazed soils and positive log fold changes correspond to greater abundance in grazed soil.

Results

Soil microbial community structure

Soil microbial community composition across the Great Plains sampling sites was directly and interactively structured by the presence of grazing bison and by differences among sites (PERMANOVA results, Table 2.2), with most of this variation (45%) explained by site alone, and the remainder either directly (1.4%) or site-specifically (7.4%) related to grazing (Table 2.2, Figure 2.2A). The best NMDS model of community dissimilarity had a stress value of 0.107 (Figure 2.2), and its two dimensions were most strongly correlated with environmental variables related to geography, climate, and soil type (Figure 2.2B). Specifically, NMDS axis 1 was best correlated with latitude, NMDS axis 2 was best correlated with longitude, annual AI, and MAP, and soil C and water content were also related to regional differences in the soil microbial community (Figure 2.2B). Soil order alone explained 14.4% of variation in community composition (Table 2.2).

Soil microbial diversity and composition

Site and grazing directly and interactively affected soil microbial richness (Graze*Site $P < 0.001$), while Shannon diversity (H') varied by site and grazing with no interaction (Graze $P = 0.002$, Site $P < 0.001$). These patterns appear to be driven by higher richness and H' where there was bison grazing across all sites, but with stronger site to site variation in richness (Figure S 2.1). SBR had the greatest, and TPNP the lowest, richness and H', and the gradient between the two includes KNZ and MAX on the low end, and NVR and SVR on the high end.

The top 10 most abundant OTUs belonged to the common soil phyla Actinobacteria, Bacteroidetes, and Proteobacteria (Figure S 2.2), with Gemmatimonadetes being the only phyla with significantly greater abundance in grazed soils. The putative native prairie indicator Verrucomicrobia DA101 was the 12^{th} most abundant phylum across all sites, and its relative abundance was not affected by grazing. To help inform the 1.3% of total community variation explained independently by grazing (Table 2.2), we identified 69 taxa across all sites that were positive or negative indicators of grazing, as defined by significant positive or negative log fold differences, respectively (Figure 2.3). Twenty taxa were significantly more abundant in bison-grazed soils, including *Methylobacterium* sp., *Nocardioides* sp., and *Sphingomonas* sp.; 59 taxa were indicators of ungrazed soils, including *Ensider* sp., *Bellilinea* sp., and *Filomicrobium* sp. (Figure 2.3).

Spatial distribution

Regionally, across all sites, the soil microbial community distance-dissimilarity slope (β) was positive across both grazed and ungrazed areas, but lower with bison grazing (Figure 2.4), as supported by the ANCOVA results ($P = 0.008$, Table 2.3). However, at individual sites, the grazing effect on the distance-dissimilarity relationship was variable in direction and magnitude,

with slope differences ($\beta_{ungrazed} - \beta_{grazed}$) ranging from -0.0546 to 0.0347 (Table 2.3). Notably, at three sites (KNZ, TPNP, and CCR) the opposite of the regional pattern emerged — i.e., β in the grazed area was steeper than in the ungrazed area — and at one site (SBR) β did not differ (Table 2.3, Figure S 2.3).

Plant and soil characteristics

Plant cover and soil characteristics varied among sites and with bison grazing. Two-way ANOVA indicated that site and grazing were both associated with differences in these variables (Table 2.4), but the ubiquitous site by grazing interaction effects indicate that the magnitude and direction of grazing impact varied among sites. At most sites, in areas with a history of grazing, graminoid cover was lower, forb cover was greater, litter cover was lower, and woody cover was unaffected. However, at a number of sites this effect was weak ($P > 0.05$), and directional exceptions to the trends were apparent in that bare ground cover was higher in ungrazed areas at KNZ and TPNP, and litter cover was lower in ungrazed areas at KNZ (Figure S 2.4). Many soil variables differed among sites, but only GWC and pH were somewhat consistently affected by grazing, in that both tended to be lower in grazed soils (Table 2.4); however, these effects were weak ($P > 0.05$) at most sites (Figure S 2.5). A grazing influence ($P < 0.05$) was also detected at KNZ, TPNP, and TGP for soil %N, C:N, and SOM, but the direction of the effect was not consistent among sites as indicated by significant interaction between site and grazing (Table 2.4, Figure S 2.5).

In areas with grazers present, graminoid cover was lower and had a weak negative correlation with bison number ($r = -0.16$, $P = 0.017$). Forb cover was greater with a history of grazing and had a weak positive correlation with bison number ($r = 0.2$, $P = 0.003$) and years of bison grazing ($r = 0.33$, $P < 0.001$), and a weak negative relationship with years since last fire (r

= -0.32, P < 0.001). Litter cover was lower with a history of grazing and had a negative correlation with bison number (r = -0.16, P = 0.018) and positive correlation with years since last fire (r = 0.38, P < 0.001). Woody cover was only affected by the interaction between site and grazing history and bare ground was different among sites, but the direction was dependent on the interaction with grazing history (Table 2.4). Neither woody cover nor bare ground was significantly correlated with the three management variables tested.

Correlations between site management attributes and soil microbial response to grazing

Grazing effect on soil microbial composition difference varied from 0.159 (CCR) to 0.494 (TPNP), but there was no consistent grazing effect on difference in community dispersion (Table S 2.1, Figure 2.5A). The final multiple linear regression model of composition difference contained four variables with no interaction: bison number (slope = 2.03 X 10^{-4}, P = 0.002), MAP (slope = 5.7 X 10^{-4}, P = 0.008), GWC difference (slope = 12.5, P = 0.001), and pH difference (slope = 0.38, P = 0.017). The multiple linear regression model assessing predictors of difference between distance-dissimilarity slopes in grazed and ungrazed areas ($\beta_{ungrazed} - \beta_{grazed}$, Table S 2.1, Figure 2.5B) contained five variables with no interactions: sample month AI (slope = -4.4 X 10^{-2}, P = 0.026), number of bison (slope = 8.0 X 10^{-6}, P = 0.041), bare ground difference (slope = -4.4 X 10^{-4}, P = 0.018), litter difference (slope = -3.8 X 10^{-4}, P = 0.055), and C:N difference (slope = 5.3 X 10^{-3}, P = 0.003) (Figure S 2.6).

Relative influence of grazing management, soil, and geographic distance on soil microbes

The best RDA models for each of the three explanatory groups − distance, environment, and management variables − were significant, with RDA axes 1 and 2 explaining a large (> 0.60)

proportion of the total constrained variance (Table 2.5). The db-MEM approach to capturing geographic distance produced 4 eigenfunctions with positive spatial autocorrelation, of which three (MEM1, the coarsest resolution, MEM4, the finest resolution, and MEM3, an intermediate resolution were chosen by forward selection for use in the distance RDA model. Years since last fire, prescribed burning, rotational grazing, bison number, years of bison grazing, and stocking rate were retained in the reduced management model, and MAP, Annual PET, soil order, GWC, pH, log of %C, and log of %N were retained in the reduced environmental model. Environmental variables explained the most variation in community composition (17% total and 4.7% independently), space explained 13% total and 2.1% independently, management variables explained 13% total and 3.3% independently, 6.5% of variation was shared among the three categories, and 77% of variation was unexplained (Figure 2.6).

Discussion

To our knowledge, this is the first study to address the role of bison grazing in structuring grassland soil microbial communities at a geographic scale large enough to approach the pre-colonial range of bison. At the regional level we found that variation among microbial communities is best explained by site specific variables (45.4%), and that grazing explained less variation (8.8%). Bison grazing did reduce the geographical distance - soil microbial community dissimilarity slope across all samples at all sites, providing support for a homogenizing effect of bison grazing on soil microbial biogeography at the regional scale. But at the site scale the community change and distance-dissimilarity patterns were variable (Figure 2.5), thus grazing impact can be mediated by bison management, aboveground vegetation, and soil chemistry. Despite the site-specific impacts, we identified microbial taxa that positively or negatively responded to bison grazing at all sites.

Because the sampling sites in this study are located across broad temperature (N-S) and aridity (E-W) gradients and different soil types (Table 2.1), we predicted that climate and soil variables would be the most important determinants of regional soil microbial community composition. Indeed, our results supported this prediction (Figure 2.2). However, contrary to studies that found soil chemical variables, such as pH, soil organic matter, and nitrogen availability, as drivers of large scale microbial diversity (Fierer and Jackson, 2006; Griffiths et al. 2011; Kaiser et al. 2016), we did not find the strongest relationships between these locally heterogeneous soil properties and microbial composition (Figure 2.2B). Although soil %C and water content were regional correlates of community variation, instead, latitude, longitude, MAP, AI, and soil order (Molisols vs. Entisols) were stronger correlates. This suggests that in this dataset, soil water and C may be more reflective of the history of soil formation, which is an integration of long-term climate regimes, plant inputs, and landscape position (Jenny 1941; Shi et al. 2012). These findings fit in the broader context that climate and soil development is an important modulator of Great Plains grassland ecosystem and community dynamics over various time scales (Knapp et al. 2002; Seager et al. 2018; Bruckerhoff et al. 2020), and has been corroborated as an important control of soil microbial diversity in many ecosystems (Wang et al. 2015; Delgado-Baquerizo et al. 2019). In addition, soil order is likely an important driver because it serves as an integrative proxy for *in situ* soil conditions that microbes have to navigate, such as water potential and nutrient diffusion, because of differences in soil texture and parent material (Carson et al. 2010; Serna-Chavez et al. 2013).

The differences in soil microbial communities between grazed and ungrazed areas at each site suggests that local factors are important in modulating any grazing effects on microbial community structure. Distance-dissimilarity is one the most common biogeographic patterns

found across the tree of life (Nekola and White 1999, Soininen et al. 2007), and a steeper slope to this relationship (i.e. higher similarity closer in space) can be due to stronger local habitat controls over competitive outcomes ("environmental filtering"), dispersal limitation (weak "mass effects"), or a combination of the two (Martiny et al. 2006). Grazing weakened soil microbial distance-dissimilarity at the regional scale for our study system (Figure 2.4), and while this cannot be due to the same herd of bison moving across the landscape and directly dispersing cells from site to site, it means that bison have a consistent direct or indirect effect on soil habitat and environmental filtering, or a consistent effect on dispersal limitation, at all sites. At any particular location, the specific mode of dispersal may make dispersal limitation (or lack thereof) more important to community assembly (Lindström and Langenheder, 2012). Also, the role of environmental filtering may be strong, given the well documented effects of grazing on soil physical and chemical variables (Ruess and McNaughton 1987; Johnson and Matchett 2001; Eldridge et al, 2017; Frank et al. 2017). Our results suggest that bison can modulate both mechanisms.

Soil pH and water availability were the most likely environmental modulators of the grazing effect on soil microbial community composition and dissimilarity differences among sites (Figure 2.5A and B, and S 2.6). Both pH and water are well-established important controls of microbial growth, activity, and survival. Water availability is integral to the growth and survival of soil microbes, as pore connectivity directly affects microbial access to nutrients and their interactions with other microbes (Carson et al. 2010, Manzoni et al. 2012), and this could contribute to the relatively weaker grazing effect at sites with lower soil water content. Further, as soil dries, nutrient diffusion and microbial dispersal decreases, and less competitive species gain an edge which leads to increased richness (Treves et al. 2003; Carson et al. 2010). In this

study, the sites on Entisols have sandier soil with lower soil water availability, and also tend to have lower soil nitrogen and greater microbial diversity in both grazed and ungrazed areas (Table 2.4, Figure S 2.5), supporting a mechanism of reduced local dominance because of increased richness across the entire site area regardless of grazer presence, resulting in a weakened grazing effect (Eldridge et al. 2017). While it is unclear how bison directly or indirectly decreased soil pH, other studies have pointed to grazer urine deposition as a cascading control on soil chemical variables (Haynes and Williams, 1992; van Groenigen et al. 2005; O'Callaghan et al. 2010). Although the difference in pH between grazed and ungrazed soil was not always large, (Figure S 2.5), soil microbial diversity can change substantially across local pH gradients, in association with greater challenges for growth and respiration of many bacterial populations at lower pH (Rousk et al. 2009, 2010). However, since microbial richness was higher in grazed soils, our results are not fully consistent with this mechanism. While a combination of soil pH and water changes could contribute to a filtering effect on microbial assembly in bison-grazed soil over time, they do not fully explain differences in spatial heterogeneity of microbial communities.

Grazing animals are known to directly alter available soil nutrients, most notably N, via dung and urine deposition that can increase N availability with a magnitude that depends on forage, soil and waste composition and elemental stoichiometry (Sitters and Venterink 2015, Liu et al. 2018). Thus, it would be expected that bison influence soil microbes through an N-availability filter, as has been observed in other grasslands, where key N-cycling functional groups and the whole microbial community showed increased activity and distinct structure in response to intensive grazing (Patra et al. 2005). Fertilizer-derived N availability also consistently promotes the competitive edge of putative copiotrophic soil microbes across many grasslands (Leff et al. 2015). In our dataset, total soil N and C:N were weakly and inconsistently

linked to bison grazing (Table 2.4, Figure S 2.4), and were unrelated to grazing effects on soil microbial composition or distance-dissimilarity (Figure 2.5A and B, Figure S 2.6). This does not imply that grazing had no effect on N availability, since total soil N concentration and stoichiometry are not measurements of biologically available N; however, it is still notable that grazing influence on soil microbes was not modulated by soil C or N status. In general, the influence of urine as a nitrogen source can be transient, as N transformation processes have been shown to proceed on the order of days in urine patches and led to fluctuations of multiple soil ions, including NH_3^+, NO_3^-, Ca_2^+, and Mg_2^+ (Haynes and Williams 1992), so may not have a sustained influence on microbial competitive outcomes. Also, dung deposition can serve as a strong microbial dispersal vector, in addition to a nutrient availability filter (Zeglin et al. 2016).

In conjunction with the direct influence of bison, changes in soil nutrient dynamics and aboveground grazing pressure might be expected to shift plant growth and composition, thus indirectly alter soil microbial communities through plant-microbe interactions. We measured a consistent plant functional group response to bison, in that grass and litter cover decreased, and forb cover increased in the presence of grazers (Table 2.4, Figure S 2.4). This is not surprising and corroborates other studies on plant community changes in the presence of large herbivores (Collins et al. 1998, Bakker et al. 2003; Frank et al. 2018). A shift in plant composition could also change soil resources (Berg and Smalla, 2009; de Vries et al. 2012), and cause concomitant shifts in soil microbial communities via changes in litter stoichiometry and rhizospheric metabolites. For example, grazing can change aboveground plant tissue N content (Ling et al. 2019), decrease total belowground production (Johnson & Matchett 2001), and increase root C exudation in the rhizosphere (Hamilton et al. 2008), all of which may allow competitive dominance of certain microbial taxa in grazed soils. Microbes also compete with plants,

especially for N, and thus a change in plant species could alter competitive interactions in the soil (Harrison et al. 2007). Management choices related to fire, as well as grazing, can also affect vegetation structure (Towne et al. 2005), which helps explain correlations of forb and litter cover with years since last fire across all study sites. However, despite the clear effects of fire and grazing management on vegetation, there were no statistical relationships between plant functional group cover and soil microbial responses to bison grazing (Figure 2.5, S 2.6).

While soil microbial communities were more similar to one another in grazed areas than in ungrazed areas across the large distances among sites (Figure 2.4), bison grazing did not consistently shift the distance-decay slope at each site (Figure S 2.3). Instead, this variability in pattern was modulated by local vegetation structure and bison management, in that the largest bison herds were associated with high distance-dissimilarity slope responses (TGP, FLR), and that sites with counterintuitively higher bare ground cover at ungrazed transects (KNZ, TPNP) were also the sites that most strongly ($P < 0.001$) contradicted the regional trend in distance-dissimilarity slope responses to grazing (Table 2.4, Figure 2.5B). It was surprising that soil microbial responses to grazing were related to herd size, not to stocking density, nor to the plant responses most indicative of herbivory impact. In addition to bison acting as a variably intense environmental filter for soil microbial community assembly, grazers could shift the balance between environmental filtering and dispersal limitation. Both bison abundance and bare ground cover are non-exclusively related to decreasing dispersal limitation by increasing passive or active movement of microbial cells into the soil. Grazers are central to both mechanisms as their activity increases the relative openness of the plant canopy, allowing for increased passive aerial dispersal, and also promotes active dispersal of microorganisms in their dung.

Aerial dispersal of microorganisms is common (Finlay and Clarke, 1999), and contributes to both stochastic assembly of microbial communities (Bottos et al. 2014, Svoboda et al. 2018) and deterministic assembly based on the differences in cell size (Wilkinson et al. 2012) and dormancy abilities (Locey 2010) that allow a cell to move long distances over long periods of time. Even though our data does not provide direct evidence of passive dispersal, the process is critical for further study, considering recent calls to characterize dispersal mechanisms in microbial systems (Hanson et al. 2012; Lindström and Langenheder 2012) and to understand importance for microbial function (Elliot et al. 2019; Evans et al. 2020). An alternative dispersal route of microbial cells is more direct – they could be 'hitch-hiking' on the bison, or through the bison via dung deposition. Because all bison are not moving among all sites, a dung vector is the a more likely explanation for patterns we observed here. One other recent study found convergence of soil microbial communities with bison dung additions regardless of prairie restoration status (Chantos 2017), strengthening the need for further exploration of bison-mediated dispersal. Either passive or active dispersal could promote deterministic community assembly, and there were some microbial taxa that consistently responded to bison grazing at all sites (Figure 2.3), further suggesting that there are taxa that predictably respond to bison grazing activity across Great Plains prairies regardless of local climate, soil, and management variables. Unfortunately, taxonomic annotation of 16S rRNA genes does not provide reliable inference of soil microbial traits (Sun et al. 2020), so we cannot speculate on the common functional attributes of these putative grazed-soil indicator taxa.

Drivers of β-diversity, and the underlying mechanisms responsible for biogeographic patterns, often differ with spatial scale (Martiny et al. 2011, Sreekar et al. 2018), because both mechanisms that control diversity across space − environmental filtering and dispersal limitation

− are at play (Martiny et al. 2006; Lindström and Langenheder, 2012). When comparing between grazed and ungrazed areas within a site, the relative importance of local factors like soil chemistry and management might be higher, but when comparing samples across sites the relative importance of dispersal, and geographic distance, might be higher. Variance partition of the amount of soil microbial community variation explained by local environment and management factors or geographic distance did not resolve any one set of explanatory factors to be most important; instead, there was a large overlap in variation explained by all three variable categories (Figure 2.6). Thus, it is especially important to learn how much variation can be attributed to variables that we did not measure, including both dispersal rate and soil N availability. The simultaneous operation of multiple drivers (Figure 2.6) is an important consideration for future research on grassland microbial biogeography and working toward isolating distinct mechanisms must be a priority.

Disentangling the drivers of soil microbial biogeography has been a challenge, especially when trying to parse of out the relative importance of factors across scale. With this observational study we show that environmental variables (climate and soil) explained the most variation in soil microbial composition, but a large portion of this was shared with geographic distance and management decisions (Figure 2.6), pointing to underlying spatial structure in soil properties and the impact that site level grazing management has on soil and plant properties, which feedback onto soil communities. Clearly, bison grazing has a strong effect on local and regional soil microbial community assembly, but findings from individual locations will not necessarily extrapolate well to other sites, and we cannot say how bison are altering the soil environment or dispersing cells. More attention should be given both to experimentally testing biogeographical mechanisms, and to cultivating more soil microorganisms to understand which

life history traits are expressed by certain taxa. Further studies should also examine how temporal climatic variation influences a standardized effect of grazing pressure, and how these patterns change over time. Overall, this research supports the importance of native grazers in mediating top-down control of soil microbial biogeography in Great Plains Prairies, and further exploration of this topic will help us better manage the microbially mediated ecosystem services that grasslands provide.

References Cited

Albright, M. B. N., R. C. Mueller, L. V. Gallegos-Graves, J. Belnap, S. C. Reed, and C. R. Kuske. 2019. Interactions of microhabitat and time control grassland bacterial and fungal composition. Frontiers in Ecology and Evolution 7:367.

Allred, B. W., S. D. Fuhlendorf, and R. G. Hamilton. 2011. The role of herbivores in Great Plains conservation: comparative ecology of bison and cattle. Ecosphere 2:1-17.

Aldezabal, A., L. Moragues, I. Odriozola, and I. Mijangos. 2015. Impact of grazing abandonment on plant and soil microbial communities in an Atlantic mountain grassland. Applied Soil Ecology 96:251–260.

Arguez A., I. Durre, S. Applequist, M. Squires, R. Vose, X. Yin, and R. Bilotta. 2010. NOAA's U.S. Climate Normals (1981-2010). [precipitation, temperature]. NOAA National Centers for Environmental Information. DOI:10.7289/V5PN93JP

Asner, G. P., A. J. Elmore, L. P. Olander, R. E. Martin, and A. T. Harris. 2004. Grazing systems, ecosystem responses, and global change. Annual Review of Environment and Resources 29:261–299.

Augustine, D. J., and S. J. McNaughton. 2006. Interactive effects of ungulate herbivores, soil fertility, and variable rainfall on ecosystem processes in a semi-arid savanna. Ecosystems 9:1242–1256.

Bailey, A.W., and C.E. Poultan. 1968. Plant communities and environmental interrelationship in a portion of the Tillamook Burn, Northwestern Oregon. Ecology 49, 1-13.

Bakker, C., J. M. Blair, and A. K. Knapp. 2003. Does resource availability, resource heterogeneity or species turnover mediate changes in plant species richness in grazed grasslands? Oecologia 137:385–391.

Bardgett, R. D., J. L. Mawdsley, S. Edwards, P. J. Hobbs, J. S. Rodwell, and W. J. Davies. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology 13:650–660.

Bardgett, R. D., and D. A. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258–2268.

Beguería S., and S.M Vicente-Serrano. 2017. SPEI: Calculation of the standardised precipitation-evapotranspiration index. R package version 1.7. https://CRAN.R-project.org/package=SPEI

Bengtsson, J., J. M. Bullock, B. Egoh, C. Everson, T. Everson, T. O'Connor, P. J. O'Farrell, H. G. Smith, and R. Lindborg. 2019. Grasslands-more important for ecosystem services than you might think. Ecosphere 10:e02582.

Berg, G., and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere: Plant species, soil type and rhizosphere communities. FEMS Microbiology Ecology 68:1–13.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M.

Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37:852–857.

Bond, W. J., F. I. Woodward, and G. F. Midgley. 2004. The global distribution of ecosystems in a world without fire. New Phytologist 165:525–538.

Borer, E. T., E. W. Seabloom, D. S. Gruner, W. S. Harpole, H. Hillebrand, E. M. Lind, P. B. Adler, J. Alberti, T. M. Anderson, J. D. Bakker, L. Biederman, D. Blumenthal, C. S. Brown, L. A. Brudvig, Y. M. Buckley, M. Cadotte, C. Chu, E. E. Cleland, M. J. Crawley, P. Daleo, E. I. Damschen, K. F. Davies, N. M. DeCrappeo, G. Du, J. Firn, Y. Hautier, R. W. Heckman, A. Hector, J. HilleRisLambers, O. Iribarne, J. A. Klein, J. M. H. Knops, K. J. La Pierre, A. D. B. Leakey, W. Li, A. S. MacDougall, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, B. Mortensen, L. R. O'Halloran, J. L. Orrock, J. Pascual, S. M. Prober, D. A. Pyke, A. C. Risch, M. Schuetz, M. D. Smith, C. J. Stevens, L. L. Sullivan, R. J. Williams, P. D. Wragg, J. P. Wright, and L. H. Yang. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508:517–520.

Bottos, E. M., A. C. Woo, P. Zawar-Reza, S. B. Pointing, and S. C. Cary. 2014. Airborne bacterial populations above desert soils of the McMurdo Dry Valleys, Antarctica. Microbial Ecology 67:120–128.

Briggs, J. M., A. K. Knapp, J. M. Blair, J. L. Heisler, G. A. Hoch, M. S. Lett, and J. K. McCarron. 2005. An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. BioScience 55:243-254.

Bruckerhoff, L. A., R. K. Connell, J. P. Guinnip, E. Adhikari, A. Godar, K. B. Gido, A. W. Boyle, A. G. Hope, A. Joern, and E. Welti. 2020. Harmony on the prairie? Grassland plant and animal community responses to variation in climate across land-use gradients. Ecology 101:e02986.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J. A. Gilbert, G. Smith, and R. Knight. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal 6:1621–1624.

Carson, J. K., V. Gonzalez-Quiñones, D. V. Murphy, C. Hinz, J. A. Shaw, and D. B. Gleeson. 2010. Low pore connectivity increases bacterial diversity in soil. Applied and Environmental Microbiology 76:3936–3942.

Chantos, K. 2017. Microbial community dynamics in tallgrass prairie soil affected by native grazer feces (*Bison bison*). Master's thesis. Department of Biological Sciences, Northern Illinois University.

Collins, S. L., A. K. Knapp, J. M. Briggs, J. M. Blair, and E. M. Steinauer. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. Science 280:745–747.

Collins, S. L., and M. D. Smith. 2006. Scale-dependent interaction of fire and grazing on community heterogeneity in tallgrass prairie. Ecology 87:2058–2067.

Delgado-Baquerizo, M., F. T. Maestre, P. B. Reich, T. C. Jeffries, J. J. Gaitan, D. Encinar, M. Berdugo, C. D. Campbell, and B. K. Singh. 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nature Communications 7:10541.

Delgado-Baquerizo, M., R. D. Bardgett, P. M. Vitousek, F. T. Maestre, M. A. Williams, D. J. Eldridge, H. Lambers, S. Neuhauser, A. Gallardo, L. García-Velázquez, O. E. Sala, S. R. Abades, F. D. Alfaro, A. A. Berhe, M. A. Bowker, C. M. Currier, N. A. Cutler, S. C. Hart, P. E. Hayes, Z.-Y. Hseu, M. Kirchmair, V. M. Peña-Ramírez, C. A. Pérez, S. C. Reed, F. Santos, C. Siebe, B. W. Sullivan, L. Weber-Grullon, and N. Fierer. 2019. Changes in belowground biodiversity during ecosystem development. Proceedings of the National Academy of Sciences 116:6891–6896.

de Vries, F. T., P. Manning, J. R. B. Tallowin, S. R. Mortimer, E. S. Pilgrim, K. A. Harrison, P. J. Hobbs, H. Quirk, B. Shipley, J. H. C. Cornelissen, J. Kattge, and R. D. Bardgett. 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters 15:1230–1239.

Dray, S., P. Legendre, and P. R. Peres-Neto. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecological Modelling 196:483–493.

Dray, S., D. Bauman, G. Blanchet, D. Borcard, S. Clappe, G. Guenard, T. Jombart, G. Larocque, P. Legendre, N. Madi, H.H. and Wagner. 2020. adespatial: multivariate multiscale spatial analysis. R package version 0.3-8. https://CRAN.R-project.org/package=adespatial

Eldridge, D. J., A. G. B. Poore, M. Ruiz-Colmenero, M. Letnic, and S. Soliveres. 2016. Ecosystem structure, function, and composition in rangelands are negatively affected by livestock grazing. Ecological Applications 26:1273–1283.

Eldridge, D. J., M. Delgado-Baquerizo, S. K. Travers, J. Val, I. Oliver, K. Hamonts, and B. K. Singh. 2017. Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores. Ecology 98:1922–1931.

Eldridge, D. J., S. K. Travers, J. Val, J.-T. Wang, H. Liu, B. K. Singh, and M. Delgado-Baquerizo. 2019. Grazing regulates the spatial heterogeneity of soil microbial communities within ecological networks. Ecosystems.

Elliott, D. R., A. D. Thomas, C. L. Strong, and J. Bullard. 2019. Surface stability in drylands is influenced by dispersal strategy of soil bacteria. Journal of Geophysical Research: Biogeosciences 124:3403–3418.

Esch, E. H., D. L. Hernández, J. R. Pasari, R. S. G. Kantor, and P. C. Selmants. 2013. Response of soil microbial activity to grazing, nitrogen deposition, and exotic cover in a serpentine grassland. Plant and Soil 366:671–682.

Evans, S. E., L. P. Bell-Dereske, K. M. Dougherty, and H. A. Kittredge. 2020. Dispersal alters soil microbial community response to drought. Environmental Microbiology 22:905–916.

Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences 103:626–631.

Fierer, N., J. Ladau, J. C. Clemente, J. W. Leff, S. M. Owens, K. S. Pollard, R. Knight, J. A. Gilbert, and R. L. McCulley. 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the united states. Science 342:621–624.

Finlay, B. J., and K. J. Clarke. 1999. Ubiquitous dispersal of microbial species. Nature 400:828–828.

Frank, D. A., S. J. McNaughton, and B. F. Tracy. 1998. The ecology of the earth's grazing ecosystems. BioScience 48:513–521.

Frank, D. A., R. L. Wallen, E. W. Hamilton, P. J. White, and J. D. Fridley. 2017. Manipulating the system: How large herbivores control bottom-up regulation of grasslands. Journal of Ecology 106:434–443.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E. A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global consequences of land use. science 309:570–574.

Griffiths, R. I., B. C. Thomson, P. James, T. Bell, M. Bailey, and A. S. Whiteley. 2011. The bacterial biogeography of British soils. Environmental Microbiology 13:1642–1654. doi:10.1111/j.1462-2920.2011.02480.x

Hallett L., M. Avolio, I. Carroll, S. Jones , A. MacDonald, D. Flynn, P. Slaughter, J. Ripplinger, S. Collins, C. Gries, and M. Jones. 2020. codyn: Community Dynamics Metrics. doi: 10.5063/F1N877Z6 (URL: https://doi.org/10.5063/F1N877Z6), R package version 2.0.4, <URL: https://github.com/NCEAS/codyn>.

Hamilton, E. W., D. A. Frank, P. M. Hinchey, and T. R. Murray. 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. Soil Biology and Biochemistry 40:2865–2873.

Hanson, C.A., J.A. Fuhrman, M.C. Horner-Devine, and J.B. Martiny. 2012. Beyond biogeographic patterns: process shaping the microbial landscape. Nature Reviews, Microbiology 10:497-506.

Harrison, K. A., R. Bol, and R. D. Bardgett. 2007. Preferences for different nitrogen forms by coexisting plant species and soil microbes. Ecology 88:989–999.

Haynes, R.J., and P.H. Williams, P.H. 1992. Changes in soil solution composition and pH in urine-affected areas of pasture. Journal of Soil Science, 43: 323-334. doi:10.1111/j.1365-2389.1992.tb00140.x

Hijmans, R.J. 2019. geosphere: Spherical Trigonometry. R package version 1.5-10. https://CRAN.R-project.org/package=geosphere

Jenny, H. 1941. Factors of soil formation. McGraw-Hill, New York, New York, USA.

Johnson, L. C., and J. R. Matchett. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. Ecology 82:3377–3389.

Kaiser, K., B. Wemheuer, V. Korolkow, F. Wemheuer, H. Nacke, I. Schöning, M. Schrumpf, and R. Daniel. 2016. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. Scientific Reports 6.

Knapp, A. K., J. M. Blair, J. M. Briggs, S. L. Collins, D. C. Hartnett, L. C. Johnson, and E. G. Towne. 1999. The keystone role of bison in north american tallgrass prairie. BioScience 49:39-50.

Knapp, A. K., P. A. Fay, J. M. Blair, S. L. Collins, M. D. Smith, J. D. Carlisle, C. W. Harper, B. T. Danner, M. S. Lett, and J. K. McCarron. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. Science 298:2202–2205.

Koerner, S.E., Smith, M.D., Burkepile, D.E. et al. 2018. Change in dominance determines herbivore effects on plant biodiversity. Nature Ecology and Evolution 2, 1925–1932.

Larsson, J. 2020. eulerr: area-proportional euler and venn diagrams with ellipses. R package version 6.1.0, <URL: https://cran.r-project.org/package=eulerr>.

Leff, J. W., R. D. Bardgett, A. Wilkinson, B. G. Jackson, W. J. Pritchard, J. R. De Long, S. Oakley, K. E. Mason, N. J. Ostle, D. Johnson, E. M. Baggs, and N. Fierer. 2018. Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. The ISME Journal 12:1794–1805.

Legendre, P., and L. Legendre. 2012. Numerical ecology. 3rd English edition. Elsevier Science, Amsterdam.

Lindström, E. S., and S. Langenheder. 2012. Local and regional factors influencing bacterial community assembly: Bacterial community assembly. Environmental Microbiology Reports 4:1–9.

Locey, K. J. 2010. Synthesizing traditional biogeography with microbial ecology: the importance of dormancy: Synthesizing traditional biogeography with microbial ecology. Journal of Biogeography.

Love M.I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15: 550

Ling, B., E. J. Raynor, D. G. Goodin, and A. Joern. 2019. Effects of fire and large herbivores on canopy nitrogen in a tallgrass prairie. Remote Sensing 11:1364.

Liu, C., L. Wang, X. Song, Q. Chang, D. A. Frank, D. Wang, J. Li, H. Lin, and F. Du. 2018. Towards a mechanistic understanding of the effect that different species of large grazers have on grassland soil N availability. Journal of Ecology 106:357–366.

Manzoni, S., P. Taylor, A. Richter, A. Porporato, and G. I. Ågren. 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196:79–91.

Martiny, J. B. H., B. J. M. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske, P. J. Morin, S. Naeem, L. Øvreås, A.-L. Reysenbach, V. H. Smith, and J. T. Staley. 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4:102–112.

Martiny, J. B. H., J. A. Eisen, K. Penn, S. D. Allison, and M. C. Horner-Devine. 2011. Drivers of bacterial β-diversity depend on spatial scale:5.

McMurdie, P. J., and S. Holmes. 2013. phyloseq: An r package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE 8:e61217.

Mitchell, R. J., A. J. Hester, C. D. Campbell, S. J. Chapman, C. M. Cameron, R. L. Hewison, and J. M. Potts. 2010. Is vegetation composition or soil chemistry the best predictor of the soil microbial community? Plant and Soil 333:417–430.

Nekola, J. C., and P. S. White. 1999. The distance decay of similarity in biogeography and ecology. Journal of Biogeography 26:867–878.

O'Callaghan, M., E. M. Gerard, P. E. Carter, R. Lardner, U. Sarathchandra, G. Burch, A. Ghani, and N. Bell. 2010. Effect of the nitrification inhibitor dicyandiamide (DCD) on microbial communities in a pasture soil amended with bovine urine. Soil Biology and Biochemistry 42:1425–1436.

Oggioni, S. D., R. Ochoa-Hueso, and B. Peco. 2020. Livestock grazing abandonment reduces soil microbial activity and carbon storage in a Mediterranean Dehesa. Applied Soil Ecology 153:103588.

Oksanen J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R. B. O'Hara, G.L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs and H. Wagner. 2019. vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Parada, A. E., D. M. Needham, and J. A. Fuhrman. 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples: Primers for marine microbiome studies. Environmental Microbiology 18:1403–1414.

Patra, A. K., L. Abbadie, A. Clays-Josserand, V. Degrange, S. J. Grayston, P. Loiseau, F. Louault, S. Mahmood, S. Nazaret, L. Philippot, F. Poly, J. I. Prosser, A. Richaume, and X. L. Roux. 2005. e\Effects of grazing on microbial functional groups involved in soil n dynamics. Ecological Monographs 75:65–80.

Pérez-Valera, E., M. Goberna, K. Faust, J. Raes, C. García, and M. Verdú. 2017. Fire modifies the phylogenetic structure of soil bacterial co-occurrence networks: Soil bacterial co-occurrence networks. Environmental Microbiology 19:317–327.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rousk, J., P. C. Brookes, and E. Bååth. 2009. c\Contrasting soil ph effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology 75:1589–1596.

Rousk, J., E. Bååth, P. C. Brookes, C. L. Lauber, C. Lozupone, J. G. Caporaso, R. Knight, and N. Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4:1340–1351.

Ruess, R. W., and S. J. McNaughton. 1987. Grazing and the dynamics of nutrient and energy regulated microbial processes in the Serengeti grasslands. Oikos 49:101–110.

Samson, F. B., F. L. Knopf, and W. R. Ostlie. 2004. Great Plains ecosystems: past, present, and future. Wildlife Society Bulletin 32:6–15.

Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386–1394.

Seager, R., N. Lis, J. Feldman, M. Ting, A. P. Williams, J. Nakamura, H. Liu, and N. Henderson. 2018. Whither the 100th meridian? The once and future physical and human geography of america's arid–humid divide. part I: The story so far. Earth Interactions 22:1–22.

Serna-Chavez, H. M., N. Fierer, and P. M. van Bodegom. 2013. Global drivers and patterns of microbial abundance in soil. Global Ecology and Biogeography 22:1162–1172.

Shade, A., J. G. Caporaso, J. Handelsman, R. Knight, and N. Fierer. 2013. A meta-analysis of changes in bacterial and archaeal communities with time. The ISME journal 7:1493–1506.

Shi, Y., F. Baumann, Y. Ma, C. Song, P. Kühn, T. Scholten, and J.-S. He. 2012. Organic and inorganic carbon in the topsoil of the Mongolian and Tibetan grasslands: pattern, control and implications. Biogeosciences 9:2287–2299.

Sitters, J., and H.O. Venterink. 2015. The need for a novel integrative theory on feedbacks between herbivores, plants and soil nutrient cycling. Plant and Soil 396:421–426.

Soininen, J., R. McDonald, and H. Hillebrand. 2007. The distance decay of similarity in ecological communities. Ecography 30:3–12.

Sreekar, R., M. Katabuchi, A. Nakamura, R. T. Corlett, J. W. F. Slik, C. Fletcher, F. He, G. D. Weiblen, G. Shen, H. Xu, I.-F. Sun, K. Cao, K. Ma, L.-W. Chang, M. Cao, M. Jiang, I. A. U. N. Gunatilleke, P. Ong, S. Yap, C. V. S. Gunatilleke, V. Novotny, W. Y. Brockelman, W. Xiang, X. Mi, X. Li, X. Wang, X. Qiao, Y. Li, S. Tan, R. Condit, R. D. Harrison, and L. P. Koh. 2018. Spatial scale changes the relationship between beta diversity, species richness and latitude. Royal Society Open Science 5:181168.

Stebbins, G. L. 1981. Coevolution of grasses and herbivores. Annals of the Missouri Botanical Garden 68:75-86.

Stevens, B. M., D. Lee Sonderegger, and N. C. Johnson. 2020. Biotic and abiotic factors predict the biogeography of soil microbes in the Serengeti. preprint, Microbiology. bioRxiv 2020.02.06.936625; doi: https://doi.org/10.1101/2020.02.06.936625

Sun, B., S. Zhou, and Q. Zhao. 2003. Evaluation of spatial and temporal changes of soil quality based on geostatistical analysis in the hill region of subtropical China. Geoderma 115:85–99.

Sun, S., R. B. Jones, and A. A. Fodor. 2020. Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. Microbiome 8:46.

Svoboda, P., E. S. Lindström, O. Ahmed Osman, and S. Langenheder. 2018. Dispersal timing determines the importance of priority effects in bacterial communities. The ISME Journal 12:644–646.

Towne, E. G., D. C. Hartnett, and R. C. Cochran. 2005. Vegetation trends in tallgrass prairie from bison and cattle grazing. Ecological Applications 15:1550–1559.

Treves, D. S., B. Xia, J. Zhou, and J. M. Tiedje. 2003. A two-species test of the hypothesis that spatial isolation influences microbial diversity in soil. Microbial Ecology 45:20–28.

Truett, J. C., M. Phillips, K. Kunkel, and R. Miller. 2001. managing bison to restore biodiversity. Great Plains Research: A Journal of Natural and Social Sciences 11:123-144.

van Groenigen, J. W., P. J. Kuikman, W. J. M. de Groot, and G. L. Velthof. 2005. Nitrous oxide emission from urine-treated soil as influenced by urine composition and soil physical conditions. Soil Biology and Biochemistry 37:463–473.

Wang, X., J. D. Van Nostrand, Y. Deng, X. Lü, C. Wang, J. Zhou, and X. Han. 2015. Scale-dependent effects of climate and geographic distance on bacterial diversity patterns across northern China's grasslands. FEMS Microbiology Ecology:fiv133.

Wilcox, K. R., Z. Shi, L. A. Gherardi, N. P. Lemoine, S. E. Koerner, D. L. Hoover, E. Bork, K. M. Byrne, J. Cahill, S. L. Collins, S. Evans, A. K. Gilgen, P. Holub, L. Jiang, A. K. Knapp, D. LeCain, J. Liang, P. Garcia-Palacios, J. Peñuelas, W. T. Pockman, M. D. Smith, S. Sun, S. R. White, L. Yahdjian, K. Zhu, and Y. Luo. 2017. Asymmetric responses of primary productivity to precipitation extremes: A synthesis of grassland precipitation manipulation experiments. Global Change Biology 23:4376–4385.

Wilkinson, D. M., S. Koumoutsaris, E. A. D. Mitchell, and I. Bey. 2012. Modelling the effect of size on the aerial dispersal of microorganisms. Journal of Biogeography 39:89–97.

Zeglin, L. H., B. Wang, C. Waythomas, F. Rainey, and S. L. Talbot. 2016. Organic matter quantity and source affects microbial community structure and function following volcanic eruption on Kasatochi Island, Alaska. Environmental Microbiology 18:146–158.

Zhao, F., C. Ren, S. Shelton, Z. Wang, G. Pang, J. Chen, and J. Wang. 2017. Grazing intensity influence soil microbial communities and their implications for soil respiration. Agriculture, Ecosystems & Environment 249:50–56.

Tables and Figures

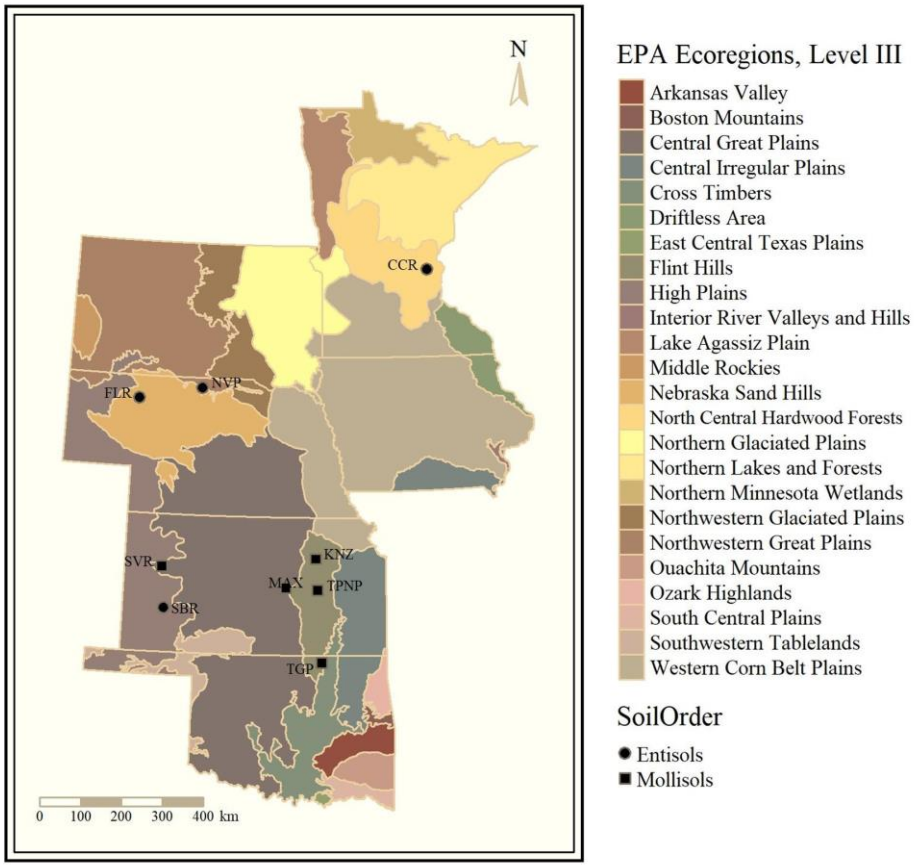

Figure 2.1 Map of site headquarters with Environmental Protection Agency (EPA) ecoregions level III. The site symbols are coded by soil order.

Table 2.1 Sampling site information, including bison and fire management, soil, and climate characteristics, ordered from west to east. Abbreviations: TNC = The Nature Conservancy, KDWPT = Kansas Departmenet of Wildlife, Parks, and Tourism, KSU= Kansas State University, NPS = National Parks System, UMN = University of Minnesota, lat = latitude in decimal degrees, long = longitude in decimal degrees, MAP = mean annual precipitation, MAT = mean annual temperature, AI = aridity index

Site *Date sampled*	Location (lat/long)	Management (Type)	Bison reintroduction (year)	Stocking Density (acres/animal)	Rotational Grazing	Prescribed Burns	Soil Order	MAP (mm)	MAT (°C)	AI (P/PET)
Fawn Lake Ranch (FLR) 31 July 2019	Gordan, NE (42.499, -101.902)	Turner Enterprise, Inc. (Private Ranch)	2006	1 to 3	Yes	No	Entisols	463	8	0.77
Smoky Valley Ranch (SVR) *11 July 2019*	Oakley, KS (38.861, -100.983)	TNC (Ranch Preserve)	2013	21	Yes	Yes	Mollisols	501	11	0.71
Sandsage Bison Range (SBR) *24 July 2019*	Garden City, KS (37.949, -100.88)	KDWPT (Wildlife Refuge)	1924	73	Yes	No	Entisols	486	12	0.67
Niobrara Valley Preserve (NVP) *18 July 2019*	Johnstown, NE (42.784, -100.028)	TNC (Ranch Preserve)	1985	36	No	Yes	Entisols	599	9	0.94
Maxwell Wildlife Refuge (MAX) *5 July 2019*	Canton, KS (38.477, -97.451)	KDWPT (Wildlife Refuge)	1951	13	No	Yes	Mollisols	833	13	1.08
Konza Prairie Biological Station (KNZ) *28 June 2019*	Manhattan, KS (39.107, -96.609)	TNC and KSU (Experimental Station & Preserve)	1987	8	No	Yes	Mollisols	899	12.5	1.19
Tallgrass Prairie National Preserve (TPNP) *9 July 2019*	Strong, KS (38.432, -96.558)	TNC and NPS (Preserve)	2014	12	No	Yes	Mollisols	907	12.5	1.18
Tallgrass Prairie Preserve (TGP) *16 July 2019*	Pawhuska, OK (36.846, -96.423)	TNC (Ranch Preserve)	1989	9	No	Yes	Mollisols	1192	15	1.39
Cedar Creek Ecosystem Science Reserve (CCR) *23 September 2019*	East Bethel, MN (45.402, -93.199)	UMN (Experimental Station & Preserve)	2018	8	No	Yes	Entisols	814	7	1.31

Table 2.2 PERMANOVA results across all sites for soil microbial community composition (two-way test for Graze and Site and one-way test for SoilOrder: Entisols vs. Mollisols)

Factor	Sum of Squares	F	R^2	P
Site	36.13	34.75	0.454	0.001
Graze	1.08	8.313	0.014	0.001
Site*Graze	5.85	5.63	0.074	0.001
Residuals	36.52	-	0.459	-
SoilOrder	11.5	50.15	0.144	0.001
Residuals	68.08	-	0.856	-

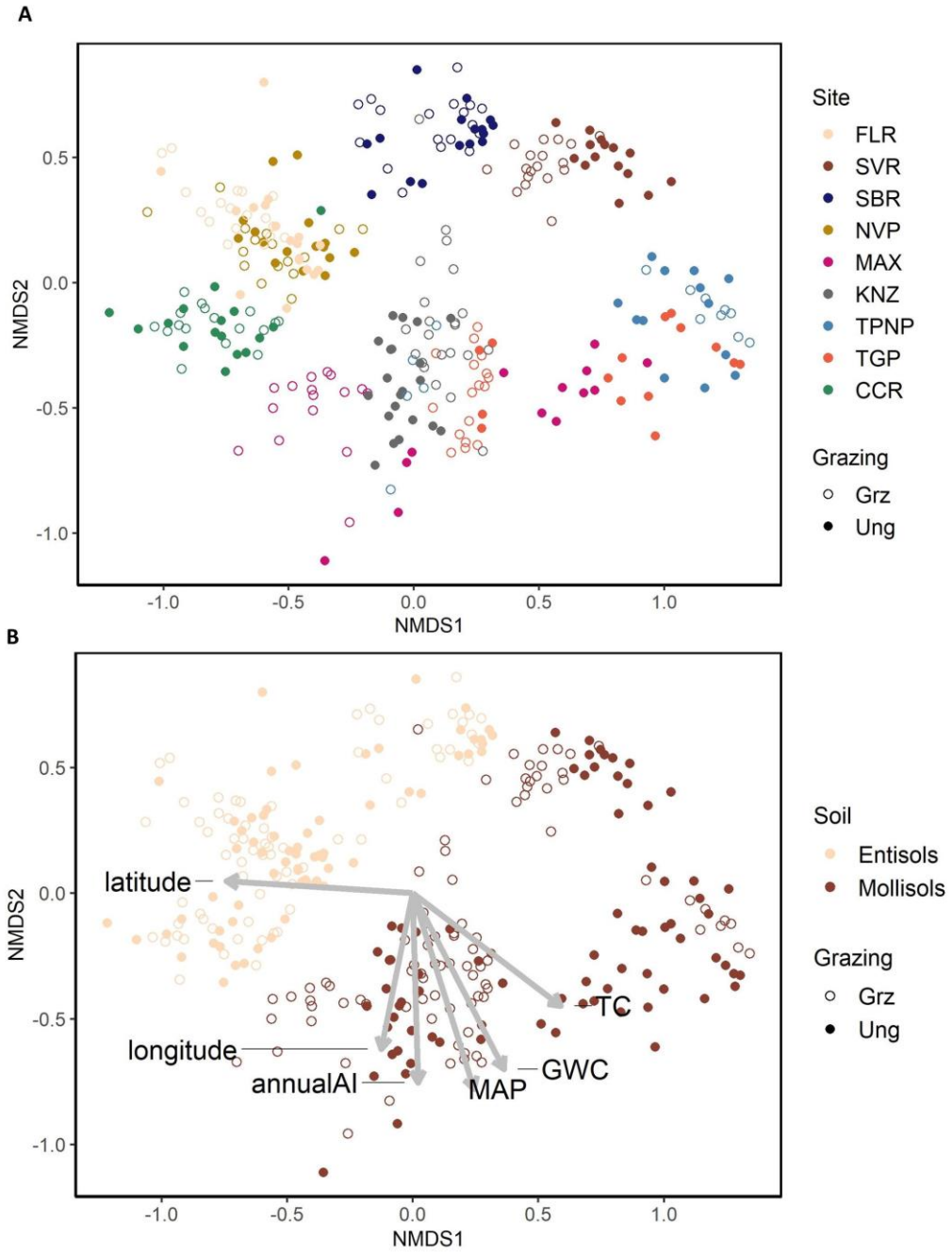

Figure 2.2 NMDS ordination models of 16s rRNA gene community composition for all samples A) with colors representing sites and symbols grazing history, and B) with colors representing soil order and symbols grazing history, with the top environmental vectors fitted on the ordination. Abbreviations: ung = no bison, grz = bison grazing, annualAI = annual aridity index, MAP = mean annual precipitation, GWC = gravimetric water content, and TC = total carbon.

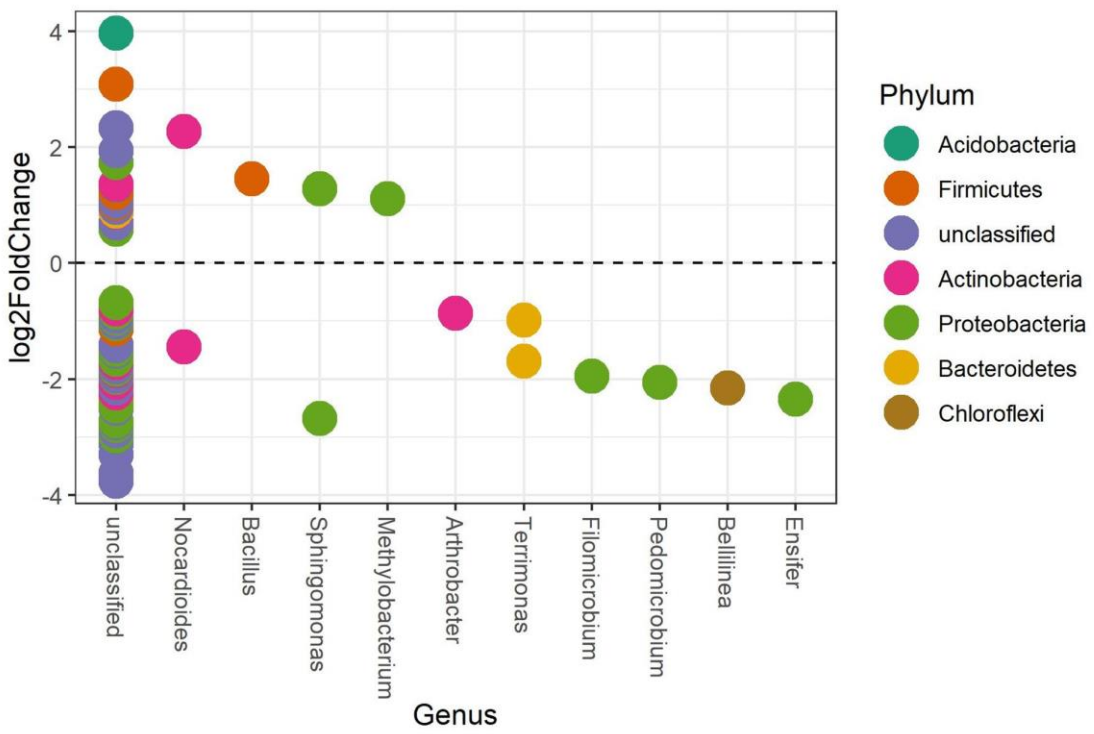

Figure 2.3 Differentially abundant taxa calculated using negative binomial generalized linear models with DESeq2 package and defined at a significance level of 0.01. Positive log2FoldChange values indicated greater abundance in grazed areas.

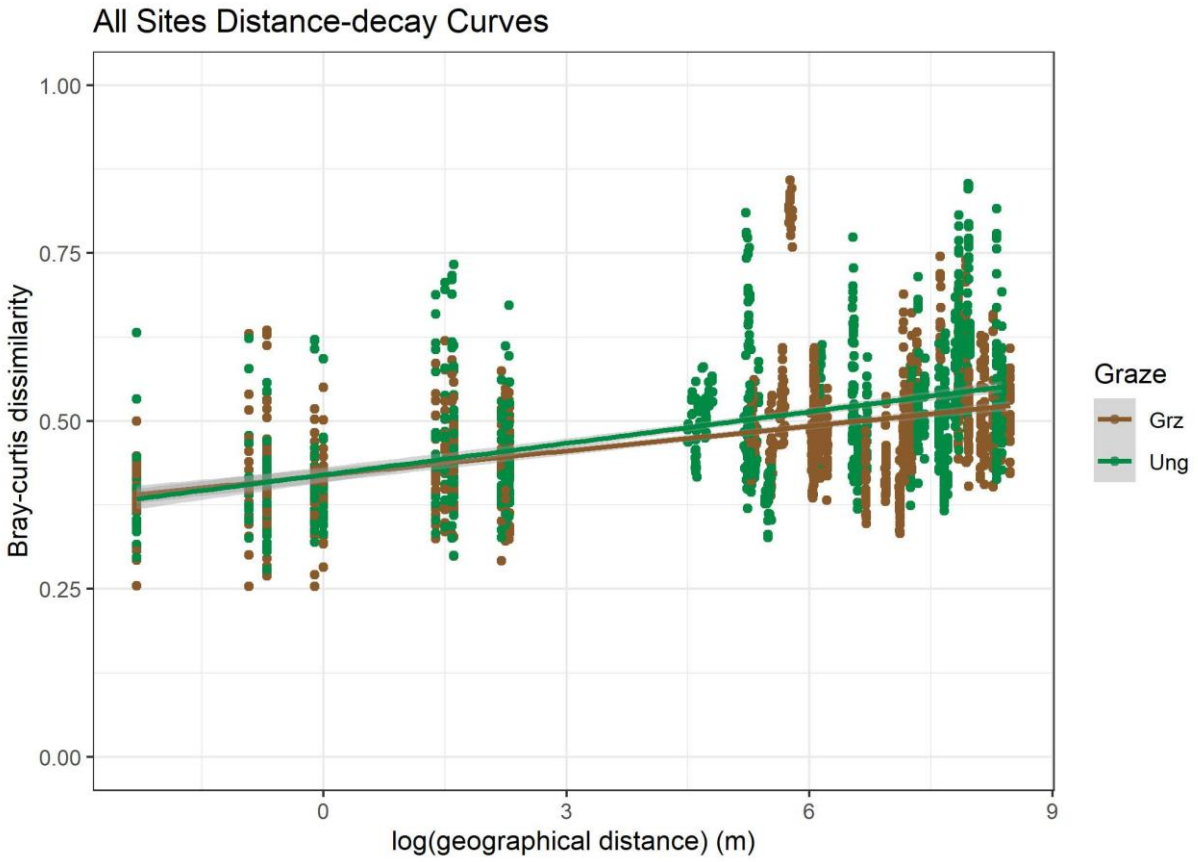

Figure 2.4 Plot of distance-dissimilarity curves using all sites, with the linear regression lines displayed for both grazed (grz, brown line) and ungrazed (ung, green line) soils.

Table 2.3 Summary of individual and full dataset (FULL) distance decay linear regression models for both grazed and ungrazed samples and analysis of covariance (ANCOVA) results to compare slopes within sites. Bolded values indicated significant results ($P < 0.05$), and $\beta_{difference}$ is calculated as $\beta_{ungrazed} - \beta_{grazed}$.

	Grazed				Ungrazed				Comparison	
Site	β	Y-int	R2	P	β	Y-int	R2	P	$\beta_{difference}$	P
FLR	0.0062	0.4552	0.0834	**< 0.001**	0.0159	0.4465	0.214	**< 0.001**	0.0097	**0.001**
SVR	0.0091	0.407	0.2947	**< 0.001**	0.0211	0.349	0.5496	**< 0.001**	0.012	**< 0.001**
SBR	0.0124	0.3894	0.3839	**< 0.001**	0.0138	0.4253	0.3637	**< 0.001**	0.0014	0.501
NVP	0.0043	0.4691	0.0503	**0.009**	0.0178	0.3996	0.3458	**< 0.001**	0.0135	**< 0.001**
MAX	0.012	0.4311	0.317	**< 0.001**	0.0305	0.4674	0.4526	**< 0.001**	0.0185	**< 0.001**
KNZ	0.0199	0.3542	0.2736	**< 0.001**	0.0069	0.4069	0.1404	**< 0.001**	-0.013	**< 0.001**
TPNP	0.079	0.3412	0.861	**< 0.001**	0.0244	0.3833	0.5905	**< 0.001**	-0.0546	**< 0.001**
TGP	0.0141	0.3711	0.4947	**< 0.001**	0.0488	0.2746	0.5586	**< 0.001**	0.0347	**< 0.001**
CCR	0.017	0.4333	0.4195	**< 0.001**	0.0092	0.491	0.0552	**0.006**	-0.0078	**0.0334**
FULL	0.0533	-1.02	0.5336	**< 0.001**	0.0785	-1.3218	0.33	**< 0.001**	0.0252	**0.008**

Table 2.4 ANOVA results for soil and plant characteristics. Abbreviations: ung = no bison, grz = bison grazing; values in bold are significant and group noted in parenthesis represents the category with a greater mean value for the response variable.

Variable	Factors		
	Site F, P	Graze F, P	Site*Graze F, P
Grass cover (%)	39.42, < 0.001	32.93, < 0.001 *(ung)*	7.58, < 0.001
Forb cover (%)	5.71, < 0.001	27.62, < 0.001 *(grz)*	4.21, < 0.001
Woody cover (%)	1.84, 0.071	1.04, 0.309	3.16, 0.002
Litter cover (%)	48.78, < 0.001	21.19, < 0.001 *(ung)*	12.04, < 0.001
Bare ground cover (%)	18.39, < 0.001	1.40, 0.238	12.81, < 0.001
GWC	839.32, < 0.001	68.68, < 0.001 *(ung)*	7.39, < 0.001
pH	148.42, < 0.001	62.1, < 0.001 *(ung)*	21.53, < 0.001
SOM	299.80, < 0.001	1.07, 0.303	4.33, <0.001
TC	459.34, < 0.001	0.666, 0.415	2.079, 0.069
TN	351.51, < 0.001	1.46, 0.228	11.61, < 0.001
C:N	36.812, < 0.001	0.001, 0.981	8.1, < 0.001

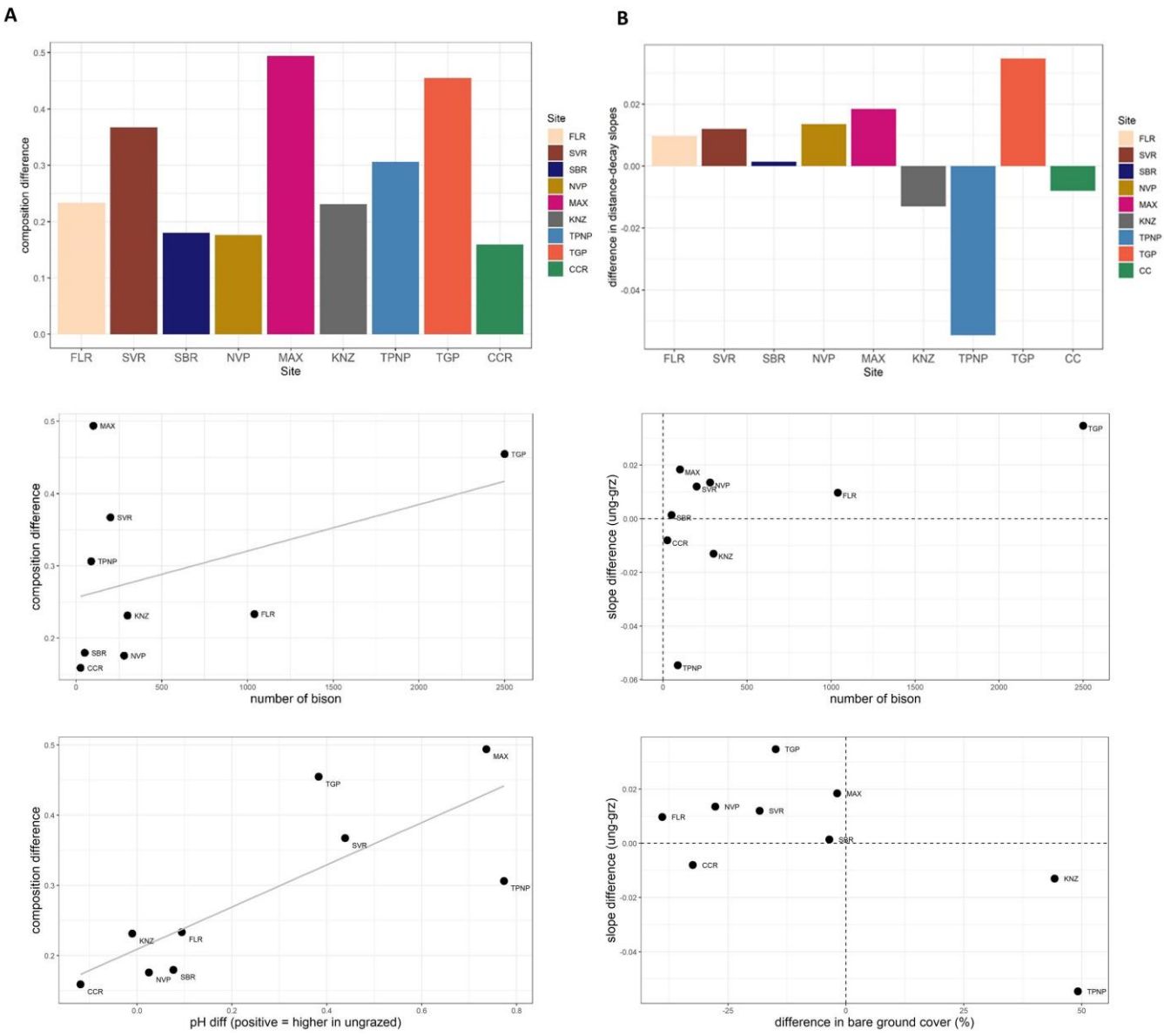

Figure 2.5 Grazing effect graphs A) bar plots of composition difference measured as difference between treatment centroids and the values plotted against number of bison and difference in pH between grazed and ungrazed samples and B) bar plots of difference in slope and the values plotted against number of bison and difference in bare ground cover between grazed and ungrazed samples. Sites are ordered west to east in the bar plots.

Table 2.5 Best RDA models for each of the explanatory groups. Forward selection was employed to keep the best variables for each group. RDA axes represent constrained variation while PC axes represent unconstrained and thus variation not attributed to the explanatory variables tested. Variation explained by each explanatory group independently was found using variance portioning.

				Proportion of total variance explained by RDA			Proportion of total explained (constrained) variance	
Explanatory group	Full constrained adj-R2	Reduced Constrained adj-R2	Number of reduced constrained axes	RDA1	RDA2	PC1	RDA1	RDA2
Space (X1)	0.1456	0.1291	3	0.095	0.031	0.1046	0.690	0.227
Environmental (X2)	0.1711	0.1673	7	0.093	0.047	0.058	0.497	0.271
Management (X3)	0.1382	0.1287	6	0.085	0.021	0.1126	0.583	0.140
X1 | X2 + X3		0.0207						
X2 | X1 + X3		0.0471						
X3 | X1 + X2		0.0334						

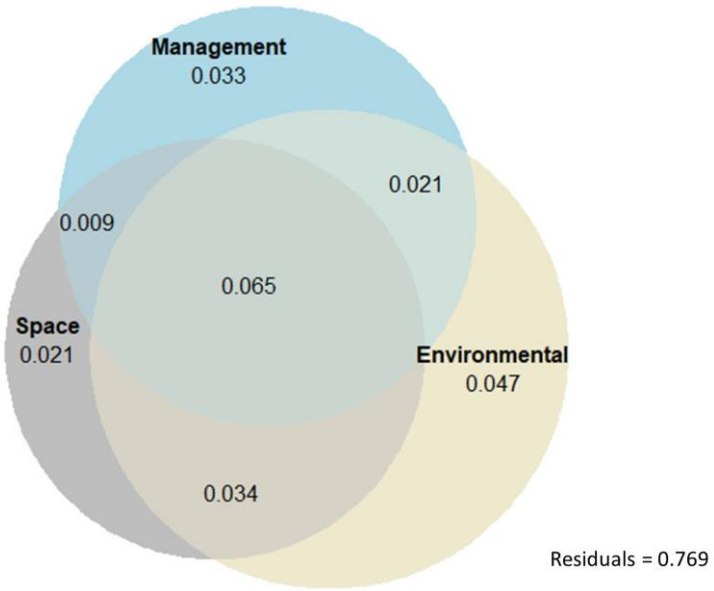

Figure 2.6 Plot of variance partitioning results with Venn diagram components proportional to the amount of variance explained by geographic distance, environmental characteristics, and management attributes.

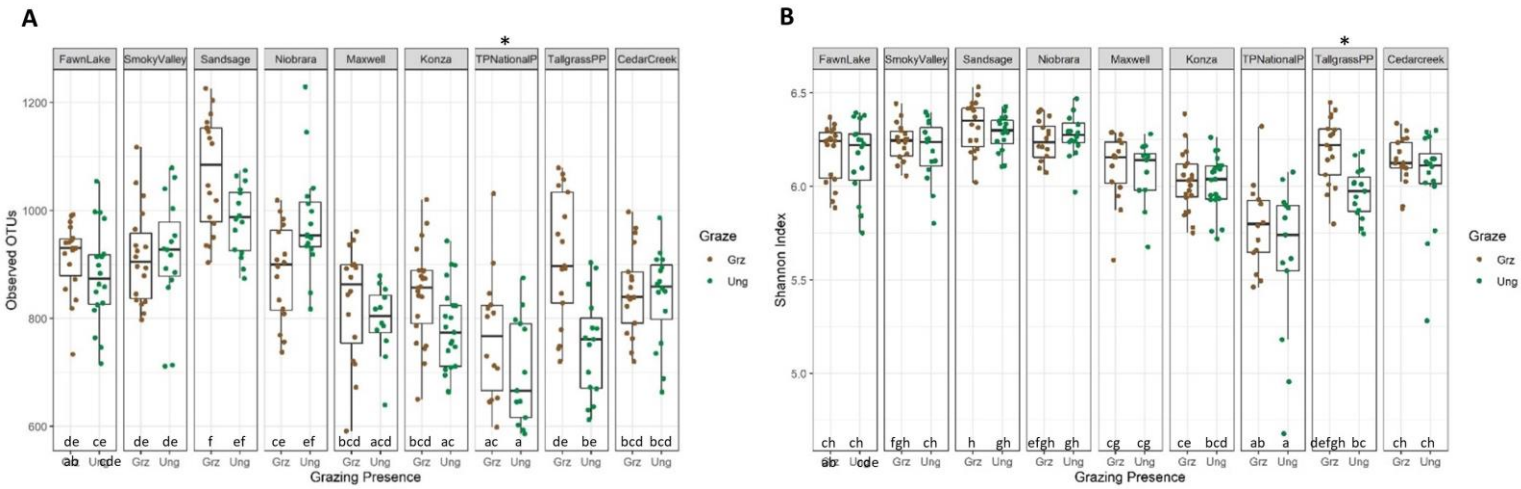

Figure S 2.1 Boxplots of A) soil microbial OTU richness and B) Shannon index for all samples faceted by site. Asterisk (*) indicates significant grazing effect at that site and letter indicates groups for site-specific grazing effects.

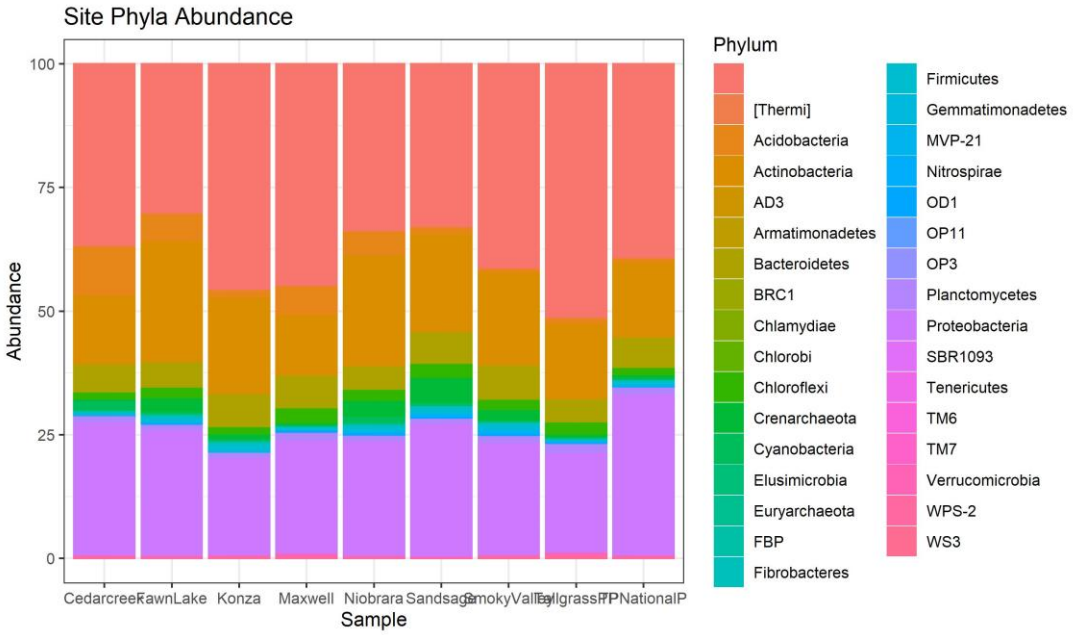

Figure S 2.2 Relative abundance of all taxa pooled by phyla at each site.

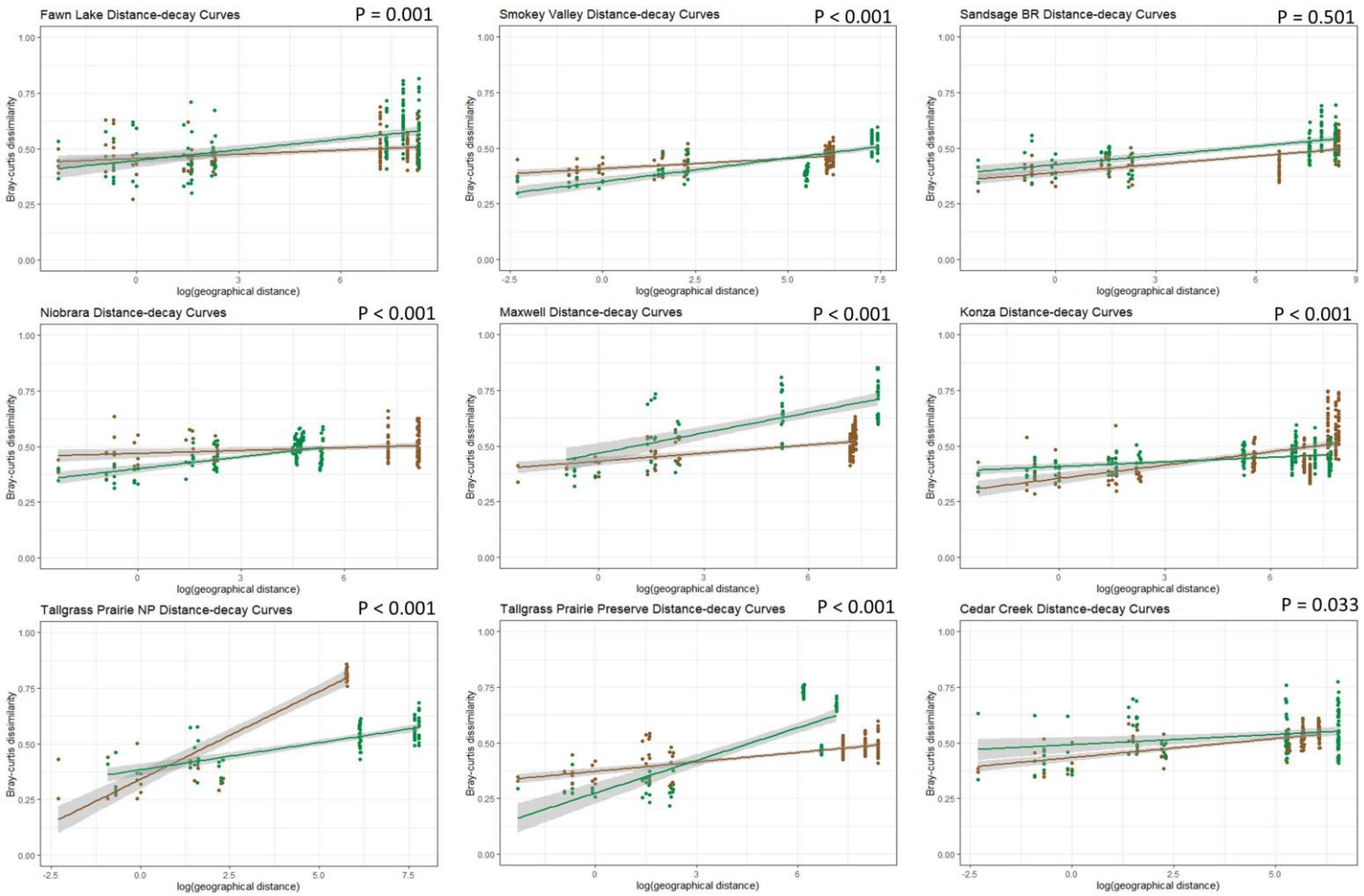

Figure S 2.3 Plots of individual site distance-decay curves, with the linear regression lines displayed for both grazed (grz, brown line) and ungrazed (ung, green line) soils. Site specific P-values for ANCOVA results are reported in the top right corner of each plot.

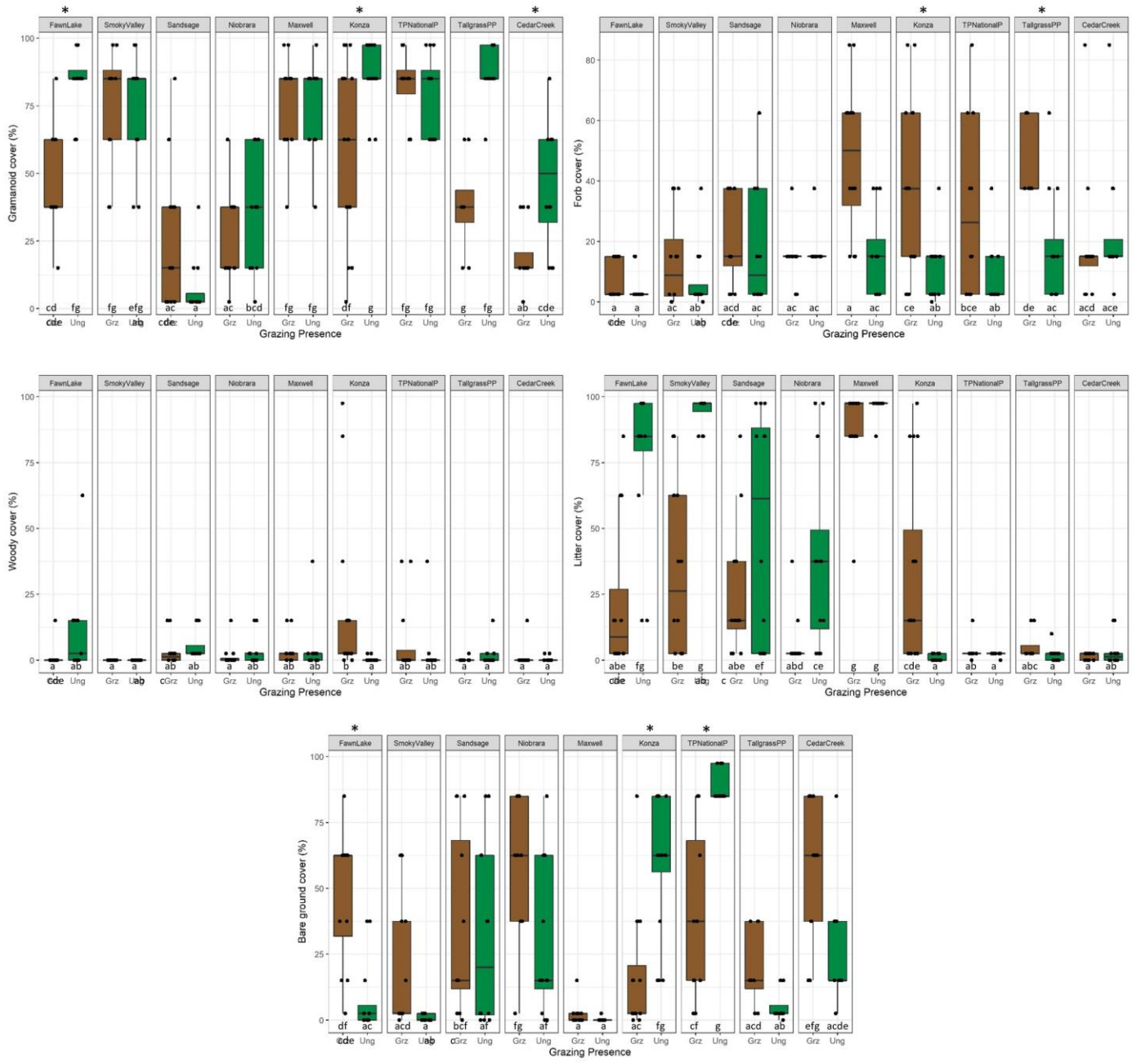

Figure S 2.4 Boxplots of vegetation cover for all samples faceted by site. Asterisk (*) indicates significant grazing effect at that site and letter indicates groups for site-specific grazing effects.

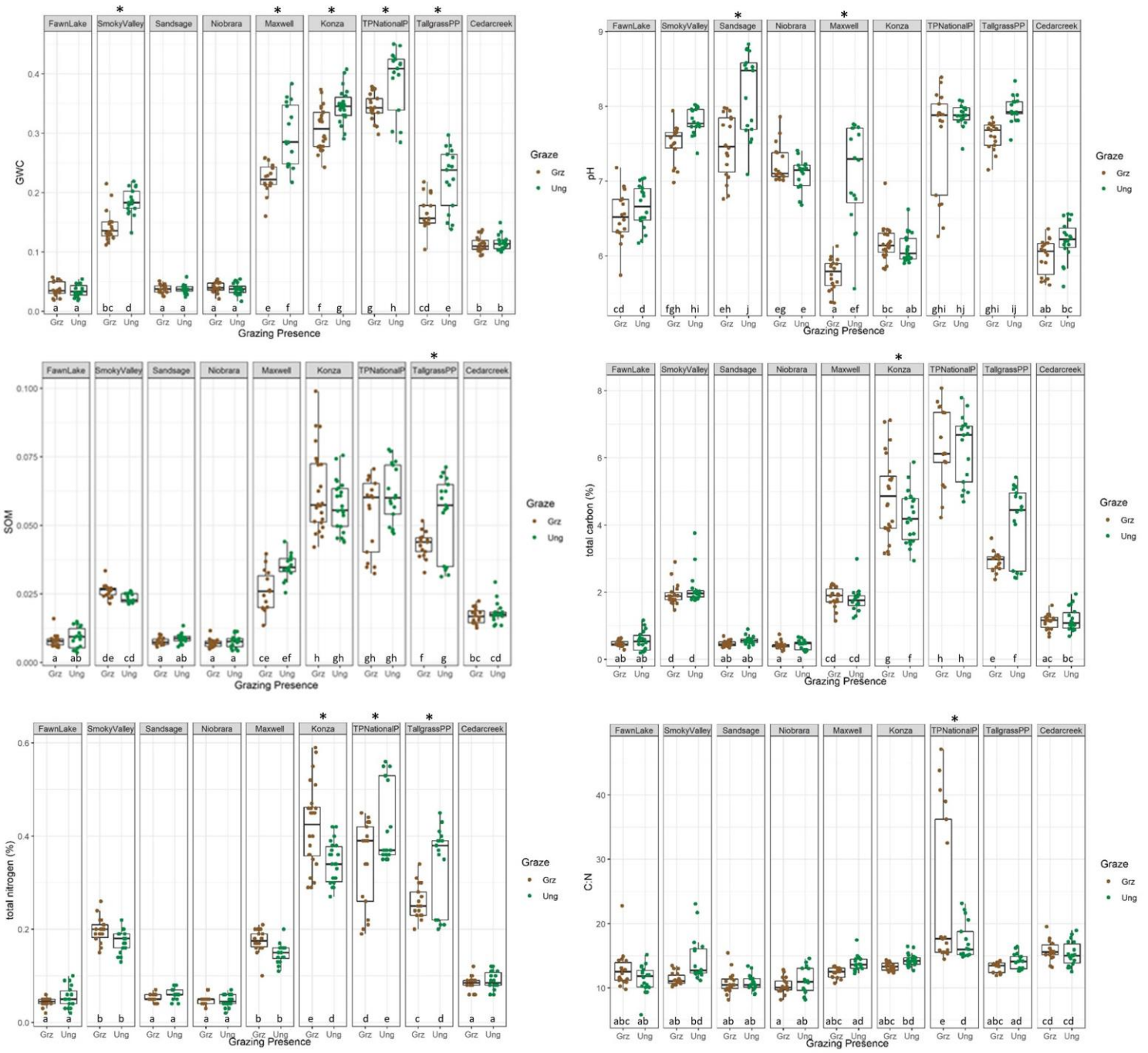

Figure S 2.5 Boxplots of soil variables for all samples faceted by site. Asterisk (*) indicates significant grazing effect at that site and letter indicates groups for site-specific grazing effects.

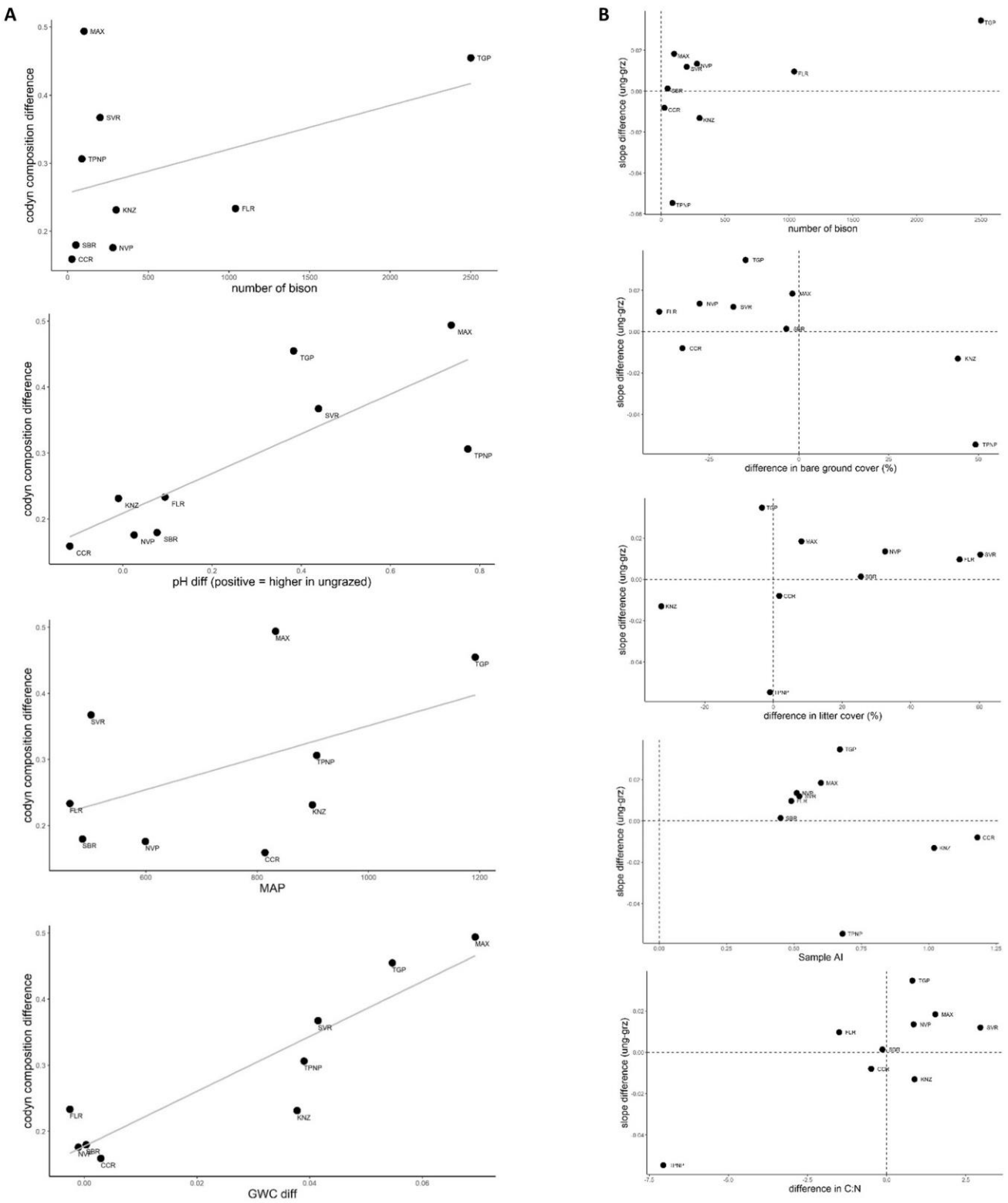

Figure S 2.6 Scatterplots of retained covariates for best linear regression models predicting A) codyn composition and B) difference in distance decay slopes.

Table S 2.1 Output for the 'codyn' multivariate_difference function showing within site comparisons only. Sites are ordered west to east.

Site	Composition difference	[dispersion difference]	Treatment with greater dispersion
FLR	0.233427	0.032848	Ung
SVR	0.367328	0.003664	Ung
SBR	0.179708	0.029038	Ung
NVP	0.175861	0.017373	Grz
MAX	0.494099	0.092302	Ung
KNZ	0.231318	0.016773	Grz
TPNP	0.306267	0.100333	Grz
TGP	0.45483	0.051134	Ung
CCR	0.158937	0.013782	Ung

Chapter 3 - Experimental manipulation of passive dispersal and active dispersal via bison dung affects soil microbial diversity and composition similarly across different land uses in a tallgrass prairie

Abstract

Microbial communities display biogeographical patterns that are driven by local environmental conditions and dispersal limitation, but the relative importance of underlying dispersal mechanisms and their consequences on community structure are not well described. High dispersal rates can cause soil microbial communities to become more homogenous across space and therefore it is important to identify factors that make dispersal more likely. This study experimentally manipulated soil microbial dispersal during the growing season at a single tallgrass prairie site by changing the relative openness of soil to passive dispersal and active dispersal via bison dung in areas that have a history of bison grazing or no grazing and annual fire or infrequent fire. We expected dispersal to be highest in grazed and annually burned areas and that the addition of bison dung would increase overall microbial richness and lead to homogenization of communities over time. We found passive dispersal rates to be similar across land use histories, but the influx of taxa to the soil was greater if communities began with low richness. Additionally, bison dung significantly increased overall richness and made communities across divergent grazing and fire histories more similar. These results are the first to show that passive dispersal and active dispersal through bison dung can alter grassland soil microbial diversity and composition.

Introduction

Microorganisms are the most diverse group of organisms on the planet (Locey and Lennon, 2016) and are integral to ecosystem functions such as nutrient cycling, biomass production, and carbon storage (Schimel and Schaeffer 2012; Colman and Schimel 2013; Glassman et al. 2018; Kuypers et al. 2018). Yet, a mechanistic understanding of the biogeography of microbial taxa lags behind the extensive research for other organisms, such as plants and animals (Hanson et al. 2012). Accumulated evidence that microbial taxa can be dispersal limited and subject to legacy effects and current environmental conditions (Martiny et al, 2006, van der Gast 2015), has created new questions about microbial community dynamics and the resulting compositional patterns that emerge.

It is hypothesized that microbial communities are assembled through similar mechanisms as macro-organismal communities – selection, drift, dispersal, and mutation, (Hanson et al. 2012; Nemergut et al. 2013) and that the relative importance of each of these factors will depend on the environment and the target organisms. In some cases, the patterns of microbial communities that emerge from these mechanisms are similar to those for macro-organisms, while in other contexts they are different. For example, microbial taxa often display the same broad spatial scaling patterns that are found among plants and animals (Green and Bohannan 2006, Locey and Lennon, 2016), but the strength of these patterns can be weaker for microbial life due to biological and methodological differences, such as dormancy and sampling extent (Locey 2010, Meyer et al. 2018). One biogeographical pattern that has been found across macrobial and microbial communities is that of distance-dissimilarity, or the decrease in community similarity with geographical distance (Soininen et al. 2007). The strength of the distance-dissimilarity relationship (i.e. the slope of regression line of community dissimilarity

against geographic distance) depends on the balance between two main mechanisms of community assembly -- environmental filtering and dispersal limitation (Nekola and White 1999, Leibold et al. 2004, Soininen et al. 2007, Hanson et al. 2012). Environmental filtering results when local environmental conditions allow for the survival and perpetuation of certain taxa and this increases the similarity of locations closest to one another (Hanson et al. 2012). Dispersal limitation also increases similarity at close locations, while higher dispersal rates might increase similarity at farther locations, weakening the relationship between distance and dissimilarity. Furthermore, environmental conditions that constrain dispersal and competitive interactions can have profound effects on how communities assemble (Chase 2003), and it is therefore critical to understand how different ecosystem attributes and land management affect the interplay of underlying biogeographical mechanisms.

Grasslands are diverse ecosystems that provide numerous services worldwide, (Bengtsson 2019) and are subject to change due to shifting management practices, such as varying fire and grazing intensities (Bond et al. 2004; Briggs et al. 2005; Borer et al. 2014). While work on microbial ecology is increasing, the effects of grassland management on the relative importance of environmental filtering and dispersal limitation in structuring soil microbial communities is still not clear. In northern China, Cao et al. (2016) determined that environmental filtering was the main process shaping microbial community distribution, mainly related to soil pH and climate factors, but Richter-Heltman et al. (2020) found the opposite, that stochastic assembly processes impacted diversity in temperate prairie in Germany. Overall, even if the mechanisms are unknown, soil communities display strong responses to herbivore grazing via shifts in activity (Esch et al. 2013; Cline et al. 2017; Eldridge et al. 2017) and composition (Patra et al. 2005; Cline et al. 2017). Prescribed fires are also a common management practice in

grasslands that alter the soil environment from direct heat, removal of plant, and subsequent changes to soil nutrient availability (Docherty et al. 2011). Although responses vary by grassland type, frequent fire can lead to increased soil microbial activity and shifts in composition (Perez-Valera 2017; Carson and Zeglin 2018; Yang et al. 2020). Many more studies exist that measure and describe factors related to environmental filtering of soil microbes, but dispersal could also have important consequences for grassland soil microbial community assembly.

Dispersal is the least understood microbial biogeography mechanism in most ecosystems (Albright & Martiny 2017), but could be affected by fire and grazing. In all grasslands, including North American tallgrass prairies, grazing would be expected to be important for microbial community dynamics, given the evolutionary relationship between large herbivores and grasses (Stebbins 1981), and the keystone role of bison both historically and contemporarily (Knapp et al. 1999). At sites across the Great Plains, bison grazing tends to decrease the strength of the soil microbial distance-dissimilarity relationship (Allenbrand in prep.; Chapter 2), and bison reintroduction to Tallgrass prairie can cause convergence of soil microbial communities with varied management backgrounds, with their dung implicated as an important mechanism (Chantos 2017). Also, North American bison have a distinct gut microbiome (Bergmann et al. 2015), as do most megaherbivores in more ancient grasslands (Kartzinel et al. 2019). Therefore, as herbivores move around the landscape, they may be actively dispersing cells via dung deposition. Concurrently, passive dispersal of some microbial cells via aerial deposition is also likely (Finlay and Clarke, 1999; Bottos et al. 2014; Elliott et al. 2019), and fire and grazing could influence how readily air borne cells reach the soil since both create bare soil patches open to aerial inputs (Bakker et al. 2003; Henry et al. 2006). This mechanism has been suggested at one grassland site, where a history of annual burning decreased dissimilarity across the landscape

(Zeglin et al., unpublished data). Further, fire can promote aerial dispersal of microbes, by aerosolizing viable soil microbial cells and spores (Kobziar et al. 2018, Moore et al. 2020). In sum, both bison grazing and annual burning could decrease dispersal limitation of soil microorganisms.

Although observation of these patterns provides important insights, even combined with environmental data, it does not directly measure to what degree active or passive dispersal mechanisms are operating (Green and Bohannan 2006). To overcome this shortcoming, it is necessary to experimentally alter the dominant factors predicted to be responsible for the pattern. If a relationship between microbial community composition, environmental correlates, and variation explained by distance is already established, two main research avenues, excluding modeling, can be taken for experimental evaluation of distance-dissimilarity mechanisms: environmental manipulation or altering dispersal rates (Hanson et al. 2012). The few studies that have manipulated microbial dispersal have been successful in altering rates and composition of dispersed taxa, and have shown that altered dispersal has a significant effect on community dynamics (Bell 2010; Berga et al. 2015; Albright and Martiny 2018). Therefore, in a grassland ecosystem, we designed an experiment to ask how environmental manipulation (via differences in fire and grazing management), and alteration of dispersal rates (via manipulation of soil openness to aerial dispersal, and active addition of bison dung as a dispersal vector) influence dispersal limitation and subsequent assembly dynamics of soil microbial communities.

We hypothesized that microbial dispersal would be higher in burned and grazed areas than in unburned and ungrazed areas, but that open canopy (burned area) communities will display stochastic assembly from passive wind dispersal, while communities in grazed areas would show more determinism due to active dispersal by bison and converge over time. To test

the hypotheses, we manipulated the potential rate of passive dispersal using soil bags with open or closed mesh, and the potential rate of active dispersal using addition of fresh bison dung, to sterilized and non-sterilized ("live") soil, and deployed these experiments in replicates across grazed, burned, and neither grazed nor burned watersheds at Konza Prairie Biological Station (KPBS, Manhattan, KS). Specific predictions included: 1) passive dispersal rates, or accumulation of new microbial taxa over time in sterilized soil open to dispersal, will be apparent everywhere but will be highest in burned areas and lowest in unburned and ungrazed areas, and 2) active dispersal, via the addition of bison dung, will increase the number of new microbial taxa over in all areas, and lead to microbial community convergence across land uses (Figure 3.1).

Methods

Study location

This experiment was performed at Konza Prairie Biological Station (KPBS), located in northeastern Kansas (39°05'N, 96°35'W), part of the Flint Hills region of KS and OK. KPBS is located on one of the few remaining native tracts of tallgrass prairie, was established as an ecological research station in 1971, and became part of the Long-Term Ecological Research (LTER) Network in 1980. Watershed scale treatments of differing fire intervals have been in place since the 1970s, and bison were reintroduced to a subset of these watersheds in the 1980s, thus large areas with contrasting land management treatment have been maintained for decades. For this study, experimental research was restricted to upland soils (Florence series, Udic Argiustolls) in three of the long-term treatments: ungrazed and infrequently burned (20 year fire interval), bison-grazed and infrequently burned, and ungrazed and frequently burned (annual fire

interval). No infrequently burned watersheds experienced fire in the study year, so are referred to as “unburned” treatments hereafter.

Experimental design

Dispersal manipulations were installed across the experimental landscape, with four field replicates in each of three different land use histories: annually burned and ungrazed, infrequently burned and grazed, and infrequently burned and ungrazed. Each experimental unit contained five different treatments randomly assigned in a checkerboard pattern: sterilized soil closed to dispersal (minimal dispersal), sterilized soil open to dispersal (passive dispersal), live soil open to dispersal (live soil control), sterilized soil open to dispersal from bison dung (active dispersal), and live soil open to dispersal from bison dung (dispersal + filtering) (Figure 3.2). The open versus closed dispersal contrast was achieved using nylon mesh (Tisch Scientific, North Bend, OH, USA) bags with pore sizes of 20 μm and 0.22 μm, respectively. These mesh sizes have been shown to successfully manipulate bacterial and archaeal migration rate (Albright and Martiny 2018). The soil inside the bag was either live (unsterilized) or sterilized (via autoclaving at 121°C degrees for 20 minutes), and bags were always deployed to the same land use from which the soil was collected. In the active dispersal treatments, recently collected bison dung was deposited on top of the live and sterile soil bags in the field after the bags were placed into the ground. To allow for temporal sampling, each treatment included four bags that corresponded to one day (T1), one week (T2), one month (T3), and three months (T4) post deployment, plus subsampling of the overlying bison dung at each time point.

Treatment preparation and installment

Fresh bison fecal samples were collected into gallon Ziploc bags using aseptic technique on 4 June 2020. Areas of the dung touching soil or vegetation was avoided, and was only

collected from bison 2 years and older to insure they had weaned and were eating a representative diet. The samples were kept on ice until transported back to the lab where they were stored at -20°C until further analysis. A subsample of approximately 50 mL was separated and the remaining dung was divided in half to process for treatments.

Experimental unit locations were established and soil for the dispersal bags were collected from each sampling point within the unit using a 2 cm diameter soil auger to a depth of 2 cm. Cores were homogenized into one composite sample for each land use history by sieving through 4 mm mesh using aseptic technique. A subsample for live soil and sterilized soil from each of the land use histories was collected and stored at -20°C for initial characterization of microbial communities.

Open and closed dispersal soil bags were made with two different materials: nylon mesh with a pore size of 20 μm and a nylon membrane mesh with a pore size of 0.22 μm, respectively. Each bag had a dimension of 2 cm x 2 cm, but open and closed bags were constructed with two different methods. Using aseptic technique, the open bags were sewn using weather resistant nylon thread stitched along three edges with a folded edge to decrease the amount of stitching. Using aseptic technique, the closed bags were glued using Gorilla Glue Clear Grip along three edges with a folded edge. A small opening was left in each bag for filling, which was then closed with the corresponding method and bags were further processed according to dispersal treatment. All bags used for live soil treatments were sterilized by autoclaving prior to filling and closed using aseptic technique and placed in UV-sterilized 1-L Nalgene bottles according to land use history. All bags used for sterile soil treatments were sterilized after filling by placing in UV-sterilized 1-L Nalgene bottles according to land use history and autoclaved with the lids loosely on. This allowed for aseptic transport to the field site for installation.

Soil bags were deployed back into sampling locations according to land use history and treatment assignment on 14 June 2019. Live bison dung was deposited in equal amounts on top of soil bags according to treatment assignment. All soil bags and dung were deposited underneath any litter that was present. On 15 and 21 June, 12 July, and 6 September 2019, the appropriate soil bags were extracted, transported to the lab, and stored at -20°C until further processing. Before DNA extraction, soil was transferred from the nylon bags to pre-labeled gamma-sterilized centrifuge tubes for long term-storage.

DNA extraction and polymerase chain reaction (PCR)

Total genomic DNA (gDNA) was extracted from approximately 0.5 g of homogenized soil or dung per sample using the Qiagen DNeasy PowerSoil kit (Qiagen Sciences, Germantown, MD, USA) following manufacturer's instructions but with the following modifications: PowerBead Tubes were disrupted by bead beating for 20 s using a MP Biomedicals (Santa Ana, CA, USA) sample disruptor set at 4 m/s velocity, supernatant was transferred using the recommended minimum volume, and for final DNA elution step 50 μL of solution C6 was added and incubated for 5 min at room temperature before spinning down and repeated using the flow-through. In addition, since dung collected at later time points was markedly more desiccated and thus absorbed water, 500 μL of extraction buffer was added to the PowerBead column and then filled to capacity with dung even if dung mass was below 0.5 g. Genomic DNA (gDNA) was stored at -20°C until further analysis. Yield of gDNA was measured using a ThermoFisher Quant-iT PicoGreen dsDNA Assay Kit and quantified $gram^{-1}$ dry soil (Thermo Fisher Scientific Inc., Waltham, MA, USA). From the gDNA extracts, the 16S rRNA gene was targeted for Illumina bacterial sequencing using universal bacterial primers (515F/926R) following established protocols (Caporaso et al. 2012, Parada et al. 2016) with one modification: PCR was

run for 25 cycles instead of 35. Three technical replicates were run for each barcoded sample and each reaction was confirmed with 1% agarose gel electrophoresis. Upon successful PCR, technical replicates were pooled, cleaned using Exo-SapIT (Applied BioSystems, Foster City, CA, USA), and amplicon pools quantified using the Quant-iT PicoGreen assay kit (Life Technologies, Grand Island, NY, USA). Amplicon amounts were then normalized to 75 ng per barcoded sample, combined into one library and cleaned using a QIAquick Gel Extraction Kit (Qiagen, Germantown, MD, USA). The library was sequenced on a 2 x 250 paired-end read Ilumina MiSeq run with 15% PhiX at the Kansas State University Integrated Genomics Facility.

Bioinformatics

Raw Illumina sequence data was processed using the QIIME2 software package (Boylen et al., 2019). Sequences were demultiplexed, joined and quality filtered using the q-score filtering method set to a PHRED score threshold of 20. Proceeding with only the forward reads, they were trimmed to 250 base pairs and denoised using deblur. The remaining sequences were clustered to 97% sequence similarity and assigned to operational taxonomic units (OTUs) using the open-source workflow. After removing chimeric sequences using vsearch, OTUs were aligned to the GreenGenes v 13.18 16s rRNA gene reference database and taxonomy assigned using a Naïve-Bayes classifier trained at 97% similarity. Singletons, doubletons, and non-prokaryotic OTUs (i.e. chloroplasts and mitochondria) and control and blank samples were removed using filter functions before further analysis.

The remaining pre-processing, statistical analysis and visualizations were performed in R version 3.6.2 (R Core Team, 2017). The sequence library was further pre-processed using phyloseq version 3.10 (McMurdie and Holmes, 2013) by creating a phyloseq object and removing samples that did not have at least 3000 reads and removing OTUs that did not have at

least 5 reads per sample, resulting in a dataset with 253 samples and 2,088,873 total sequences with 5156 unique OTUs. From this, two separate datasets were created: a rarefied dataset with all samples trimmed to 3000 sequences by random sampling resulting in 253 samples and 506,000 total sequences with 5096 unique OTUs, and a normalized data set by proportional transformation of each sample using total sequence counts resulting in 253 samples and 2,530,000 total sequences with 5156 unique taxa. The low sequence count for the rarefied dataset was selected as the best balance for maintaining samples from early sampling points and relative diversity among treatments with deeper sequencing (Figure S 3.1).

Dispersal analysis

All alpha diversity metrics were calculated using phyloseq and the statistical testing done with base R version 3.6.2 (R Core Team, 2017) and R package vegan (Oksanen et al. 2019). The alpha diversity metrics of observed OTUs and evenness were calculated using the rarefied dataset with the estimate_richness function from phyloseq (McMurdie and Holmes 2013). To test the effect of dispersal treatment, land use history, and the interaction between the two on DNA yield and microbial richness for all time points, we used two-way analysis of variance (ANOVA) models and lsmeans function for post-hoc pairwise comparison of groups. To test the effect of treatment and land use history on DNA yield and microbial richness as a function of time, we used general linear models to perform analysis of covariance (ANCOVA) with the *lm* function (R Core Team 2019) and *Anova* function (Fox and Weisberg 2019). The models tested time, treatment, land use history and the pairwise interactions between all three (time by treatment, time by land use, treatment by land use, and time by treatment by land use). Separate ANCOVA models were performed on different subset of treatment levels to test specific hypotheses. For significant effects, least square means for all pairwise comparisons were used as

the post-hoc test for identification of significantly different levels using the *lsmeans* function ('lsmeans', Length 2016) and *cld* function ('multcompView', Graves et al. 2019).

Community composition

A Bray-Curtis dissimilarity matrix was calculated from the normalized dataset to evaluate beta diversity and community differences were visualized with non-metric multidimensional scaling (NMDS). A 3-way permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the effect on community composition of treatment, watershed history, time, and their interaction using the adonis function in vegan with 999 permutations (Oksanen et al. 2019). Average distance from centroid for each treatment within each time point was calculated using betadisper function in vegan (Oksanen et al. 2019). Treatment community dispersion were compared between land use and across time using the codyn multivariate_change function (Hallett et al. 2020).

Results

DNA yield

DNA yield was undetectable at time zero in sterile soil treatments, remained lower over the incubation period for all originally sterile treatments relative to live soil treatments, and increased but remained lowest in the "closed" (0.2 μM) mesh treatment (Figure 3.3A, Table 3.1, Table 3.2). DNA yield did not change significantly across the sampling times for any other treatments except for live dung, which increased substantially over time, and also had the highest overall yield (Table 3.1, Table 3.2, Figure 3.3A). In unburned and grazed areas, sterile soil treatments had higher DNA yield and were not different from open live soil treatments, and in addition, open live soil had the highest yield from unburned, ungrazed watersheds (Table 3.1).

Richness

Microbial richness increased over time in all dispersal treatments but was not affected by grazing or fire (Figure 3.3B, Table 3.1, Table 3.2). While all slopes of OTU accumulation over time were statistically similar, indicating a constant dispersal rate of approximately 2-4 OTUs per day, there were significant differences in the intercepts of each model, reflecting dispersal treatment effects on total richness (Table 3.2). The live soil control bags had lowest richness, and never reached the level of richness measured in intact field soil reference samples (Figure 3.3B). In contrast, the initially sterile soil treatments exposed to passive dispersal accumulated higher richness, reaching intact field soil reference levels after 3 months (Figure 3.3B, Table 3.2). The active dispersal of microbes in live dung increased richness by hundreds of OTUs immediately, an effect that persisted for the duration of the experiment, maintained the highest richness overall, and weakened the significance of the slope of OTU accumulation over time (Figure 3.3B, Table 3.1 and 3.2). Also, pure live dung had fewer observed OTUs than the soils with dung added (Figure 3.3B, Table 3.2).

Community composition

Overall microbial community composition was affected by dispersal treatment (23.8% of variation explained), time (9.4%), and land-use context (2.2%), with additional time by treatment interaction (11.8%) and land-use by treatment and by time (5.1%, 1.8%) effects (PERMANOVA, Table 3.3). In the NMDS ordination of all data, time effects created shifts in community composition along Axis 1, and soil and dung effects separated along Axis 2 (Figure 3.4). The live control and initially sterile open soil treatments on day one had low to no sequencing success and are therefore not displayed in the NMDS ordination. Untreated soil reference samples had the most similar community composition to the soil with dung added

dispersal treatments at the end of the dispersal experiment. Dung amended soil treatments also showed consistently lower dispersion and greater convergence of microbial communities over time when compared to the soil only treatments (Figure 3.5, Table 3.4). Additionally, the trajectory of dispersion around treatments was similar among land uses (Table 3.4). The intact field soil reference samples were most similar in community composition to the combined passive and active dispersal treatments (open soil with dung added) at the end of the experiment.

Discussion

This is the first study to show that passive microbial dispersal and active dispersal from bison dung contributes to soil microbial richness and composition. The experiment revealed that passive microbial dispersal occurs at similar rates across areas with different fire and grazing management (Figure 3.3B, Table 3.1) and that the relative openness of the soil does not affect the passive dispersal rate, rather it affects the total number of taxa present (Figure 3.3B). Furthermore, the active dispersal of bison dung to sterile and live soil resulted in an additive effect of hundreds of taxa from the initiation of the experiment, taxa that may have contributed to community convergence over time (Figure 3.4 and 3.5, Table 3.4).

It proved difficult to cut off microbial dispersal completely, despite DNA and richness levels below detection at the beginning of the experiment in the "closed" (0.2 μM mesh) bags. This treatment successfully decreased immediate (within 24 h) dispersal into the soil, however, DNA and richness still rose over time at similar rates to the "open" (20 μM mesh) bags, suggesting that either new cells were consistently coming in or there was growth after colonization (Table 3.2). Colonizers within or after 24 hours of exposure could establish if carbon and nutrient sources left after the sterilization death of pre-existing microbial populations provided better environmental conditions for activity and growth. The slope of OTU change over

time was significantly positive and similar for all dispersal treatments (Figure 3.3B) and the trajectory of community change over time was similar (Figure 3.4), so while the DNA load in "closed" dispersal bags tended to be lower than that of the "open" soil treatments (Figure 3.3A), the source pool of colonizers appeared similar across all treatments and experimental units. In contrast, the slope of DNA yield increased substantially over time in the pure dung control samples, pointing to a greater role for growth of existing populations in that highly rich environment (Figure 3.3, Table 3.2).

We found passive dispersal to be ubiquitous across land use types and evident within 24 hours, providing evidence that dispersal mechanisms are important contributors to soil microbial richness and composition at our study location and partly supporting our first prediction. Passive dispersal routes include aerial movement from wind and rain (Bottos et al. 2014) or movement through the soil matrix, with both likely happening in our system. Microbial cells can be transported via wind-blown dust at local and regional scales, with distance traveled dependent on wind direction, speed, and soil type (Acosta et al. 2015, Elliot et al. 2019). The Great Plains are persistently windy, so it would not be unexpected for wind erosion and subsequent deposition to be moving microbes around the landscape. Secondly, microbial cells can move within the soil through water-filled pore spaces, which could result in dispersal to neighboring soil when water content is sufficiently high (Carson et al. 2010; Kravchenko et al. 2012). Movement of microbes within soil can also be driven by biotic interactions, as bacterial cells have been shown to use fungal hyphae as "highways" to navigate the soil matrix (Furuno et al. 2010; Warmink et al. 2011). More experimentation would be needed to parse contributions from these non-exclusive mechanisms.

Surprisingly, the live soil control treatments had the lowest richness throughout the experiment, substantially less than the intact soil reference samples or the sterilized dispersal treatments (Figure 3.3B, Table 3.2). This suggests that removal of the soil from the field for experimental bag construction changed the microbial community, and that the 20 μM mesh barrier prevented the experimentally manipulated soils from recovering to a reference state. Biotic interactions may be important for microbial community assembly, such that modification or suppression of interactions limits microbial richness in our study system. Microbial community dynamics have been shown to be dependent on priority effects, where the first colonizers gain an advantage over later arriving taxa (Svoboda et al. 2018). In our experiment, the taxa remaining in the live soil control after lab handling could have a competitive advantage over dispersers in the field, making it more challenging for new colonizers to establish. This is also in line with metacommunity theory where increased local competition results in decreased diversity from resource limitation (Mouquet and Loreau, 2002). Also, organisms larger than 20 μm would have been unable to disperse into either dispersal treatment bags, thus removing important multi-trophic interactions. For example, predation, which has been shown to increase microbial richness by reducing the survival of dominant taxa and allowing more rare or subordinate taxa to survive (Saleem et al. 2012; Jiang et al. 2017), would have been absent. Additionally, competition and cooperation with plant roots (Berg and Smalla, 2009; Haichar et al. 2014), fungi (Deveau et al. 2018), and invertebrates (Wardle 2006; Bray et al. 2019) are well known biotic factors structuring microbial communities. Experimental investigation of these interactions for microbial community assembly should be explored further.

Microbial richness was always higher, and community composition different, when bison dung was added, providing evidence for the importance of direct active dispersal (Figure 3.3B,

Figure 3.4, Table 3.2). Bison dung could serve as an important source of microbes to the soil, given that thousands of bacterial and hundreds of archaeal taxa have been identified in bison fecal samples (Bergmann et al. 2015). Additionally, a field bison dung incubation experiment conducted at a different tallgrass prairie site observed increased similarity among soil microbial communities within a single restoration year after 3 weeks of exposure to the dung (Chantos, 2017). Microbial dispersal to surface soil could also result from increased activity of dung-affiliated invertebrates with their own host-associated microbiomes in and around the bison dung. Dung beetles are of specific importance (Slade et al. 2015), and their abundance and diversity increases with bison presence and recent fire (Barber et al. 2020). While it is possible that the dung is altering the nutrient status of the soil below and around it (Sitters and Venterink 2015), another study saw no effect of bison dung addition on the C:N status of adjacent soil (Chantos, 2017), and therefore it is not clear whether environmental filtering is happening in our experiment. As predicted, soil microbial communities converged more strongly under bison dung, in a more temporally consistent manner than convergence within soil only treatments (Figure 3.5, Table 3.4,). However, because we did not include a sterile dung treatment, we cannot say if this is from active dispersal only, or is happening in combination with environmental filtering. The latter could be important, as suggested by a weaker dispersal pattern in dung added treatments that may be indicative of OTUs being filtered after the initial dispersal that happened within 24 hours (Figure 3.3A, Table 3.2). Abiotically, environmental filtering could result from the chemical composition of dung favoring specific groups of microbes, especially decomposers (Sitters et al. 2014). In order to test this, sterilized dung or a representative inoculum from fresh bison dung would need to be used; the sterilized dung would

test the importance of nutrient influx and quality, while the inoculum would test dispersal and biotic interactions in the absence of nutrient alteration.

Contrary to our predictions, land management, specifically bison grazing and fire, did not affect microbial dispersal rates, although there was indication that it affected community structure (Figure 3.4, Table 3.3). The lack of dispersal differences could be because proximate effects, such as soil openness to dispersal (Albright et al. 2019) and influx of microbial populations from neighboring soil and dung, might matter more than watershed scale management for overall dispersal rates. Also, the soil factors that fire and grazing are known to alter, i.e. annual fire tends to decrease N availability and N cycling activity over time (Blair 1997) and bison grazing tends to increase N availability and speed up nutrient cycling (Johnson and Matchett 2001; Allenbrand and Zeglin, unpublished data), may not be relevant for microbial dispersal. Alternatively, the effects of fire and grazing on dispersal might shift with time and our three-month experiment may not have been long enough to capture the temporal variation. For example, transiently high aerial dispersal rates may have occurred immediately after spring burning, when soil was most exposed and more aerosolized cells were mobile (Kobziar et al. 2018), combined with higher dispersal impact in spring when soil had lower microbial biomass (Wang et al. 2012). This experiment was installed in early June, about six weeks after the annual fire by this time peak fire-driven dispersal might ceased. Also, bison are known to preferentially graze recently burned areas during the months that our experiment was deployed, and then shift their use to unburned areas during the dormant season (Raynor et al. 2016; Raynor et al. 2017). Therefore, we may see increased effects of bison on dispersal outside of the growing season in the area where the experiment was located. A dispersal experiment would need to be extended in

time to evaluate the potential for temporal variation in the impact of fire and grazing management on dispersal.

The finding that dispersal is important for microbial dynamics does not reveal the relative importance of stochastic versus deterministic assembly. Although dispersal can be stochastic if probabilities of cell movement are equal among taxa, it can also be deterministic if cell size and life history strategies make it more likely for certain taxa to passively disperse (Hanson et al. 2012). Therefore, which taxa are dispersing, their chances of survival in a new location, and the role of priority effects also need to be considered. Additionally, the dual role for aerial and soil dispersal makes identifying the regional versus local signal challenging, but nonetheless, a constant rate of passive aerial dispersal could maintain higher soil diversity high across the landscape. In metacommunity theory, mass effects − the constant immigration of individuals because of high dispersal rates – can homogenize communities and maintain the presence of rare taxa in communities (Leibold et al. 2004; Lindström and Langenheder 2012). In microbial communities this effect might be stronger because of microorganisms' ability to enter dormancy and effectively serve as a 'seed bank' if dispersed into initially unfavorable conditions (Locey et al. 2020; Wisnoski et al. 2020). Therefore, a pertinent future direction is defining the prevalence of dormancy in immigrating microbial populations, and learning whether the active community displays similar biogeographical patterns.

Overall, this experiment provides strong evidence that soil microbial dispersal is happening passively throughout the growing season in both grazed and burned land management areas in tallgrass prairie. Furthermore, active dispersal through bison dung also increases microbial community diversity and affects composition, but more work needs to be done to better understand the role that dung plays in environmental filtering. Microbial dispersal can

have real and important consequences on community composition (Albright and Martiny 2018) and function (Mallon et al. 2015; Evans et al. 2020), which will affect ecosystem management, conversation, and restoration. Our results contribute to both an increased understanding of grassland soil microbial community dynamics, and to a growing body of literature on soil microbial biogeography.

References Cited

Acosta-Martínez, V., S. Van Pelt, J. Moore-Kucera, M. C. Baddock, and T. M. Zobeck. 2015. Microbiology of wind-eroded sediments: Current knowledge and future research directions. Aeolian Research 18:99–113.

Albright, M. B. N., and J. B. H. Martiny. 2018. Dispersal alters bacterial diversity and composition in a natural community. The ISME Journal 12:296–299.

Albright, M. B. N., R. C. Mueller, L. V. Gallegos-Graves, J. Belnap, S. C. Reed, and C. R. Kuske. 2019. Interactions of microhabitat and time control grassland bacterial and fungal composition. Frontiers in Ecology and Evolution 7:367.

Bakker, C., J. M. Blair, and A. K. Knapp. 2003. Does resource availability, resource heterogeneity or species turnover mediate changes in plant species richness in grazed grasslands? Oecologia 137:385–391.

Bell, T. 2010. Experimental tests of the bacterial distance–decay relationship. The ISME Journal 4:1357–1365.

Bengtsson, J., J. M. Bullock, B. Egoh, C. Everson, T. Everson, T. O'Connor, P. J. O'Farrell, H. G. Smith, and R. Lindborg. 2019. Grasslands-more important for ecosystem services than you might think. Ecosphere 10:e02582.

Berg, G., and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere: Plant species, soil type and rhizosphere communities. FEMS Microbiology Ecology 68:1–13.

Berga, M., Ö. Östman, E. S. Lindström, and S. Langenheder. 2015. Combined effects of zooplankton grazing and dispersal on the diversity and assembly mechanisms of bacterial metacommunities: Grazing and dispersal effects on bacterial metacommunities. Environmental Microbiology 17:2275–2287.

Bergmann, G. T., J. M. Craine, M. S. Robeson, and N. Fierer. 2015. Seasonal shifts in diet and gut microbiota of the american bison (*Bison bison*). PLOS ONE 10:e0142409.

Blair, J. M. 1997. Fire, N availability, and plant response in grasslands: A test of the transient maxima hypothesis. Ecology 78:2359–2368.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M.

Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37:852–857.

Bond, W. J., F. I. Woodward, and G. F. Midgley. 2004. The global distribution of ecosystems in a world without fire. New Phytologist 165:525–538.

Borer, E. T., E. W. Seabloom, D. S. Gruner, W. S. Harpole, H. Hillebrand, E. M. Lind, P. B. Adler, J. Alberti, T. M. Anderson, J. D. Bakker, L. Biederman, D. Blumenthal, C. S. Brown, L. A. Brudvig, Y. M. Buckley, M. Cadotte, C. Chu, E. E. Cleland, M. J. Crawley, P. Daleo, E. I. Damschen, K. F. Davies, N. M. DeCrappeo, G. Du, J. Firn, Y. Hautier, R. W. Heckman, A. Hector, J. HilleRisLambers, O. Iribarne, J. A. Klein, J. M. H. Knops, K. J. La Pierre, A. D. B. Leakey, W. Li, A. S. MacDougall, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, B. Mortensen, L. R. O'Halloran, J. L. Orrock, J. Pascual, S. M. Prober, D. A. Pyke, A. C. Risch, M. Schuetz, M. D. Smith, C. J. Stevens, L. L. Sullivan, R. J. Williams, P. D. Wragg, J. P. Wright, and L. H. Yang. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508:517–520.

Bottos, E. M., A. C. Woo, P. Zawar-Reza, S. B. Pointing, and S. C. Cary. 2014. Airborne bacterial populations above desert soils of the McMurdo Dry Valleys, Antarctica. Microbial Ecology 67:120–128.

Bray, N., J. Kao-Kniffin, S. D. Frey, T. Fahey, and K. Wickings. 2019. Soil macroinvertebrate presence alters microbial community composition and activity in the rhizosphere. Frontiers in Microbiology 10.

Briggs, J. M., A. K. Knapp, J. M. Blair, J. L. Heisler, G. A. Hoch, M. S. Lett, and J. K. McCARRON. 2005. An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. BioScience 55:243.

Cao, P., J.-T. Wang, H.-W. Hu, Y.-M. Zheng, Y. Ge, J.-P. Shen, and J.-Z. He. 2016. Environmental filtering process has more important roles than dispersal limitation in shaping large-scale prokaryotic beta diversity patterns of grassland soils. Microbial Ecology 72:221–230.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6, 1621–1624. http://doi.org/10.1038/ismej.2012.8

Carson, C. M., and L. H. Zeglin. 2018. Long-term fire management history affects N-fertilization sensitivity, but not seasonality, of grassland soil microbial communities. Soil Biology and Biochemistry 121:231–239.

Carson, J. K., V. Gonzalez-Quiñones, D. V. Murphy, C. Hinz, J. A. Shaw, and D. B. Gleeson. 2010. Low pore connectivity increases bacterial diversity in soil. Applied and Environmental Microbiology 76:3936–3942.

Chantos, K. 2017. Microbial community dynamics in tallgrass prairie soil affected by native grazer feces (BISON BISON). Department of Biological Sciences, Northern Illinois University.

Chase, J. M. 2003. Community assembly: when should history matter? Oecologia 136:489–498.

Cline, L. C., D. R. Zak, R. A. Upchurch, Z. B. Freedman, and A. R. Peschel. 2017. Soil microbial communities and elk foraging intensity: implications for soil biogeochemical cycling in the sagebrush steppe. Ecology Letters 20:202–211.

Colman, B. P., and J. P. Schimel. 2013. Drivers of microbial respiration and net N mineralization at the continental scale. Soil Biology and Biochemistry 60:65–76.

Deveau, A., G. Bonito, J. Uehling, M. Paoletti, M. Becker, S. Bindschedler, S. Hacquard, V. Hervé, J. Labbé, O. A. Lastovetsky, S. Mieszkin, L. J. Millet, B. Vajna, P. Junier, P. Bonfante, B. P. Krom, S. Olsson, J. D. van Elsas, and L. Y. Wick. 2018. Bacterial–fungal interactions: ecology, mechanisms and challenges. FEMS Microbiology Reviews 42:335–352.

Docherty, K. M., T. C. Balser, B. J. M. Bohannan, and J. L. M. Gutknecht. 2012. Soil microbial responses to fire and interacting global change factors in a California annual grassland. Biogeochemistry 109:63–83.

Eldridge, D. J., M. Delgado-Baquerizo, S. K. Travers, J. Val, I. Oliver, K. Hamonts, and B. K. Singh. 2017. Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores. Ecology 98:1922–1931.

Elliott, D. R., A. D. Thomas, C. L. Strong, and J. Bullard. 2019. Surface stability in drylands is influenced by dispersal strategy of soil bacteria. Journal of Geophysical Research: Biogeosciences 124:3403–3418.

Esch, E. H., D. L. Hernández, J. R. Pasari, R. S. G. Kantor, and P. C. Selmants. 2013. Response of soil microbial activity to grazing, nitrogen deposition, and exotic cover in a serpentine grassland. Plant and Soil 366:671–682.

Evans, S. E., L. P. Bell-Dereske, K. M. Dougherty, and H. A. Kittredge. 2020. Dispersal alters soil microbial community response to drought. Environmental Microbiology 22:905–916.

Finlay, B. J., and K. J. Clarke. 1999. Ubiquitous dispersal of microbial species. Nature 400:828–828.

Fox J., and S. Weisberg. 2019. An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Furuno, S., K. Päzolt, C. Rabe, T. R. Neu, H. Harms, and L. Y. Wick. 2010. Fungal mycelia allow chemotactic dispersal of polycyclic aromatic hydrocarbon-degrading bacteria in water-unsaturated systems. Environmental Microbiology 12:1391–1398.

Glassman, S. I., C. Weihe, J. Li, M. B. N. Albright, C. I. Looby, A. C. Martiny, K. K. Treseder, S. D. Allison, and J. B. H. Martiny. 2018. Decomposition responses to climate depend on microbial community composition. Proceedings of the National Academy of Sciences 115:11994–11999.

Graves, S., H. Piepho, and L. Selzer with help from S. Dorai-Raj (2019). multcompView: visualizations of paired comparisons. R package version 0.1-8. https://CRAN.R-project.org/package=multcompView

Green, J., and B. J. M. Bohannan. 2006. Spatial scaling of microbial biodiversity. Trends in Ecology & Evolution 21:501–507.

Haichar, F. el Z., C. Santaella, T. Heulin, and W. Achouak. 2014. Root exudates mediated interactions belowground. Soil Biology and Biochemistry 77:69–80.

Hallett L, Avolio M, Carroll I, Jones S, MacDonald A, Flynn D, Slaughter P, Ripplinger J, Collins S, Gries C, Jones M (2020). codyn: Community Dynamics Metrics_. doi: 10.5063/F1N877Z6 (URL: https://doi.org/10.5063/F1N877Z6), R package version 2.0.4, <URL: https://github.com/NCEAS/codyn>.

Hanson, C.A., J.A. Fuhrman, M.C. Horner-Devine, and J.B. Martiny. 2012. Beyond biogeographic patterns: process shaping the microbial landscape. Nature Reviews, Microbiology 10:497-506.

Henry H, N.R. Chiariello, P.M. Vitousek, H.A. Mooney, C.B. Field (2006) Interactive effects of fire, elevated carbon dioxide, nitrogen deposition, and precipitation on a California annual grassland. Ecosystems 9:1066–1075.

Jiang, Y., M. Liu, J. Zhang, Y. Chen, X. Chen, L. Chen, H. Li, X.-X. Zhang, and B. Sun. 2017. Nematode grazing promotes bacterial community dynamics in soil at the aggregate level. The ISME Journal 11:2705–2717.

Johnson, L. C., and J. R. Matchett. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. Ecology 82:3377–3389.

Kartzinel, T. R., J. C. Hsing, P. M. Musili, B. R. P. Brown, and R. M. Pringle. 2019. Covariation of diet and gut microbiome in African megafauna. Proceedings of the National Academy of Sciences 116:23588–23593.

Knapp, A. K., J. M. Blair, J. M. Briggs, S. L. Collins, D. C. Hartnett, L. C. Johnson, and E. G. Towne. 1999. The keystone role of bison in north american tallgrass prairie. BioScience 49:39–50.

Kobziar, L. N., M. R. A. Pingree, H. Larson, T. J. Dreaden, S. Green, and J. A. Smith. 2018. Pyroaerobiology: the aerosolization and transport of viable microbial life by wildland fire. Ecosphere 9:e02507.

Kravchenko, A., H.-C. Chun, M. Mazer, W. Wang, J. B. Rose, A. Smucker, and M. Rivers. 2013. Relationships between intra-aggregate pore structures and distributions of Escherichia coli within soil macro-aggregates. Applied Soil Ecology 63:134–142.

Kuypers, M. M. M., H. K. Marchant, and B. Kartal. 2018. The microbial nitrogen-cycling network. Nature Reviews Microbiology 16:263–276.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters 7:601–613.

Length, R. V. (2016). Least-Squares Means: The R Package lsmeans. Journal of Statistical Software, 69(1), 1-33. doi:10.18637/jss.v069.i01

Lindström, E. S., and S. Langenheder. 2012. Local and regional factors influencing bacterial community assembly: Bacterial community assembly. Environmental Microbiology Reports 4:1–9.

Locey, K. J. 2010. Synthesizing traditional biogeography with microbial ecology: the importance of dormancy: Synthesizing traditional biogeography with microbial ecology. Journal of Biogeography:no-no.

Locey, K. J., and J. T. Lennon. 2016. Scaling laws predict global microbial diversity. Proceedings of the National Academy of Sciences 113:5970–5975.

Locey, K. J., M. E. Muscarella, M. L. Larsen, S. R. Bray, S. E. Jones, and J. T. Lennon. 2020. Dormancy dampens the microbial distance–decay relationship. Philosophical Transactions of the Royal Society B: Biological Sciences 375:20190243.

Mallon, C. A., J. D. van Elsas, and J. F. Salles. 2015. Microbial invasions: the process, patterns, and mechanisms. Trends in Microbiology 23:719–729.

Martiny, J. B. H., B. J. M. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske, P. J. Morin, S. Naeem, L. Øvreås, A.-L. Reysenbach, V. H. Smith, and J. T. Staley. 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4:102–112.

McMurdie, P. J., and S. Holmes. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE 8:e61217.

Meyer, K. M., H. Memiaghe, L. Korte, D. Kenfack, A. Alonso, and B. J. M. Bohannan. 2018. Why do microbes exhibit weak biogeographic patterns? The ISME Journal 12:1404–1413.

Moore, R. A., C. Bomar, L. N. Kobziar, and B. C. Christner. 2020. Wildland fire as an atmospheric source of viable microbial aerosols and biological ice nucleating particles. The ISME Journal.

Mouquet, N., and M. Loreau. 2002. Coexistence in metacommunities: the regional similarity hypothesis. The American Naturalist 159:420–426.

Nekola, J. C., and P. S. White. 1999. The distance decay of similarity in biogeography and ecology. Journal of Biogeography 26:867–878.

Nemergut, D. R., S. K. Schmidt, T. Fukami, S. P. O'Neill, T. M. Bilinski, L. F. Stanish, J. E. Knelman, J. L. Darcy, R. C. Lynch, P. Wickey, and S. Ferrenberg. 2013. Patterns and processes of microbial community assembly. Microbiology and Molecular Biology Reviews 77:342–356.

Oksanen J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R. B. O'Hara, G.L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs and H. Wagner. 2019. vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Parada, A. E., D. M. Needham, and J. A. Fuhrman. 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18:1403–1414.

Patra, A. K., L. Abbadie, A. Clays-Josserand, V. Degrange, S. J. Grayston, P. Loiseau, F. Louault, S. Mahmood, S. Nazaret, L. Philippot, F. Poly, J. I. Prosser, A. Richaume, and X. L. Roux. 2005. Effects of grazing on microbial functional groups involved in soil n dynamics. Ecological Monographs 75:65–80.

Pérez-Valera, E., M. Goberna, K. Faust, J. Raes, C. García, and M. Verdú. 2017. Fire modifies the phylogenetic structure of soil bacterial co-occurrence networks: Soil bacterial co-occurrence networks. Environmental Microbiology 19:317–327.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raynor, E. J., A. Joern, J. B. Nippert, and J. M. Briggs. 2016. Foraging decisions underlying restricted space use: effects of fire and forage maturation on large herbivore nutrient uptake. Ecology and Evolution 6:5843–5853.

Raynor, E. J., A. Joern, A. Skibbe, M. Sowers, J. M. Briggs, A. N. Laws, and D. Goodin. 2017. Temporal variability in large grazer space use in an experimental landscape. Ecosphere 8:e01674.

Richter-Heitmann, T., B. Hofner, F.-S. Krah, J. Sikorski, P. K. Wüst, B. Bunk, S. Huang, K. M. Regan, D. Berner, R. S. Boeddinghaus, S. Marhan, D. Prati, E. Kandeler, J. Overmann, and M. W. Friedrich. 2020. Stochastic dispersal rather than deterministic selection explains the spatio-temporal distribution of soil bacteria in a temperate grassland. Frontiers in Microbiology 11.

Saleem, M., I. Fetzer, C. F. Dormann, H. Harms, and A. Chatzinotas. 2012. Predator richness increases the effect of prey diversity on prey yield. Nature Communications 3:1305.

Schimel J.P. and S.M. Schaeffer. Microbial control over carbon cycling in soil. 2012. Frontiers in Microbiology 3:1–11.

Sitters, J., M.J. Maechler, P. J. Edwards, W. Suter, and H. O. Venterink. 2014. Interactions between C : N : P stoichiometry and soil macrofauna control dung decomposition of savanna herbivores. Functional Ecology 28:776–786.

Sitters, J., and H. Olde Venterink. 2015. The need for a novel integrative theory on feedbacks between herbivores, plants and soil nutrient cycling. Plant and Soil 396:421–426.

Slade, E. M., T. Roslin, M. Santalahti, and T. Bell. 2016. Disentangling the 'brown world' faecal–detritus interaction web: dung beetle effects on soil microbial properties. Oikos 125:629–635.

Soininen, J., R. McDonald, and H. Hillebrand. 2007. The distance decay of similarity in ecological communities. Ecography 30:3–12.

Stebbins, G. L. 1981. Coevolution of grasses and herbivores. Annals of the Missouri Botanical Garden 68:75.

Svoboda, P., E. S. Lindström, O. Ahmed Osman, and S. Langenheder. 2018. Dispersal timing determines the importance of priority effects in bacterial communities. The ISME Journal 12:644–646.

van der Gast, C. J. 2015. Microbial biogeography: the end of the ubiquitous dispersal hypothesis?: Microbial biogeography. Environmental Microbiology 17:544–546.

Wang, Q., M. Zhong, and S. Wang. 2012. A meta-analysis on the response of microbial biomass, dissolved organic matter, respiration, and N mineralization in mineral soil to fire in forest ecosystems. Forest Ecology and Management 271:91–97.

Wardle, D. A. 2006. The influence of biotic interactions on soil biodiversity. Ecology Letters 9:870–886.

Warmink, J. A., R. Nazir, B. Corten, and J. D. van Elsas. 2011. Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. Soil Biology and Biochemistry 43:760–765.

Wisnoski, N. I., M. E. Muscarella, M. L. Larsen, A. L. Peralta, and J. T. Lennon. 2020. Metabolic insight into bacterial community assembly across ecosystem boundaries. Ecology 101:e02968.

Yang, S., Q. Zheng, Y. Yang, M. Yuan, X. Ma, N. R. Chiariello, K. M. Docherty, C. B. Field, J. L. M. Gutknecht, B. A. Hungate, A. Niboyet, X. L. Roux, and J. Zhou. 2020. Fire affects the taxonomic and functional composition of soil microbial communities, with cascading effects on grassland ecosystem functioning. Global Change Biology 26:431–442.

Tables and Figures

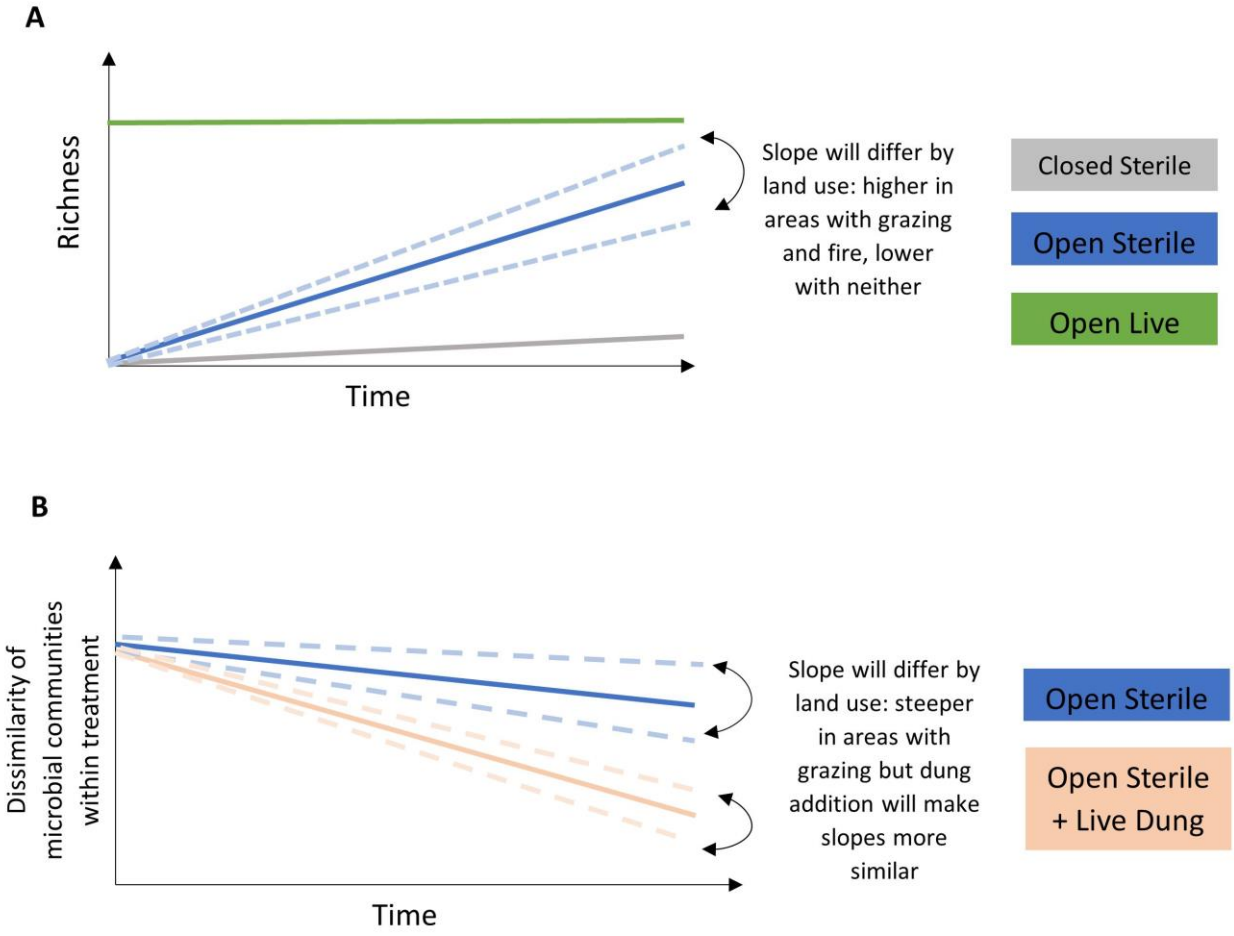

Figure 3.1 Conceptual model of A) predicted OTU accumulation over time for each treatment with indication of land use effect for the open sterile slope, and B) predicted microbial community dissimilarity of passive dispersal treatment and active dispersal treatment (dung amended sterile soil) across land uses over time.

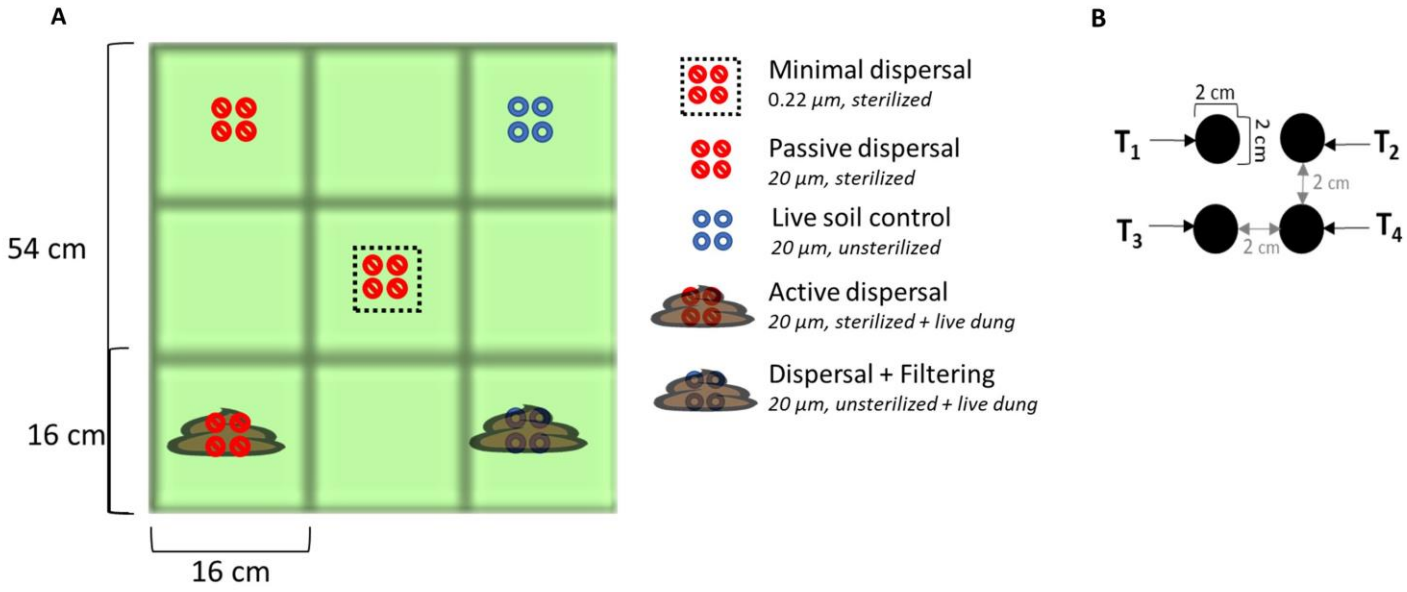

Figure 3.2 A) Layout of experimental unit with treatments randomly assigned in a checkerboard pattern. There were four replicates in each of the three watershed histories. Live = unsterilized. B) Enlarged diagram of individual treatment layout, each treatment has four individual soil bag samples corresponding to sampling time points.

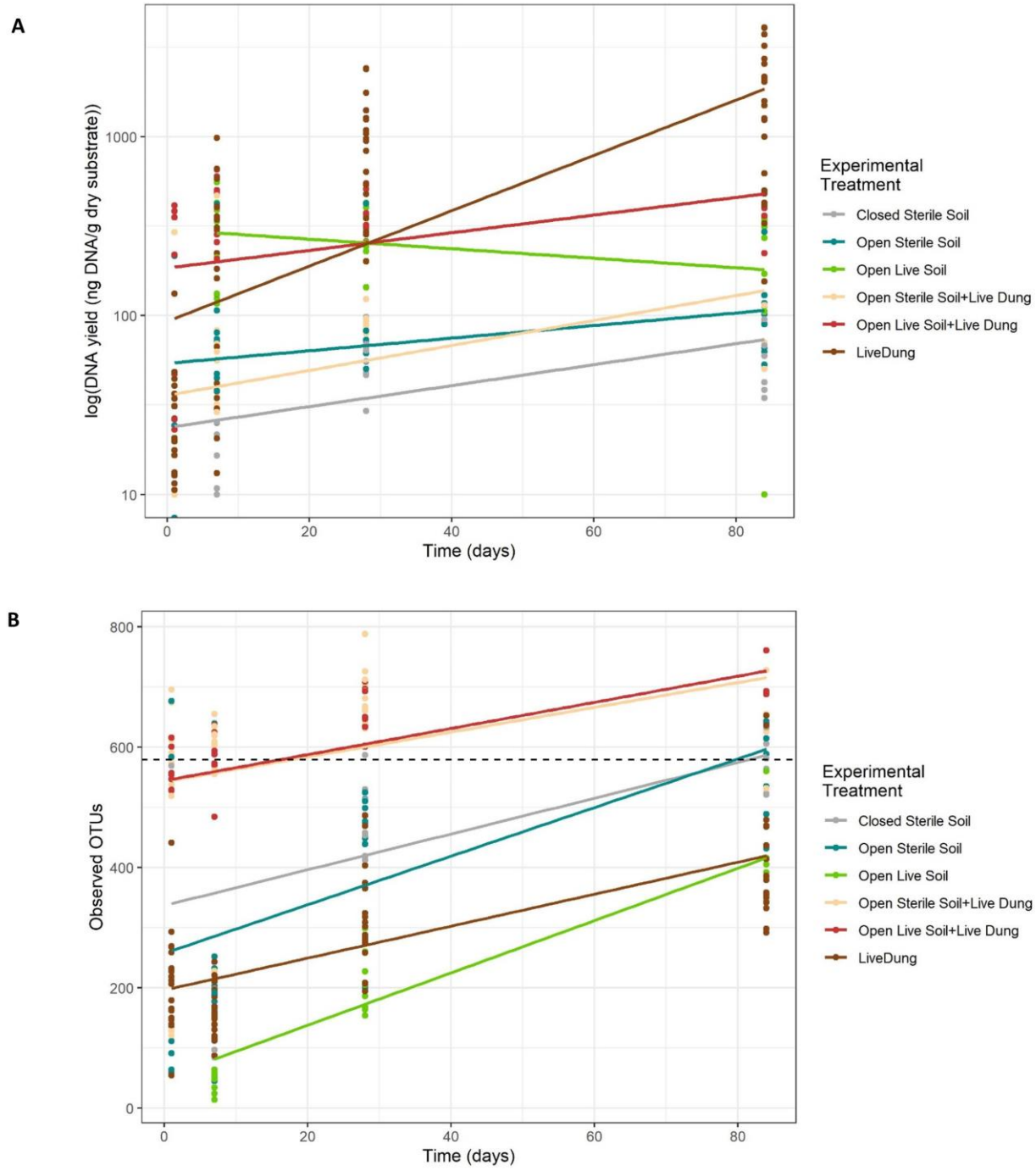

Figure 3.3 A) DNA yield (ng g^{-1} dry substrate) across time and B) microbial richness (observed OTUs) across time with reference soil richness indicated by black dashed line. Ordinary least squares regression lines displayed and colored by treatment.

Table 3.1 ANCOVA results for DNA yield (g g^{-1} dry substrate) and microbial richness (observed OTUs) for models comparing the treatment levels A: closed sterile soil, open sterile soil; B: open sterile soil, open live soil, open sterile soil + live dung, and open live soil + live dung; C: open sterile soil + live dung, open live soil + live dung, and live dung.

Factors	Closed vs. Open Sterile Soil		Open soil vs. Soil + Dung		Dung vs. Soil + Dung	
	DNA yield F, P	Richness F, P	DNA yield F, P	Richness F, P	DNA yield F, P	Richness F, P
Time	0.187, 0.666	4.556, 0.036*	0.141, 0.708	17.832, <0.0001*	0.007, 0.935	1.436, 0.233
Treatment	8.288, 0.0005*	5.091, 0.008*	3.374, 0.021*	22.088, <0.0001*	0.523, 0.594	38.581, <0.0001*
Land Use	0.076, 0.927	2.718, 0.072	1.583, 0.21	2.397, 0.096	0.04, 0.961	2.035, 0.135
Time*Trt	0.247, 0.783	0.8311, 0.439	0.281, 0.839	1.478, 0.224	5.444, 0.005*	0.746, 0.476
Time*Land Use	0.16, 0.852	0.993, 0.375	0.157, 0.855	0.462, 0.631	0.008, 0.992	0.448, 0.64
Trt*Land Use	6.524, 0.0001*	0.993, 0.375	2.687, 0.018*	1.455, 0.2	0.407, 0.804	1.612, 0.175
Time*Trt*Land Use	1.065, 0.38	0.603, 0.662	0.372, 0.896	0.425, 0.861	0.498, 0.737	0.367, 0.832

Table 3.2 Slopes and intercepts for full linear models of all pooled across land use; model = DNA yield or OTU richness ~ Time in days*Treatment. Post-hoc groups for y-intercepts were defined using P < 0.05 significance threshold for least-squared means among group comparisons.

TREATMENT	DNA yield			OTU richness		
	slope, P-value	intercept, P-value	Post-hoc groups for y-int	slope, P-value	intercept, P-value	Post-hoc groups for y-int
Closed sterile soil (minimal dispersal)	0.52 0.002*	28.8 0.001*	a	3.0 0.0017*	336.7 < 0.0001*	c
Open sterile soil (passive dispersal)	0.56 0.301	73.6 0.003*	a	4.0 < 0.0001*	257.3 <0.0001*	c
Live soil (live soil control)	-0.97 0.21	321.6 <0.0001*	a	4.4 <0.0001*	50.7 0.005*	a
Sterile soil + dung (active dispersal)	0.43 0.455	67.2 0.001*	a	2.1 0.046*	542.9 <0.0001*	d
Live soil + dung (dispersal + filtering)	0.84 0.48	308.7 <0.0001*	a	2.2 0.003*	544.4 <0.0001*	d
Live dung (live dung control)	19.2 <0.0001*	200.6 0.082	b	2.7 <0.0001*	196.1 <0.0001*	b

Table 3.3 PERMANOVA results across all treatments, time points, and land uses for soil microbial community composition. Trt = Treatment (6 levels).

Factor	Sum of Squares	F	R^2	P
Time	9.42	14.404	0.094	0.001*
Trt	23.805	15.6	0.238	0.001*
Land Use	2.174	4.986	0.022	0.001*
Time*Trt	11.828	3.876	0.118	0.001*
Time*Land Use	1.777	1.359	0.018	0.02*
Trt*Land Use	5.115	2.133	0.051	0.001*
Time*Trt*Land Use	6.427	1.092	0.064	0.093

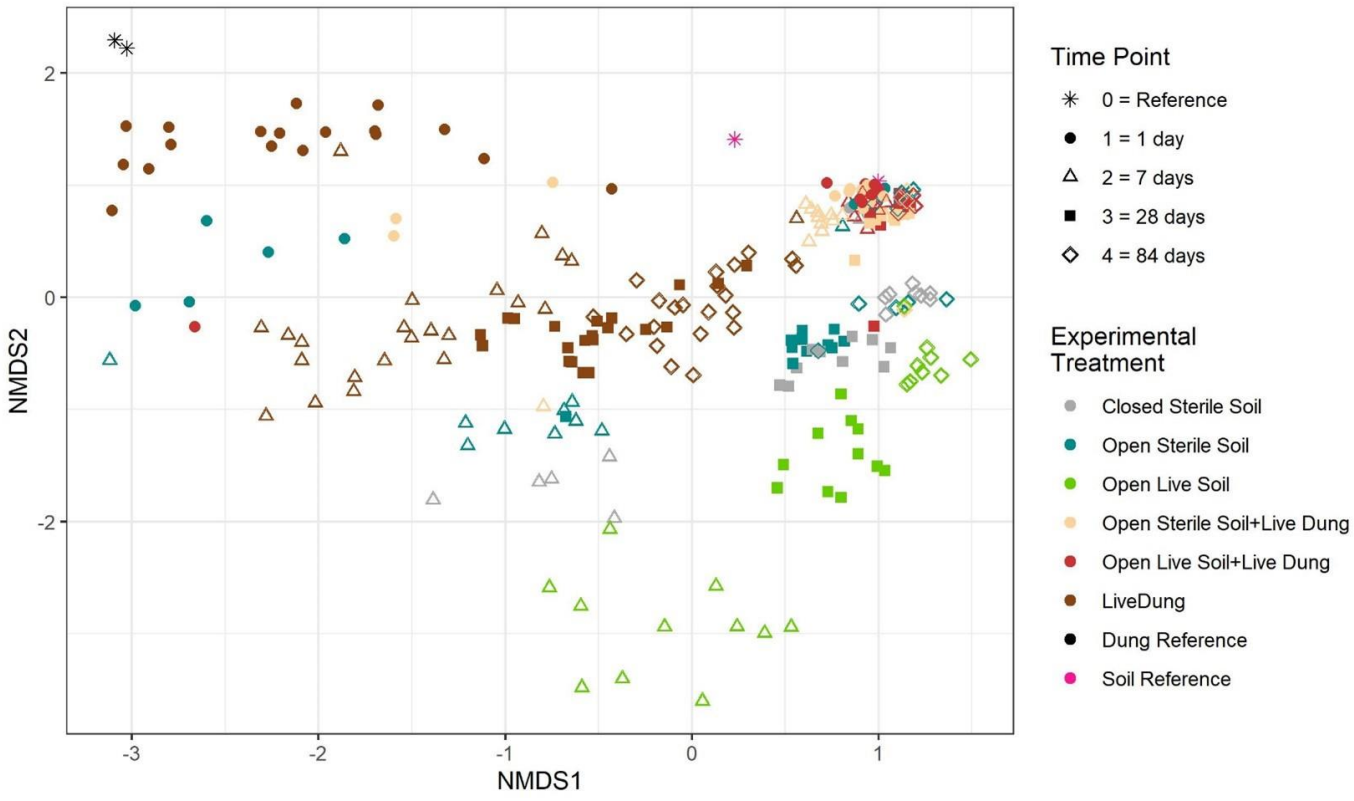

Figure 3.4 NMDS ordination models of 16S rRNA gene community composition for all samples with colors representing experimental treatment (including reference soil and dung samples) and symbols representing sampling time. Changes over time were primarily represented by NMDS Axis 1.

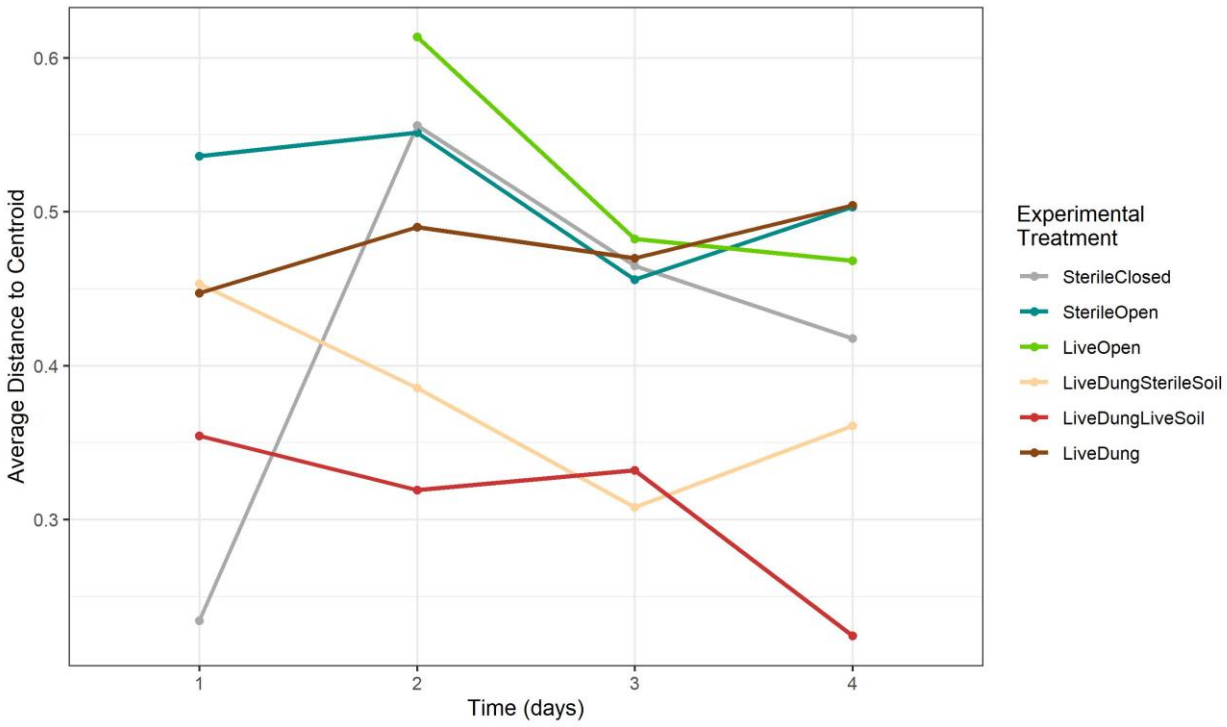

Figure 3.5 Average distance of samples to treatment centroid within each time point in multivariate space using a Bray-Curtis distance matrix. Colors represent experimental treatment.

Table 3.4 Change in dispersion of microbial communities for selected treatments. Negative values indicate the community in converging and positive values indicate the community is diverging for over time. Calculated using "codyn" multivariate_change function.

Land use	Timepoint	Open sterile	Open Live	open sterile + dung	open live + dung
Burned, Ungrazed	1-2	0.162	0.503	0.024	-0.28
Burned, Ungrazed	2-3	-0.107	-0.157	-0.028	-0.035
Burned, Ungrazed	3-4	0.072	0.007	-0.027	-0.182
Unburned, Ungrazed	1-2	-0.063	NA	-0.152	-0.027
Unburned, Ungrazed	2-3	-0.032	-0.082	-0.082	0.022
Unburned, Ungrazed	3-4	0.056	-0.016	-0.189	-0.058
Unburned, Grazed	1-2	0.038	NA	-0.09	0.102
Unburned, Grazed	2-3	-0.1	-0.075	-0.153	0.082
Unburned, Grazed	3-4	0.014	-0.367	-0.271	-0.388

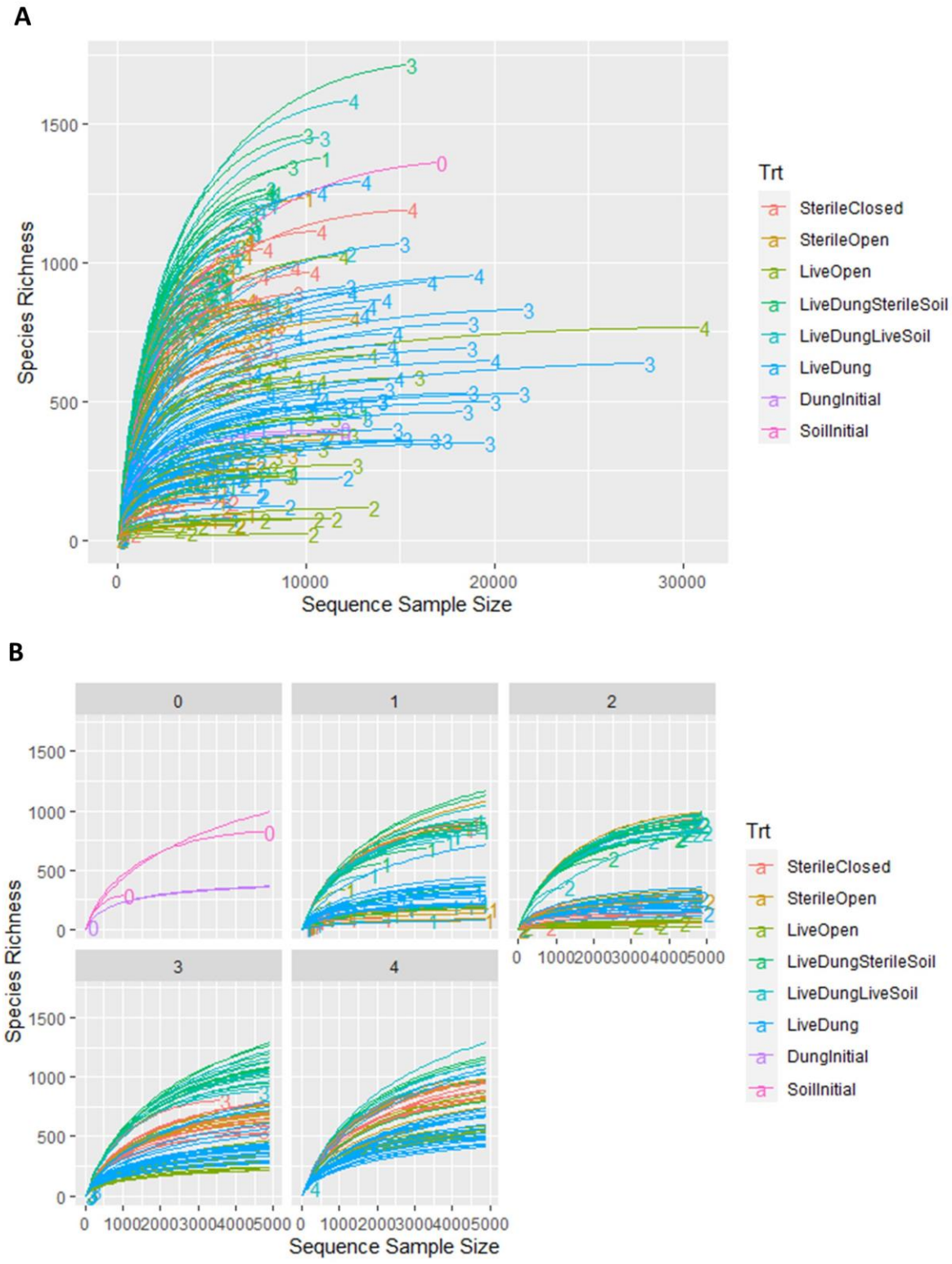

Figure S 3.1 Rarefaction curves for A) all data and B) all data faceted by time and only displaying to 5000 sequences

Chapter 4 - Conclusion

Grassland ecosystems are increasingly threatened by conversion to agriculture, climate change, altered carbon and nitrogen cycles, and loss of biodiversity (Samson et al 2004; Bengtsson et al. 2019). These alterations impact ecosystem functioning globally and imperil the environmental services humans rely upon, resulting in uncertainty around future human welfare (Cardinale et al. 2012). Great Plains prairies have been hit especially hard, with 70% reduction in area compared to that of the historical range (Samson et al. 2003). Restoration concepts expound that the best way to conserve the remaining areas and convert degraded land back to grasslands is to restore the drivers that would have existed pre-European settlement (Fuhlendorf and Engle 2001, Brockway et al. 2002), including large herbivore grazing and fire, thus fire and grazing are commonly implemented by land managers. However, it is hard to predict how alteration of grazing and fire will affect soil microbial diversity, distribution, and function because a smaller body of research exists for belowground community responses, despite the integral role of microbes in ecosystem function (Schimel and Schaeffer 2012; Glassman et al. 2018; Kuypers et al. 2018). Our research has helped to fill in this gap, and highlights the importance of bison grazing in structuring microbial communities at various scales, suggesting that bison may serve as a key factor that structures belowground communities as well as above (Knapp et al. 1999).

In my first study, I found that bison grazing can significantly decrease the distance-dissimilarity of microbial communities across sites and regions of the Great Plains that have wide ranging climate, soils, and management characteristics. Although there were pronounced differences in the strength of the grazing effect at each site, with a few showing the opposite of regional trends, we were able to identify local factors that explained this variability. Sites with more bison on the landscape, more bare soil area, and that were wetter tended to have larger

differences in microbial spatial heterogeneity between grazed and ungrazed areas. Bare ground exposure partly explained the distance-dissimilarity patterns among all sites, pointing to the potential role of aerial microbial dispersal for increasing community similarity. In addition, differences in soil pH and moisture between grazed and ungrazed areas were positively correlated with overall difference in microbial community composition, suggesting local soil factors are also important for structuring communities at a landscape level. Together, findings suggest that bison activity can shift both the relative competitive constraints of the local environment and how easily microbes disperse across the landscape, which impacts overall soil microbial biogeography.

In my second study, the experimental manipulation of microbial dispersal at one grassland site revealed that passive dispersal happens at similar rates in areas that have contrasting fire and grazing histories, and that overall microbial richness is lower both when dispersal to the soil is restricted and when the initial community starts off with higher richness. Furthermore, we found a large and consistent increase in soil microbial richness when active dispersal was applied by adding bison dung, and that regardless of land use background, dung addition caused soil microbial communities to converge over time. This dataset provides evidence that dispersal is important to grassland soil microbial community assembly, and that bison can directly alter soil microbial richness and composition through dung.

Overall, this thesis shows that bison grazing activity can directly and indirectly change soil microbial community dynamics in the central Great Plains. The historical relationship between grazers and Great Plains prairies started 7-5 MaBP when grasslands began expanding and the presence of herbivore megafauna increased, leading to a biome that developed with large herbivore grazing as an integral component (Anderson 2006). It is therefore not surprising that

bison are modulators of belowground grassland dynamics, however, the strong responses of microbial communities to bison grazing show it to be critical that more attention be given to deciphering the mechanisms behind these responses. Ultimately, knowing how belowground community dynamics are directly and indirectly linked to bison grazing will improve the management and conservation of invaluable North American grasslands.

References Cited

Anderson, R. C. 2006. Evolution and origin of the Central Grassland of North America: climate, fire, and mammalian grazers 1. The Journal of the Torrey Botanical Society 133:626–647.

Bengtsson, J., J. M. Bullock, B. Egoh, C. Everson, T. Everson, T. O'Connor, P. J. O'Farrell, H. G. Smith, and R. Lindborg. 2019. Grasslands-more important for ecosystem services than you might think. Ecosphere 10:e02582.

Brockway, D. G., R. G. Gatewood, and R. B. Paris. 2002. Restoring fire as an ecological process in shortgrass prairie ecosystems: initial effects of prescribed burning during the dormant and growing seasons. Journal of Environmental Management 65:135–152.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. Nature 486:59–67.

Fuhlendorf, S. D., and D. M. Engle. 2001. Restoring Heterogeneity on Rangelands: Ecosystem Management Based on Evolutionary Grazing Patterns. BioScience 51:625.

Glassman, S. I., C. Weihe, J. Li, M. B. N. Albright, C. I. Looby, A. C. Martiny, K. K. Treseder, S. D. Allison, and J. B. H. Martiny. 2018. Decomposition responses to climate depend on

microbial community composition. Proceedings of the National Academy of Sciences 115:11994–11999.

Knapp, A. K., J. M. Blair, J. M. Briggs, S. L. Collins, D. C. Hartnett, L. C. Johnson, and E. G. Towne. 1999. The Keystone Role of Bison in North American Tallgrass Prairie. BioScience 49:39–50.

Kuypers, M. M. M., H. K. Marchant, and B. Kartal. 2018. The microbial nitrogen-cycling network. Nature Reviews Microbiology 16:263–276.

Samson, F. B., F. L. Knopf, and W. R. Ostlie. 2004. Great Plains ecosystems: past, present, and future. Wildlife Society Bulletin 32:6–15.

Schimel J.P. and S.M. Schaeffer. Microbial control over carbon cycling in soil. 2012. Frontiers in Microbiology 3:1–11.