CAREER: How do microorganisms and grazing mammals interact at local to regional scales to regulate grassland soil nitrogen cycling processes?

OVERVIEW

Grassy ecosystems cover over a third of the planet's vegetated surface, and most grasslands that remain unconverted to row-crop agriculture are grazed with livestock. Yet, there is no coherent perspective on the generalized effects of large ungulate grazing on terrestrial N cycling processes, beyond the "grazing lawn" phenomenon, in which a higher proportion of N is in plant-available form due to consumer-driven nutrient recycling. The overarching hypothesis of this work is that **the distribution of grassland soil N cycling microbes is more strongly affected by landscape-scale than local factors, with greater functional consequences for guilds with lower functional redundancy**. In other words, the presence of large grazers at the landscape scale will affect microbial diversity more strongly than will edaphic factors at the soil core scale, and this will be associated with differences in soil nitrification potential (a more specialized N-cycling function) more than differences in denitrification or decomposition potential (sequentially less specialized functions). To evaluate the three predictions associated with this hypothesis, including collecting evidence to evaluate the key mechanism of grazer-driven microbial dispersal at the landscape scale, project personnel, students, and citizen scientists will work together to measure soil microbial diversity and activity in bison- and cattle- grazed and ungrazed areas, at distances ranging from 10-cm (local soil microbial habitat) to 1000-km (Flint Hills, KS region) scales. Temporal and experimental objectives will strengthen the inference that can be made from the work. Outcomes will include significant advancement in knowledge of the interactive biotic and abiotic factors regulating microbially-mediated N-cycling function, including factors supporting ecosystem N retention, at local to regional scales; broad and timely communication of this knowledge to students and citizens; and engagement of these audiences in the scientific research process.

INTELLECTUAL MERIT

The biotic interactions, and diversity-based mechanisms, that regulate ecosystem processes are generally less well studied than abiotic drivers. However, especially since human interactions with the N cycle have changed it dramatically, and land management strongly affects the movement of both organisms and materials into, out of, and within ecosystems, biotic interactions should be more explicitly incorporated into developing concepts in ecosystem ecology. After humans, microorganisms regulate the N cycle. This project will define the extent to which, and some mechanisms underlying how, large grazer presence affects key grassland microbial N-cycling processes. In addition to large-scale pattern evaluation, experimental work will parse the independent effects of microbial community composition and soil pH, organic matter and N availability on N cycling processes. Larger-scale spatial, temporal and conceptual knowledge gain, and dissemination of this knowledge to a broad audience, are the main goals.

BROADER IMPACTS

This proposal supports the career development of a female junior ecologist through a series of integrated outreach and educational activities with high school, undergraduate and graduate students, and government, non-profit and private citizen land managers. Data Nuggets for local high school classes will be developed and directly shared with partner teachers in the classroom, data analysis and experimental design and hypothesis-testing lectures will be incorporated into the PI's undergraduate Microbial Ecology course, a course of graduate students will learn molecular techniques by collecting and analyzing a subset of samples, and partnerships with the Nature Conservancy and USFWS Partners for Fish and Wildlife and the Kansas Grazing Lands Coalition will facilitate sampling across the Flint Hills, KS region, including sample and data collection by citizen scientists (local ranchers).

CAREER: How do microorganisms and grazing mammals interact at local to regional scales to regulate grassland soil nitrogen cycling processes?

PROJECT DESCRIPTION

Overall Significance and Integration of Career Research and Teaching Goals

Earth's ecosystems are changing, both in amounts of nutrients and rates of their transformation, and in the identity and abundance of organisms regulating these dynamics. There is an increasingly urgent need for information on the mechanisms underlying these biogeochemical changes, and an equally important need for this information to be used to manage the trajectory of change. The maintenance of ecosystem functions, including primary production, nutrient retention and removal, at levels that support sustainable secondary production and minimal biodiversity loss, is a goal of both ecologists and land managers. Concurrent with multifactorial global change, people in the societies with the capacity to control and manage this change seem to value basic science less than ever in modern history. The loss of scientific literacy and the rapid degradation of earth's ecosystems are truly alarming.

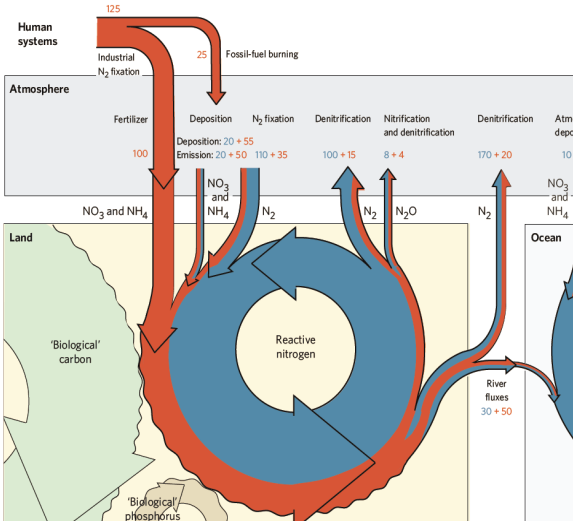
My motivation for writing this CAREER grant proposal is to help address these concurrent problems, and to motivate others to help, as well. More specifically, my **long-term career goal** is to advance the knowledge base that can be used to manage ecosystem services sustainably, and to provide students and managers with the fundamental concepts and context to do so. Towards this purpose, I will focus my efforts more on research that will lead to greater understanding of the biotic and abiotic factors that support and predict nitrogen cycling processes in a changing world (**research goal**), and I will include high school, undergraduate and graduate students, and private land managers, in these research endeavors (**educational goal**). While the research goals are big-picture, the educational activities, enabling more people to participate in small, but real, ways in the exciting process of scientific advancement, are essential to the achievement of my overall career goal. **Integrating education with research**, as I have planned, will extend the breadth of data collection and thus inference, beyond one site to a regional scale, and will also extend the impact of this research beyond the university to the current and future citizens and decision-makers of the region. The educational component of this project will increase both the amount and transfer of knowledge gained through the proposed research well beyond what would be possible without it.

Intellectual Merit

IIa. Context and motivation

Our planet's nitrogen (N) cycle (Figure 1) has been dramatically altered in recent history[1, 2], due to increasing societal requirements for food and the industrial fixation of atmospheric N to the N fertilizer that supports greater agricultural production. The consequent doubling of available N in the biosphere demonstrates the importance of human activity for global N cycling processes. However, once fertilizer hits the soil, micro-organisms control the amounts and forms of N that remain plant-available, as well as the amount of N that is removed from the biosphere and returned to the atmosphere[3]. While human activity dominates the process of global N fixation, our understanding

Figure 1. The N cycle is controlled by humans and microbes. Figure from Gruber & Galloway 2009.



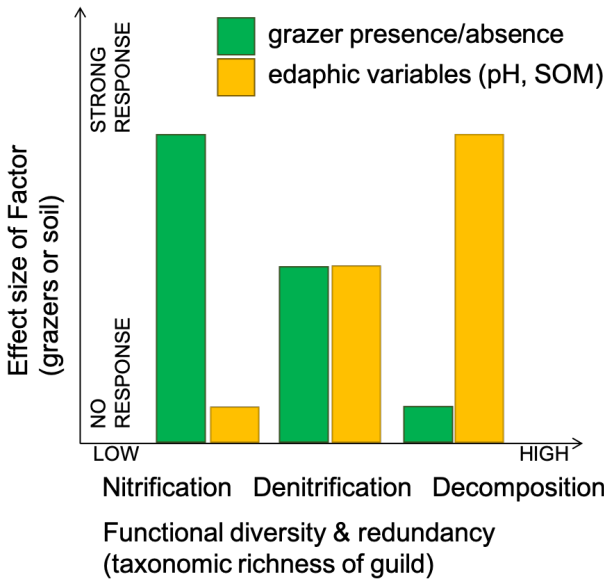
of the microbial activities that regulate terrestrial N availability and N removal is incomplete[4-7], and human control over these microbial processes is weak[8, 9].

Microbial N cycling presents a particularly challenging suite of metabolic processes to understand, because biological and environmental variability both affect N dynamics, and soils are highly heterogenous[10-15]. Fortunately, significant scientific advances in recent decades have made it possible to identify and quantify the microorganisms that embody important terrestrial N cycling processes[16-18]. It is a crucial time to learn more about the dominant controls over the distribution and activity of soil N-cycling microbes, and to empower students and citizens to participate in this endeavor.

Grassy ecosystems cover over a third of earth's vegetated terrestrial land, and most grasslands that remain unconverted to croplands are used for agricultural production in the form of range for grazing livestock[19, 20]. The tallgrass prairie in the Flint Hills region of Kansas, USA, is the last contiguous extent of remnant tallgrass prairie in North America, and also an important center of cattle production. Pre-colonialization[21], this region was habitat to abundant large mammals, including the grazing bison[22]; now, the cow is the dominant grazing mammal. The tallgrass prairie is special in many ways, including being retentive of N, due to the maintenance of an N-limited state by frequent fire and high microbial immobilization of N in the soil[23-25]. Research in many grasslands shows that grazing animals increase plant N availability[26-28]. However, we do not know how the presence of grazing mammals affects the potential for microbial N loss from tallgrass prairie ecosystems[29], or whether bison and cattle affect these soil N cycling processes similarly.

To better understand controls over grassland N cycling (**research goal**), we must know how interactions between grazing mammals and microorganisms regulate the distribution and activity of soil N-cycling processes. Because human management activities and decisions control the movement of grazers, and the conversion of low-N-input rangeland to N-input-heavy cropland, involving students and citizens in this novel research on their local ecosystem is essential to maximizing both the amount and the impact of this knowledge gain (**educational goal**).

Figure 2. Conceptual model. SOM: soil organic matter.



The overarching hypothesis of this work (Figure 2) is that **the distribution of grassland soil N cycling microbes is more strongly affected by landscape-scale than local factors, with greater functional consequences for guilds with lower functional redundancy**. In other words, I predict that the presence of grazers on the landscape will affect microbial diversity more than soil edaphic factors at the individual sample scale, and that this will be associated with differences in soil nitrification potential more than differences in denitrification or decomposition potential.

To address this hypothesis and translate the findings to a broad audience, the project's research and educational activities will include people at all levels of education from high school, undergraduate, and graduate students, a postdoctoral researcher, and private landowners. These activities will evaluate the following three predictions that underpin the broad hypothesis:

P1. *Among all sites and over time at the regional scale, the presence of large grazing animals strongly increases nitrification potential rates, weakly increases denitrification potential rates, and does not affect decomposition potential rates. Conversely, soil factors are correlated with decomposition, less strongly so with denitrification, and not correlated with nitrification.*

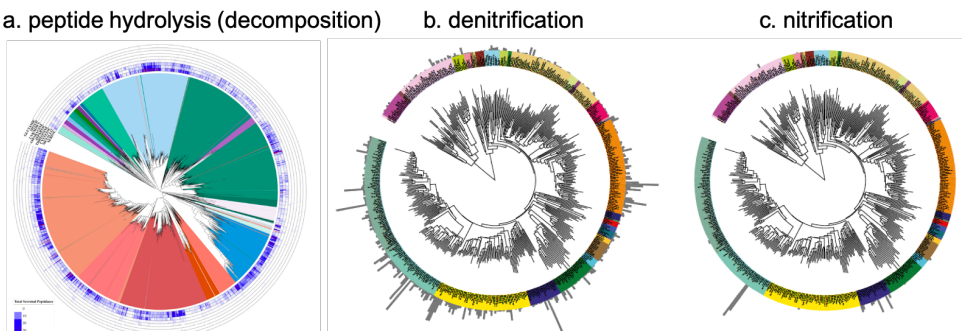
P2. *The presence of large grazing animals consistently changes the soil microbial community composition and reduces evidence of microbial dispersal limitation at the pasture or watershed scales. In turn, community composition and geographic distance are more correlated with nitrification than denitrification or decomposition potential.*

P3. *In the same local soil habitat, the different microbial communities from grazed and ungrazed soils have similar decomposition potential, but different denitrification and nitrification potentials. In contrast, soils with different pH and OM content will support different decomposition potentials for microbial communities of the same origin.*

For this project, I define "landscape-scale" factors as the grassland management attributes of prescribed burning status and presence of grazing animals, including cattle or bison, and I define "local-scale" factors as the soil edaphic characteristics, including pH, total C and N, and available N content, that are heterogeneous among individual soil samples. These "local" soil edaphic factors also more directly affect the microbial habitat and activity at the cellular scale[30-32], while the "landscape" management factors affect the microbial environment indirectly through changes in nutrient and plant litter inputs[26, 27] and vegetation composition[33, 34] at the field or watershed scales.

The project's focal grassland soil N cycling processes are decomposition, nitrification, and denitrification: The microbial metabolic activities that respectively regulate levels of plant-available N, production of the most loss-prone form of N (nitrate), and the transformation of nitrate to gaseous forms that are lost to the atmosphere and effectively removed from the biosphere. Each of these three functional groups includes different types, or taxa, of microorganisms (Figure 3). Nitrification is a specialized chemolithoautotrophic metabolism, and relatively few bacterial and archaeal taxa can nitrify[35]. Denitrification encompasses a set of enzymatic reactions that may be distributed and exchanged among a diversity of different bacterial taxa[11], though many microorganisms still do not participate in denitrification. Hydrolysis of amino acids from proteinaceous organic matter is a key process of decomposition, producing nitrogenous compounds small enough to be transported into cells, and thus limits rates of potential N assimilation into either plant or microbial biomass. Extracellular peptidase genes are widespread in the bacterial and archaeal domains[36], and many soil fungi also express peptidase genes, making decomposition a very diverse N-cycling process. In sum, nitrification, denitrification and decomposition fall along a spectrum of low to high functional diversity and redundancy[37-39], based on the relative number of microbial taxa that participate in each process.

Figure 3. Functional diversity and redundancy within microbial N cycling guilds. Graphics directly from (a) Nyugen et al. 2019 and (b, c) Nelson et al. 2016. The distribution of lineages in the bacterial phylogenetic tree that can produce extracellular serine peptidases (~1,000 genomes) is noted by blue in the central ring (a), and that can perform denitrification (b) or nitrification (c) is noted by grey bars (241 & 16 genera, respectively).



The N cycle is complex (Figure 1), which is what makes it so interesting to study. However, to make this research tractable, certain functions that are important to ecosystem N availability and retention are not included in the project scope, including N fixation and dissimilatory N reduction to ammonia, and also, while fungi are very important N-cycling microbes, the proposal will use "microbial" to refer to just bacteria and archaea from this point forward. Finally, grazing mammals (Figure 4), through their digestion of plant tissues and excretion of non-assimilated N in dung and urea, bison and cattle can convert a significant proportion of the N in the ecosystem to more plant-available forms[26, 28]. While mineralization of urea-N to ammonia-N is driven by soil microorganisms[3], that function will not be included in the proposed study, because grazing animals are the proximate cause of this increased N availability. However, soil available N is considered explicitly throughout the proposal as a key soil edaphic factor, a covariate of grazer presence (Figure 5a), and an important driver of biogeochemical function and microbial diversity.

Figure 4. Ungrazed (a), bison grazed (b) and cattle grazed prairie (c) at Konza Prairie Biological Station (KPBS).

IIb. Rationale for predictions and preliminary data

P1. *Among all sites and over time at the regional scale, the presence of large grazing animals strongly increases nitrification potential rates, weakly increases denitrification potential rates, and does not affect decomposition potential rates. Conversely, soil factors are correlated with decomposition, less strongly so with denitrification, and not correlated with nitrification.*

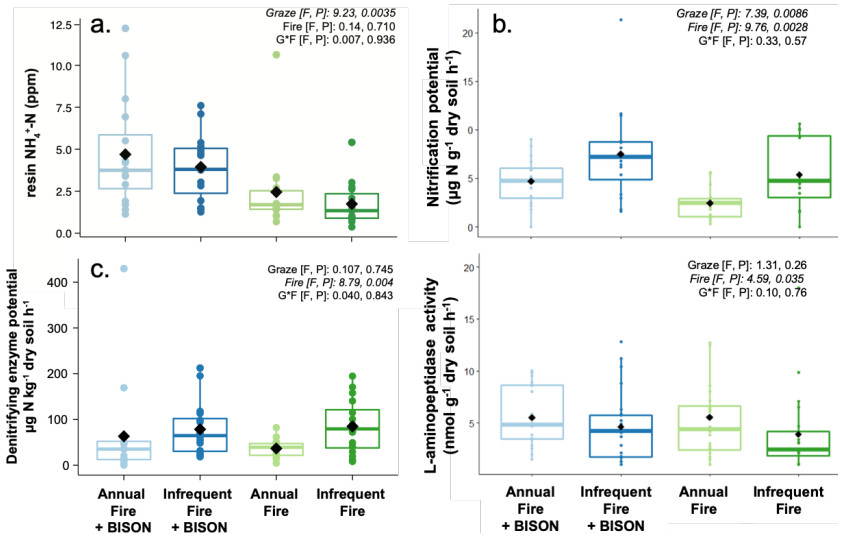
The North American tallgrass prairie is characterized by substantial above and belowground productivity, and a high capacity for N retention in perennial grasses, soil microbial biomass, and SOM[23]. Maintenance of these tallgrass prairie ecosystem services is driven in part by regular fire, because burning removes N from the ecosystem through volatilization of the N in aboveground plant litter, causing N limitation of the biota[40]. This in turn causes increased investment by plants towards foraging for N via root growth[27], and increased investment by soil microbes towards foraging for N via extracellular peptidase production (Figure 5d). The presence of large grazers like bison increases soil N availability (Figure 5a) and nitrification potential (Figure 5b), but not soil denitrification potential (Figure 5c) or decomposition potential (5d). This means that the microbial potential for N loss from the biologically available pool through denitrification, and for removal of N from the SOM pool, can be unaffected by grazers, despite higher N availability for plant uptake. These soil microbiological factors can support ecosystem N retention even in grazed prairies[41-43].

The generality of this disconnect between soil N availability, denitrification and decomposition in grazed grassland ecosystems, however, is not clear. The positive feedbacks between grazing animals and plant N availability, in the form of "grazing lawns", are generally understood to occur in grasslands worldwide[26, 28, 44-47]: Greater soil N availability causes higher grass protein content[48], which is selectively eaten by large animals[49], more N is excreted back to the soils with higher animal presence, and N remains more available in the soil. Beyond this, there does not seem to be agreement about how grazers affect each of the microbially mediated steps of the N cycle[26]. Nitrification increased and nitrifier diversity changed with higher grazing at multiple sites[50, 51], yet there is

evidence that more intense grazing can increase ammonia oxidizing archaeal numbers but not nitrification potential[52]. While some papers report increases in denitrification with grazing[41, 53, 54], many others do not ([37, 43, 51, 55], Figure 5). Grazing consequences for decomposition are very mixed[56]. In one study, shoot litter decomposed more quickly and released N, but root litter immobilized more N, in grazed areas[48]. Different levels of grazing intensity have been associated with variable decomposition and SOM accumulation or loss responses[42, 57-59], and grazing had no effect on microbial C utilization patterns[60]. It may be that grazing generally impacts nitrification more than denitrification more than decomposition, or not; but is difficult to draw any conclusions on this prediction solely from the series of case studies available in the literature.

This project's primary research goal is to expand the understanding of the N cycle in grazed grasslands to a broader conceptual and geographic scale, and to do this, we must start by measuring microbial N cycling at replicated sites and years. Like soil C content[56-59], the effects of grazing on N retention in grassland soils may be context-dependent. Possible explanations for variable increases in microbial N cycling rates, despite higher N availability, in grazed soils (Figure 5), include that either a different microbial community in grazed soils[37, 53, 61-63] (Figure 6a, P2), or different soil pH or C content[28, 55, 57] (P3), constrains activity potentials.

Figure 5. Bison grazing increases (a) N availability and (b) nitrification potential but not (c) denitrification potential or (d) peptidase activity potential.



P2. *The presence of large grazing animals consistently changes the soil microbial community composition and reduces evidence of microbial dispersal limitation at the pasture or watershed scales. In turn, community composition and geographic distance are more correlated with nitrification than denitrification or decomposition potential.*

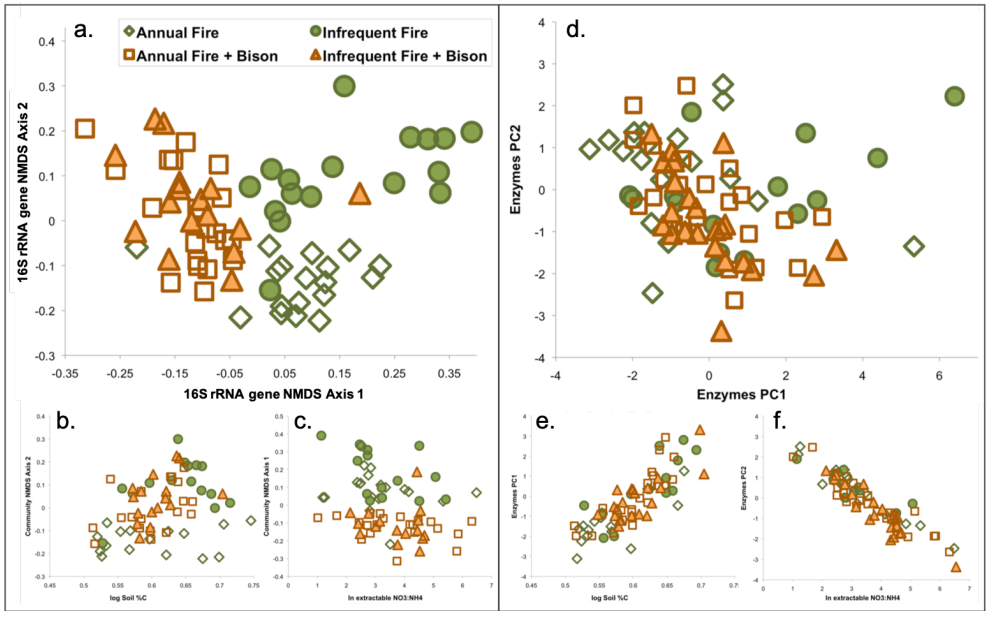
Grazing can affect grassland soil microbial community structure [64-66], often in association with changes in N cycling rates [37, 53, 61-63], but this is not always the case. It is also unclear whether links between large grazers and microbial diversity are direct or indirect. By increasing soil nutrient availability, grazing animals might indirectly promote growth of taxonomically distinct microbes with higher N cycling rate potentials; or, animal movement across the landscape may directly disperse microorganisms that also have different functional attributes. Large ungulate grazers redistribute N within the ecosystem; could they redistribute microbial cells, as well?

One study showed that both grazing, and direct increases in N availability via fertilization, affect soil microbial communities, but that these shifts are not congruent[67]. This suggests that higher N availability may not be the proximate mechanism for grazing-caused soil microbial community change. Another more recent study posits that relaxed competition between microorganisms for soil C causes the increase in microbial diversity observed with higher grazing pressure, but shows no direct evidence for this mechanism[68]. As already noted, increased soil C is hardly a universal result of

grazing[69], and often does not explain the effects of grazing on microbial community composition[62, 70]. In contrast, animal movement while foraging is a direct and consistent phenomenon.

At KPBS, bison grazing had a surprisingly clear effect on soil microbial community composition (Figure 6a), but this shift was not predicted by soil C or N (Figure 6b-c). Furthermore, geographic distance between samples was correlated with community composition among all samples, but soil factors were not (Mantel test [R, P]: distance: [0.24, <0.01], soil factors [0.080, >0.05]), suggesting dispersal limitation could have a stronger influence on community composition than local soil habitat. In fact, with grazers on the landscape, the composition of soil microbes sampled kilometers apart was more likely to be similar to soil microbes sampled centimeters apart (Figure 9).

Figure 6. Bison grazing changes soil (a) microbial community composition (MCC; PERMANOVA Grazing R^2, *P: 0.098, 0.00001;* Fire R^2, *P: 0.035, 0.0002;* G*F interaction R^2, *P: 0.030, 0.0009*) and (b) extracellular enzyme activity (EEA). However, EEA (e-f), not MCC (b-c), is correlated (*P < 0.05*) with soil %C and extractable nitrate:ammonium ratio (shown as a proxy for N availability).



There is now widespread evidence that soil microbes are dispersal-limited[71-74], particularly taxa with fewer genes, thus more specialized functional potentials[75]. Grazing by bison or cattle potentially lowers microbial dispersal limitation. Generally, the movement of micro-organisms into new environments has consequences for traits like disease or antibiotic resistance [76-78]. Still, links between microbial dispersal and terrestrial ecosystem services are largely unknown[79].

If there is a stronger dispersal-mediated link between microbial community structure and function for guilds with more restricted taxonomic diversity, then nitrification potential will respond to grazing more than soil C or N, with a weak distance-decay relationship, like community composition (Figures 6a-c, 9). In contrast, the taxonomically diverse and functionally redundant decomposition enzyme activity potential at KBPS might be predicted quite well by local-scale soil C and N levels, but not by watershed-scale grazing status, as preliminary data do show (Figure 6d-f), and decomposition potential patterns might fall in an intermediate area (Figure 2). Coordinated and distributed sampling and data analysis will be needed to address this hypothesis and predictions.

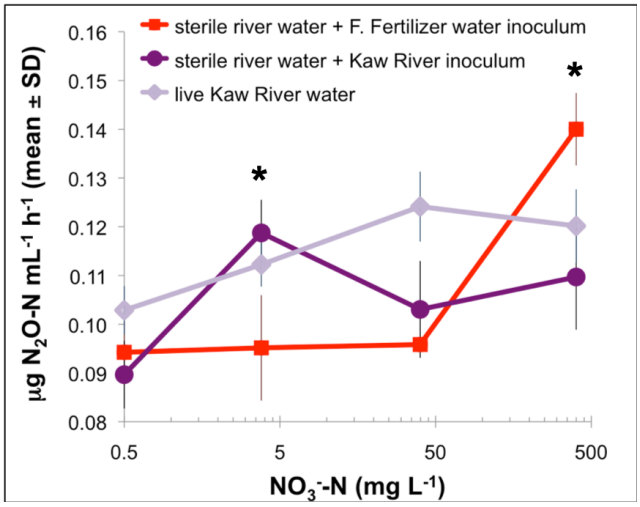
P3. *In the same local soil habitat, the different microbial communities from grazed and ungrazed soils have similar decomposition potential, but different denitrification and nitrification potentials. In contrast, soils with different pH and OM content will support different decomposition potentials for microbial communities of the same origin.*

The composition and origin of a microbial community can affect biogeochemical functions[80] like decomposition[81-83], respiration[84, 85], and denitrification[86-88](Figure 7). But, a generally decomposable substrate like grass litter might not be associated with differential activity of specialists, like pine or rhododendron litter[81]. In that case, instead of community composition, edaphic characteristics like pH[30, 89], SOM content[31] or N availability[32] may be the primary determinants of grass litter decomposition and associated extracellular enzyme activity[90]. In contrast, the realized activity of a taxonomically specialized biogeochemical function is more likely to be constrained by community composition[81]. Furthermore, major caveats to interpreting patterns seen in the preliminary data and proposed field-scale sampling grazing remain: Covariance between grazer presence and N availability is likely, and regionally different soil types may vary in SOM or pH, affecting biogeochemical activity and microbial diversity independent of grazing.

So, a set of experiments to provide evidence supporting, or against, the predicted direct importance of microbial community composition for determining soil N-cycling potentials is essential to address the project's overarching hypothesis[82]. A subset of soils representing the range of contrasting pH and grazing history from across the Flint Hills terrestrial landscape will be used for reciprocal inoculation experiments. Because a full reciprocal inoculation of soils with different pH, OM and N availability would be untenable, the experiments will include grass litter and urea amendments to evaluate the covarying importance of OM and N availability, respectively. The PI's lab has successfully used similar experiments to evaluate a similar hypothesis (Figure 7).

Finally, while the qualitative differences in microbial functional diversity and redundancy that underpin the conceptual model seem reasonable (Figures 2, 3), information confirming by directly quantifying these differences would make this research project stronger. Metagenomic analysis, which allows quantification of all functional genes affiliated with all organisms in one sample is becoming more commonplace and accessible[91-93]. The metagenomic N-cycling potential and functional diversity of these soil microbial communities will be measured[94]. In addition to substantiating the proposed functional redundancy mechanism, this will provide information on the complete biogeochemical functional diversity of the study soils, providing a foundation for continued research.

Figure 7. Experimental inoculations of DNP assays (no DOC added, different levels of nitrate added) with different-sourced microbial communities. Asterisks note significantly different activity between river and fertilizer pond water inocula ($P < 0.05$). The results support the prediction that a microbial community from a high-nutrient habitat (fertilizer waste water) has a higher capacity, but lower affinity, for denitrification than a microbial community from in a low-nutrient habitat (river water).



IIc. Integrated research and education activity plan

P1. *Among all sites and over time at the regional scale, the presence of large grazing animals strongly increases nitrification potential rates, weakly increases denitrification potential rates, and does not affect decomposition potential rates. Conversely, soil factors are correlated with decomposition, less strongly so with denitrification, and not correlated with nitrification.*

P2. *The presence of large grazing animals consistently changes the soil microbial community composition and reduces evidence of microbial dispersal limitation at the pasture or watershed scales. In turn, community composition and geographic distance are more correlated with nitrification than denitrification or decomposition potential.*

To evaluate whether the terrestrial N cycling and microbial diversity responses to bison grazing at KBPS described above are generally representative of the tallgrass prairie ecosystem at a regional scale, and to gather evidence addressing the possible dispersal mechanism, repeated sampling at broadly distributed sites is necessary, and standardized methods for comparison across locations and years must be used. To integrate this goal with my educational goals, citizen scientists (landowners) and graduate students (through graduate coursework on microbial diversity) will be directly engaged in the research. Furthermore, the data on grazer effects on grassland N-cycling will be translated into activities for local high-school classroom use.

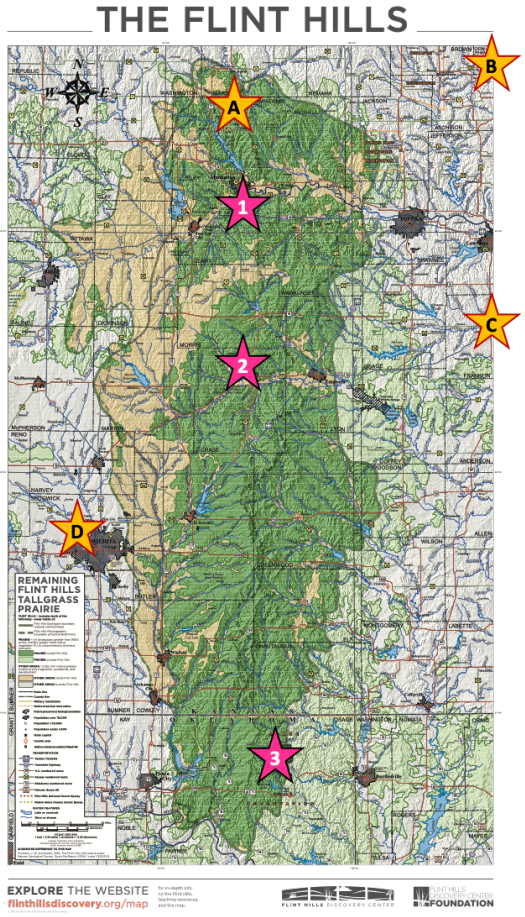


Figure 8. Map of Flint Hills, KS & OK bison grazed site locations (pink stars: 1=KPBS, 2=TNP, 3=TPP) and participating High School science classes (yellow stars: A=Blue Valley, B=Leavenworth, C=Shawnee Mission, D=Maize).

The Flint Hills region stretches ~6.36 million acres from northeastern Kansas into northern Oklahoma (Map[95], Figure 8) and is the largest extent of remnant tallgrass prairie in the world. Because of the relatively rugged hillslope topography here, very little sod was tilled during the USA's westward expansion period. Almost 60,000 acres of this prairie is owned by The Nature Conservancy (TNC), including the area managed for research by KSU as the KBPS, the Tallgrass Prairie National Preserve (TPNP, Strong City, KS) managed by the National Parks Service, and the Tallgrass Prairie Preserve (TPP, Pawhuska, OK) managed by TNC. State and federal governments own little to none of this land. Instead, most of this landscape is privately owned and operated, and while ~200,000 acres of private land is or has been left "unmanaged" under conservancy status with the support of TNC, the US Department of Agriculture Natural Resources Conservation Service (NRCS), the US Fish & Wildlife Service's Partners for Fish & Wildlife (USFWS PFW), or the Kansas Land Trust, most of the remaining tallgrass prairie in the country and the world is actively used as cattle range. Thus, any activities to understand the ecosystem dynamics of tallgrass prairie at a regional scale must include private and TNC lands.

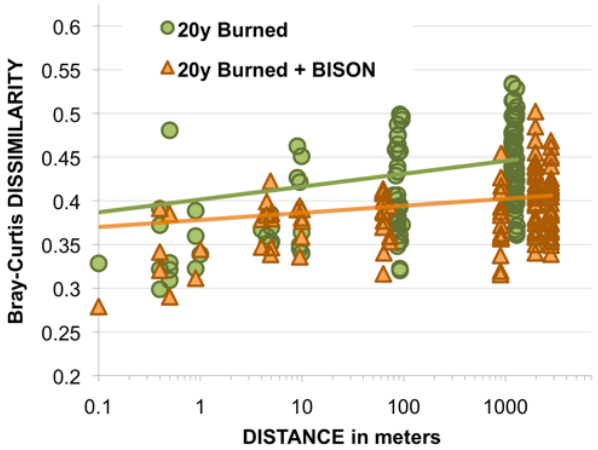
Site selection. The KPBS is an experimental landscape, with replicated watershed-scale combinatory treatments of grazing (bison, cattle or none) and prescribed fire interval that have been maintained since the 1970s. This unique facility, and the research projects it hosts, provides a valuable source of information on ecological responses to fire, grazing and climate, and a record of management history in each watershed. Much of the Flint Hills region is comparable in management history, if not data wealth: TNC's TPNP and TPP are regularly burned and include both bison and cattle grazing in separate areas, and keep records of management history. KPBS and TNC leadership, and the Director

of the KSU Cow-Calf Unit (CCU, which grazes on KPBS), understand the goals of this project and have agreed to share fire and grazing records, and host students and postdocs sampling for this project at their sites, should this proposal be funded (see letters of support from Dr. Blair (KPBS), Mr. Obermeyer (TNC-TPNP), Mr. Hamilton (TNC-TPP) and Dr. Olson (CCU)).

Citizen science activities. Most private land is cattle grazed, with some areas left ungrazed through easements or other individual management decisions, but information on management history is not readily accessible except by personal communication. Traditionally, sampling on private lands would entail requesting landowner permission and sending research personnel to collect samples. However, by coordinating with the USFWS PFW and the Kansas Grazing Lands Coalition (KGLC), this project will recruit landowners to provide samples, as well as key data on the management history of the locations sampled. A synopsis of the preliminary data, goals and hypotheses, and required data parameters has been shared with PFW and with the full Board of the KGLC, and representatives of these groups have expressed enthusiastic interest in participation (see letters of support from Mr. Crouch (KGLC) and Mr. Kramos (PFW)). The PFW and KGLC representatives are confident that at least 3 landowners each year will be willing to volunteer to collect soil and share coarse information on the management history of their property to help answer our research questions. In turn, PI Zeglin and her team will communicate research findings directly with the landowners through brief, tailored reports, and will visit field days at the KGLC sponsored range schools and other public forums, to communicate broad fundamentals about conducting science, grassland ecosystem ecology, and the ways in which grazers and soil characteristics each affect soil biogeochemistry and fertility.

Project personnel and landowners will collect mineral soils to 15cm depth using sterile technique on ungrazed, bison grazed and cattle grazed soils in all project years, at replicated transects with a semilog-distance design (Figure 9). Landowner soil samples will be collected into pre-labeled bags and a pre-paid shipping cooler with ice packs so samples remain cold overnight, and all samples will be collected on ice and nitrification and denitrification potentials analyzed within 48 hours (Figure 10). KPBS sampling will include two transect replicates at two watershed replicates of each grazing treatment. With 6 samples in each transect, collected at distances of 0m, 0.1m, 0.5m 1m, and 10m, and replicate transects and watersheds, the distance coverage reaches 5 orders of magnitude. With sites extending into northern OK, the project scope covers 10s to 100s of km, or 7 orders of magnitude scale. The non-KPBS sites, which will not include watershed scale replication, will each be sampled at two (cattle grazed: 6 total sites) or three (bison grazed: 3 total sites) replicate transects per treatment, at as far a distance as each local site's layout allows.

Figure 9. The shallower slope of the distance-dissimilarity relationship in grazed soils supports the mechanism of reduced microbial dispersal limitation across the terrestrial landscape where bison are present. ANCOVA Grazing *F, P: 6.34, 0.0134*; Fire (not shown) *F, P: 6.16, 0.0129*; Grazing*Fire *F, P:* 2.05, 0.153).

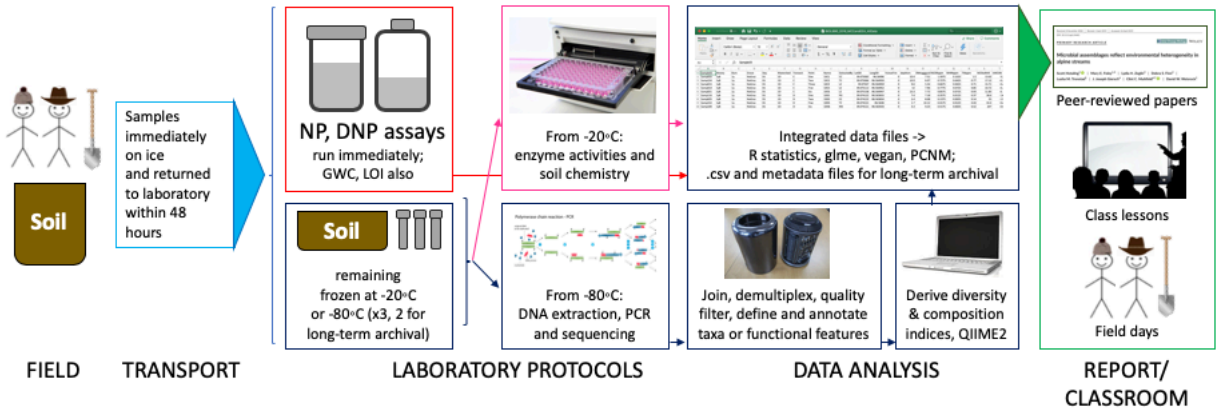


With this strategy, spatial coverage is maximized but sample numbers are kept within a logistically reasonable cap. Unfortunately, with so many more sites in the region hosting cattle than bison, and KPBS the only site in the region with defined watershed-scale replication, the statistical model comparison of grazing treatment will have an unbalanced design. However, in addition to the categorical comparison, this geographic-distance

explicit design enables evaluation of soil core-scale versus site-scale controls over terrestrial N-cycling processes and diversity. Distance-dissimilarity and distance-decay relationships can identify the scale at which spatial autocorrelation ceases, and other factors become more important[10, 96-98]. Redundancy analysis can quantify the level of variability in community composition that is explained by distance, soil, or grazing management[99-102], and linear models and Mantel (matrix) correlation tests can evaluate whether edaphic factors are correlated with activity or diversity parameters[103]. With information on geographic distance, soil characteristics, and grazing status, this project can distinguish biotic and abiotic drivers of grassland N-cycling processes and microbial diversity, and evaluate their generality across space and time.

METHODS (P1)- Soil edaphic factors. Soil field water content, pH, total C and N, and extractable nitrate and ammonium will be measured on all samples using standard methods. The KSU Soil Testing Lab will run total organic C and total N, and the Zeglin Lab will measure the other factors. Nitrate and ammonium will be measured on 2M KCl extracts of a subsample of each soil using 96-well plate versions of standard protocols[104, 105]. *Nitrification potential (NP) activity*. A subsample of fresh soil will be aerobically incubated with 100 mM phosphate buffer solution spiked with NH_4-N for 24h, and subsamples collected at three time points through the incubation[106]. The accumulation of NO_3^- in solution will be measured using the 96-well plate colorimetric assay noted above, and NP in $\mu g\ N\ g^{-1}$ dry soil h^{-1} calculated. *Denitrification potential (DNP) activity*. Soil DNP rates will be estimated using two assays, one with added C and N substrate (i.e. denitrification enzyme activity assay), and one with no amendment to estimate the substrate-limited DNP attainable under field conditions[88]. Acetylene will be added to a soil slurry in evacuated serum bottles to prevent nitrous oxide (N_2O) reduction to N_2, the final step of denitrification. N_2O concentrations will be measured after 1 and 4 hours of shaken incubation using gas chromatography (GC) with a Shimadzu 2014 GC

Figure 10. Annual research and education workflow including sample collection and analysis.



analyzer (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). The evacuation manifold and GC are located in Dr. Walter Dodds' lab (Professor in KSU Biology, letter of support attached), and are already in regular use by the Zeglin Lab (Figure 5). *Extracellular enzyme activity (EEA) potential*. Hydrolytic and oxidative enzyme potential activities will be measured using fluorometric and colorimetric substrates using well-established 96-well plate assays[90, 105, 107, 108]. Enzymes measured will include phosphatase (Phos; EC 3.1.3.1), leucine aminopeptidase (LAP; EC 3.4.11.1), serine aminopeptidase (SAP; EC 3.4.21), cellobiohydrolase (CBH; EC 3.2.1.91), β-glucosidase (βG; EC 3.2.1.21), and β-*N*-acetylglucosaminidase (NAG), peroxidase (Perox; EC 1.11.1.7), and phenol oxidase (Phenox; EC 1.10.3.2). This represents a standard suite of EEAs that assess the soil microbial community C:N:P limitation status, with the addition of the taxonomically diverse serine peptidase (SAP)[36], for direct comparison with the more commonly measured[109, 110] LAP activity.

METHODS (P2)- Soil DNA isolation and sequencing preparation. Total genomic DNA (gDNA) will be isolated from approximately 0.5 g field moist soil using a DNeasy PowerSoil Kit (Qiagen, Venlo, Netherlands), following the manufacturer's protocol with one modification (physical lysis on a MP Biomedicals FastPrep instrument (30s at 5.5 m/s)). DNA yield will be quantified using Quant-it DNA kits (Molecular Probes, Life Technologies, Grand Island, NY). An extraction blank will be run with every batch of samples, and if DNA is present in the negative, those samples will be re-extracted or dropped from subsequent analysis. *Polymerase Chain Reaction (PCR) of taxonomically informative bacterial and archaeal genes*. To prepare for amplicon sequencing, one-step PCR using 16S ribosomal RNA gene universal primers 515F and 926R, with sequencing adapters and unique barcodes included with the reverse primer[111-113], will be run. This protocol will follow the well-standardized Earth Microbiome Project protocols with one modification: The 3 technical replicate reactions will be run using 25 cycles per sample, instead of 35, to minimize impact of PCR bias on taxon relative abundance data. *Library preparation and sequencing*. ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) cleanup will be run on each combined amplicon pool, cleaned amplicons quantified as above and equimolar amounts combined into one normalized library, which will be finally purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany)[114]. Sequencing will take place using an Illumina MiSeq 2x250 PE cycle run with a 15% PhiX spike, at the KSU Integrated Genomic Facility (IGF), which has experience with this type of library.

METHODS (P2)- Sequence processing and analysis. The IGF routinely produces MiSeq raw sequence data with 12-17M, median 15M, passed reads from our library preparations. We will cap our multiplexed library preparations at 288 samples each, for an average ~52,000 raw reads per sample; even if 1/3 of this data is lost to quality control, ~35,000 reads per sample provides good coverage for community composition and diversity metrics. All gene raw sequence data will be demultiplexed, quality trimmed and denoised[115], aligned to reference ribosomal gene conformation and binned into taxonomic units (using vsearch and the GreenGenes (GG) database)[116, 117] and given phylogenetic group assignments (using a Naïve Bayesian Classifier and GG), all using the QIIME2 pipeline[118]. Since data are generated using QIIME-compatible protocols[119], this process is smoothly streamlined. QIIME2 will also be used to calculate alpha and beta diversity metrics, and relative abundance data matrices for all samples, which will be exported for community analysis in R-vegan[120], differential abundance comparison in R-DESeq2[121], or linear model testing in R-glm[103, 114].

Graduate course education. In 2019-2024, graduate students in ecology need familiarity, and better yet hands-on experience, with the conceptual and methodological background of modern microbial ecology. Environmental microbes are organisms like any other, with evolutionary histories, ecological niche differentiation and interactive dynamics that affect their potential and realized ecosystem functions. However, we can only observe and measure these organisms using biochemical assays – including both "modern molecular" measurements of intracellular informational or signal molecules, and more "traditional" measurements of substrate turnover driven by key enzymes in microbial metabolic pathways. To gain this integrated knowledge, students in Microbial Diversity (BIOL 890), a graduate course taught by PI Zeglin, read key papers in the literature, and also learn to extract DNA, run PCR, and complete the bioinformatics protocols to convert the resulting raw sequence data to quality-controlled information on taxon relative abundance and diversity. This activity is already underway, and students in this course produced the preliminary data shown in Figures 5a and 9. As these scientists move forward with their careers into different specialties, they will understand the intricacies of using molecular tools and interpreting the resulting data accurately.

During the years (2020, 2022 and 2024) when this course is held, graduate students will collect and analyze all samples from bison grazed and ungrazed watersheds at KPBS. All protocols are fully standardized to ensure consistency, and quality-controlled to allow identification of technical errors.

Specifically, samples from different treatments are equally and randomly distributed among students so that minimal loss of inferential power happens if one individual's protocol fails, and both DNA extraction controls and PCR negative controls are quantified and sequenced.

High school educational activities. A high-school outreach component will entail writing and sharing smaller directed lesson activities using the N-cycling rate data we collect for the larger project. Our research team will produce 5 Data Nuggets[122] over the course of the project (one each year) and the student or postdoc that wrote the Data Nugget will visit participating high schools each year to talk about the research itself. This will be useful in giving students a greater appreciation of the tallgrass prairie and a better understanding of what scientists do, and a greater understanding of the importance of nutrient cycles, which is typically a difficult concept for them to grasp. Four local high school teachers (Mrs. Hutson (Blue Valley), Mr. Brandt (Shawnee Mission, Mr. Harrison (Maize) and Mrs. Loeffler (Leavenworth), letters attached) are enthusiastically interested in using locally-relevant Data Nuggets to help teach both N-cycling and data analysis in their Biology and Environmental Science classes This is an essential aspect of this project that ensures that our research directly reaches students in a younger generation.

P3. *In the same local soil habitat, the different microbial communities from grazed and ungrazed soils have similar decomposition potential, but different denitrification and nitrification potentials. In contrast, soils with different pH and OM content will support different decomposition potentials for microbial communities of the same origin.*

A "reciprocal inoculation" experiment (Figure 11) will provide evidence for or against the connection between microbial community composition and function, and measure the functional diversity of decomposition, denitrification and nitrification guilds to address the underpinning conceptual framework[80, 94]. Because this is a relatively complex experiment, the postdoctoral researcher will be responsible for this activity, in Y4, with the assistance of the technician and research undergraduates. Also, data from this experiment will be incorporated into lectures and a data analysis homework activity for the undergraduate course Microbial Ecology (KSU Biology, BIOL 687), which the PI teaches every Spring semester.

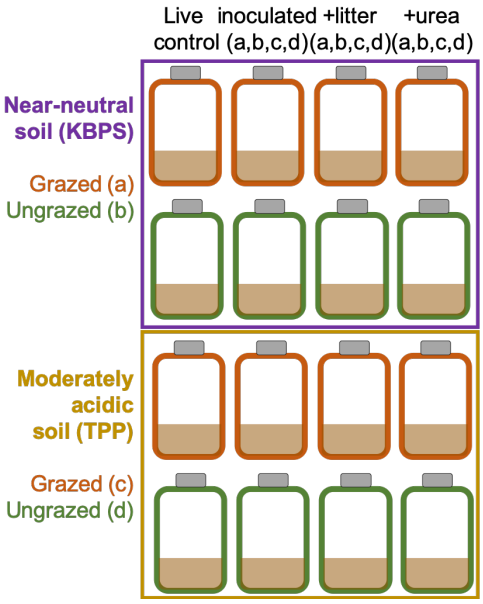


Figure 11. Inoculation experimental design. Each treatment will have 4 reps.

Soils for the experiment will be collected from grazed and ungrazed areas at KPBS in the N. Flint Hills, which has predominantly limestone parent material and near-neutral pH (6 to 7), and NPP in the S. Flint Hills, which has sandstone parent material and mildly to moderately acidic pH (5 to 6). Four field replicate soil samples, composites of the established transects, will be used as lab replicates. Microbial cells for inocula will be separated by mildly shaking a composite soil sample of each of the four field treatments (Figure 11: a, b, c, d) in phosphate-buffered saline. 10 mL of the inoculum solution will be added to ~40 g sterilized (by autoclaving twice) and air-dried soil per replicate, with only soil nutrients as substrate, or either senesced grass litter (OM) or urea (N) supplemented. The new edaphic habitat/community combinations will be incubated in the dark at room temperature for 8 weeks for active communities to establish[94]. Live control soils, with 10 mL of sterile PBS added, will

incubate in parallel. Then, soils will be destructively harvested, NP, DNP and EEA will be measured, and DNA will be extracted for microbial community analysis. Not all microbial populations will survive the separation and inoculation treatments: this experiment will elucidate the linkages between different N cycling functions and microbial community composition but will not provide data representative of field ecosystem-scale conditions[80]. These experiments complement the field data, and both are necessary to reach the project's research goals and address the overarching hypothesis.

In addition, to quantify functional diversity of nitrifying, denitrifying, polypeptide-decomposing, and other functional guilds, metagenomic data on DNA extracted in duplicate from the 16 fresh inoculum-source soils will be collected. Metagenomic libraries will be analyzed in two ways. First, the raw sequence data will be submitted to MG-RAST[123], where it is quality trimmed and annotated to multiple functional databases using a BLAT algorithm, then exported for further analysis in R (DeSeq2 package[121] for differential feature abundance comparison, which normalizes libraries to one another and uses multiple comparisons appropriate to high numbers of covarying and non-normally distributed continuous variables[124]) and STAMP (Statistical Analysis of Taxonomic and Metagenomic Profiles)[125]. Also, the raw sequence data will be demultiplexed and quality trimmed using Galaxy[126], then annotated to a protein functional database using a Hidden Markov Model such as FOAM (Functional Ontology Assignments for Metagenomes)[127], since binning sequence data by protein active site rather than genetic similarity can capture a larger amount and more accurate information on the coverage of key functional genes[128].

Finally, these data will be used to teach undergraduates about the terrestrial and global N cycles and microbial functional diversity. Thus far, students in my Microbial Ecology course have been particularly excited and inspired by case study experimental examples of research used to illustrate fundamental concepts. Their favorites include the experimental evidence that relationships between prairie grasses and fungal symbionts can be beneficial or parasitic depending on the nutrient availability status of the soil and nutrient/energy limitation status of the plant[129] , and the demonstration that motility tradeoffs drive niche differentiation in planktonic bacteria[130]. I do use some of my own work in the class, to show the links between plant primary production, soil microbial activity and growth, and organic matter accumulation[131]; however, the students would benefit from locally-relevant examples of evidence demonstrating the relationships between abiotic and biotic components of an ecosystem and the microbial functions that drive nutrient cycling. Two lectures worth of material (Functional Diversity and N-cycling II: Nitrification and Denitrification), and a homework activity for N-cycling week, will be based around these experiments. In years Y1-Y3, material for this class will be derived from the new field data.

Overall, the project will proceed in a timely manner (Figure 12), should it be funded, with research and educational activities by the PI, graduate student and postdoctoral researcher occurring regularly for the next 5 years as part of the project plan.

Figure 12. Project timeline.

	2020		2021				2022				2023				2024				2025	
	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp
1	xxx				xxx				xxx				xxxx				xxx			
2														xxx	x					
3	xxx				xxx				xxx				xxxx	xxx	x		xxx			
4		xxx	xxx			xxx	xxx			xxx	xxx			xxx	xxxx	xxx		xxx	xxx	
5			xx	xx	xx	xx	xxx	xxx	xxx	xxx	xx	xx	xx	xxx	xxx	xxx	xxx	xxx	xxxx	xxx
a	xx	x			xx	x			xx	x			xx	x			xx	x		
b		xxx	x							xxx	x							xxx	x	
c			x	xxx			x	xxx			x	xxx			x	xxx			x	xxx
d			xx	xx			xx	xx			xx	xx			xx	xx			xx	x
G	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx										
PD												xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	
UG	xx	x	x	x	xx	x	x	x	xx	x	x	x	xx	x	x	x	xx	x		
Tech	x	xxx	xx		x	xxx	xx		x	xxx	xx		x	xxx	xx		x	xxx	xx	

Summer: June-July-Aug; **F**all: Sept-Oct-Nov; **W**inter: Dec-Jan-Feb; **Sp**ring: Mar-Apr-May

1: Field soil collection (*P1, 2*), 2: Lab incubations (*P3*), 3: NP & DNP assays, 4: EEA assays & MCC library prep, 5: Data analysis & manuscript prep; a: citizen science activities, b: grad course, c: undergrad course, d: high school visits; G: grad student, PD: postdoc, UG: undergrad(s), Tech: technician

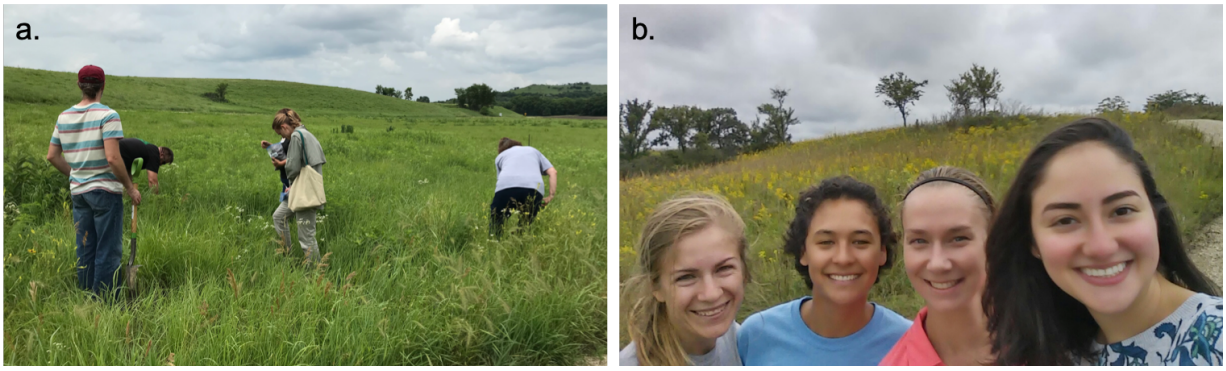
Broader Impacts

A large portion of this CAREER proposal is dedicated to Broader Impacts activities. This project will train a postdoctoral scholar, a graduate student, and several undergraduate students in the process of scientific inquiry, specifically in ecosystems science and the interdisciplinary field of microbial ecology. Further significant outreach and education will also take place in these ways:

High school education activities are a relatively smaller, but important, part of the proposed project. I have learned, through a workshop with the four intelligent and motivated teachers supporting this grant (Figure 13), that their curricular needs include opportunities to work with real data and ways to demonstrate the importance of science to locally relevant topics that students can connect with. Being able to cite a scientist from personal experience, or even meet one in person, can really help the lesson hit home. Graduate students and postdocs in particular are younger, accessible role models that can help high schoolers realize that scientists are real, and that scientific knowledge and methods will be useful skills in the students' future. The teachers I'm partnering with already teach the fundamentals; our part will be to provide digestible activities and datasets (Data Nuggets) addressing relevant topics for inclusion in lesson plans, and to visit regularly for positive reinforcement.

Undergraduate education activities are rolled into my ongoing teaching plan for the next five years. Starting in Y1 of this project, two of my scheduled lectures in Microbial Ecology (BIOL 687) each Spring semester will center around data analysis and interpretation on functional diversity and N-cycling functions, as described earlier. This course is required for Microbiology majors, but has had enrollment from 13 majors in 11 departments across 4 colleges at KSU. Most of these students will go on to work in the health sciences, and this course is their only thorough exposure to the discipline of ecology and the reality that most microorganisms are not pathogens. Further, of the 107 students taking this course over the past 3 years, ~20% were non-white, reflecting the growing Hispanic population in the state of Kansas and the USA. This course is a key source of information about the natural world to students who will be important contributing members of society for decades into the future, even if they don't become ecologists.

Figure 13. Pictures of (a) high school teachers deploying soil respiration chambers for the "Soil is Alive" lesson and (b) graduate course (BIOL 890) students in the field to collect soil for DNA extraction and library prep.



The *graduate education* portion of this proposal is already part of my regular teaching activities, as data collection by my Microbial Diversity (BIOL 890) graduate-level course. This alternating Fall course has had robust enrollment of 8-10 students from 4 departments in 3 colleges. As described earlier, this course provides graduate students with the knowledge and hands-on experience to critically interpret primary research papers that incorporate microbial diversity and functional data. Student participation in creating a novel publishable dataset, and directly adding to knowledge via this larger research project, is also a valuable part of their education and research career trajectories.

Finally, the predominance of private lands in the Flint Hills region presents an obvious outreach and *citizen science education* opportunity. It is essential for my career development, and for the impact of

this research and of science in general, to communicate and translate the process of scientific inquiry, and pertinent research findings, to the folks who live on the tallgrass prairie. Through conversations in preparation for this proposal, it is clear that governmental and private land managers are very interested in learning the same things as our academic research team, they just use different language to discuss and frame these questions. For example, there is an awareness among landowners that prairie plants are "conservative" of nutrients; this is analogous to the ecosystem concept of nutrient retention. I think that ecologists can connect with some of these folks, if they try. I think that there is strong potential that public education in this relatively targeted area can be quite successful. Many people have seemed tickled that I'm interested in teaching and learning from them at all – and answering the insightful and challenging questions they often ask can only improve my communication and teaching skills for audiences of any composition.

These broader impacts activities comprise an important effort, small but hopefully meaningful, towards increasing public awareness of ecology and biogeochemistry, and improving scientific literacy in general, now and into the future. My goal is for activities like this to become part of my professional identity, and CAREER funding would help fully realize that goal.

Results from Prior NSF Support

Zeglin is a co-Investigator on two ongoing large multi-institutional NSF grants, co-PI of one ongoing NSF grant, and lead PI of one NSF grant currently in no-cost extension.

DEB-ES-1822960: RAPID: Are Biogeochemical Responses Linked to the Microbial Composition of a Defined Nutrient and Microbial Input to a Large River? PI Zeglin, co-PI Burgin; $200,000; 03/01/2018- 02/28/2020. In one project year, this funding helped support 2 graduate students and one part-time technician, led to 1 completed MS thesis (M. Kelly), 1 invited[132] and 4 contributed conference talks, 2 conference posters, 3 manuscripts in preparation, and ongoing interaction with local stakeholders and agencies[133] on the impacts of a fertilizer waste release into the Kansas River. Main lesson: A taxonomically and functionally distinct microbial community and a high load of N did enter the river, but few novel microbial taxa seemed to thrive, so the significant increases in N uptake and removal were likely supported by resident biota.

EAR-GG-1753436: Collaborative Research: Biogeochemical Drivers of Interspecies Electron Transfer from Iron Reducers to Methanogens. PI Kirk, co-PIs Zeglin and Jin, $196,330; 08/01/2018-07/31/2021. In year 1, we recruited 1 graduate and 2 undergraduate students, produced 1 published manuscript[134] and 1 conference talk[135], and participated in GROW and LSAMP summer programs. The proposed laboratory iron reducer plus methanogen competition vs. cooperation experiments are underway, data acquisition and analysis is proceeding, and we anticipate more products and continued student and outreach engagement in years 2 and 3.

OIA-EPSCoR-1656006: RII Track-1: Microbiomes of Aquatic, Plant and Soil Systems (MAPS) Mediating Sustainability: An Observational and Experimental Network Across Kansas. PI Bowman-James; 30 co-Investigators including Zeglin. $11,826,478; 09/01/2017- 08/31/2022. To date, Zeglin's lab has a part-time technician, graduate student partial support, and undergraduates on this grant, and has produced 3 conference talks and 3 posters. Important associated outreach activities for Zeglin have included authoring a Fact Sheet[136], giving a public seminar at KPBS, and participation in a workshop with high school teachers.

DEB-LTER-1440484: Konza Prairie LTER VII: Long-Term Research on Grassland Dynamics: Assessing Mechanisms of Sensitivity and Resilience to Global Change. PI Nippert; 11 co-Investigators including Zeglin. $5,684,998; 11/01/2014- 10/31/2020. Zeglin and her students, with the help of some summer salary and data analysis costs covered by KNZ LTER, have produced 2 published manuscripts[103, 114], 1 in review[137] and 1 in preparation, 1 completed MS thesis (C. Carson), 6 conference talks and 15 posters related directly to this grant.

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134. Marquart, K. A., B. R. Haller, J. M. Paper, T. M. Flynn, M. I. Boyanov, G. Shodunke, C. Gura, Q. Jin, and M. F. Kirk. 2019. Influence of pH on the balance between methanogenesis and iron reduction. Geobiology **17**:185-198.
135. Kirk, M. F., Q. Jin, T. M. Flynn, and L. H. Zeglin. 2019. PH dependent interactions between iron reduction and methanogenesis suggest a new model for methanogenesis.*in* Geological Society of America Annual Meeting, Indianapolis, IN.
136. Zeglin, L. H., J. Haukos, M. F. Kirk, and J. Allenbrand. 2018. Microbes of the Prairie Fact Sheet. Konza Environmental Education Program (KEEP).
137. Veach, A., and L. H. Zeglin. 2018. Historical drought affects prevalence of drought sensitive, resistant and resilient soil microbial populations. Microbial Ecology **in review**.

Biographical Sketch July 2019

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a. Professional Preparation.

University of Wisconsin	Madison, WI	Zoology	BS, 2002
University of Wisconsin	Madison, WI	Biological Aspects of Conservation	BS, 2002
University of Wisconsin	Madison, WI	Environmental Studies	Certificate, 2002
University of New Mexico	Albuquerque, NM	Biology	PhD, 2008
Oregon State University	Corvallis, OR	Soil Science	Postdoctoral, 2013
USGS Alaska Science Center	Anchorage, AK	Molecular Ecology	Postdoctoral, 2014

b. Appointments.

2014-present Assistant Professor, Division of Biology, Kansas State University
2013-2014 Research Ecologist (Mendenhall Postdoctoral Fellow), Alaska Science Center, U.S. Geological Survey
2008-2013 Faculty Research Associate, Department of Soil Science, Oregon State University
2005-2008 NSF Graduate Research Fellow, Department of Biology, U. of New Mexico
2002-2005 Freshwater Sciences IGERT Trainee and Teaching Assistant (Microbiology), Department of Biology, University of New Mexico
1999-2002 Student ranger and naturalist, University of Wisconsin Arboretum
1999-2002 Student assistant and intern, Bureau of Endangered Resources, Wisconsin Department of Natural Resources and Natural Heritage Inventory
Summer 2001 Undergraduate field research assistant, North Temperate Lakes Field Station, University of Wisconsin Center for Limnology

c. Products

i. (5 most closely related of 35 peer-reviewed publications).

C. M. Carson, A. Jumpponen, J. M. Blair and **L. H. Zeglin.** 2019. Soil fungal community changes in response to long-term fire cessation and N fertilization in tallgrass prairie. Fungal Ecology, 41: 45-55.

C. M. Carson, **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.

L. H. Zeglin, B. Wang, F. Rainey, C. Waythomas, 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(1): 146-158.

A. E. Taylor, **L. H. Zeglin**, T. Wanzek, D. D. Myrold and P. J. Bottomley. 2012. Dynamics of ammonia-oxidizing archaea and bacteria populations and contributions to soil nitrification potentials. ISME Journal, 6: 2024-2032.

L. H. Zeglin, A. E. Taylor, D. D. Myrold and P. J. Bottomley. 2011. Bacterial and archaeal amoA gene distribution covaries with soil nitrification properties across a range of land uses. Environmental Microbiology Reports, 3(6): 717-726.

ii. (5 other significant products).

L. H. Zeglin. 2015. Stream microbial diversity responds to environmental changes: Review and synthesis of existing research. Frontiers in Microbiology, 6: 454.

D. D. Myrold, **L. H. Zeglin** and J. K. Jansson. 2014. The potential of metagenomic approaches for understanding soil microbial processes. Soil Science Society of America Journal, 78: 3-10.

L. H. Zeglin, P. J. Bottomley, A. Jumpponen, C. Rice, M. Arango, A. Lindsley, A. McGowan, P. Mfombep and D. D. Myrold. 2013. Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. Ecology, 94(10): 2334-2345.

R. L. Sinsabaugh, C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. L. Crenshaw, A. R. Contosta, D. Cusack, S. Frey, M. E. Gallo, T. B. Gartner, S. E. Hobbie, K. Holland, B. L. Keeler, J. S. Powers, M. Stursova, C. Takacs-Vesbach, M. P. Waldrop, M. D. Wallenstein, D. R. Zak and **L. H. Zeglin.** 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters, 11 (11): 1252-1264.

L. H. Zeglin, M. Stursova, R. L. Sinsabaugh and S. L. Collins. 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. Oecologia, 154 (2): 349-359.

d. Synergistic activities.

1. Soil Ecology Section Secretary, Ecological Society of America (2017 – present).
2. Scientific Steering Committee, Biogeochemistry group co-leader: Konza Prairie Long-Term Ecological Research (LTER) Program VII (2014 – present). Responsibilities include leading discussion to guide future integrated research directions and bring together researchers from diverse backgrounds around key shared interests and goals.
3. Member/Participant: National Ecological Observatory Network (NEON) Observational Sampling Strategy Technical Working Group (2017 – present), Microbial Working Group (2011 – 2017), NEON Steam Experiment Working Group (2008 – 2015). Responsibilities include participating in discussions and contributing feedback as requested by NEON.
4. Mentor: Research mentor for one postdoctoral scholar (0 current) and 6 graduate students at KSU (4 current), and undergraduate students at UNM (3 undergraduates including one co-author), OSU (3 undergraduates including one co-author), USGS (2 undergraduates) and KSU (12 undergraduates including 4 REUs). Of the 6 graduate students at KSU, 4 are women and 2 are underrepresented minorities. Of the 20 undergraduate students, 9 are women (7 at KSU) and 3 are underrepresented minorities (2 at KSU).
5. Outreach: Konza Prairie Biological Station (KPBS) Advisory Committee Member, 2019-present; KBPS Fire Crew volunteer, 2015-present; KS-NSF-EPSCoR High School Teachers Summer Institute, 17-21 June 2019, "Soil and water" group leader; Rock Creek High School Girls in STEM Camp, 4 May 2019, "Disturbance and biological interactions maintain tallgrass prairie"; Microbes of the Prairie Public Seminar, 2 April 2019, KPBS; *Microbes of the Prairie Fact Sheet*, 2018, distributed by the Konza Environmental Education Docent Program at KPBS; KPBS tour guide for groups including Grassland Restoration National Meeting, The Nature Conservancy International Prairie Celebration, National Soil Judging Competition; Alaska Maritime Fish & Wildlife Refuge (AMNWR) "Wednesday Walkabout" fieldwork interpretation with local kids in Adak, AK, August 2017; Field research in local newspaper: The Adak Eagle's Call September 2015 Volume 4 Issue 9, p. 3; AMNWR R/V Tiglâx open house science interpretation, summer 2015 (Adak, AK and Dutch Harbor, AK).