

PROJECT SUMMARY

Overview:

Microbiome science is an emergent discipline recently recognized as a critical pursuit for understanding ecosystem function. The challenge of simultaneously feeding a growing population, sustaining agriculture, maintaining soil quality, and minimizing greenhouse gases and water contaminants requires new data-driven solutions based in a fundamental understanding of the role and dynamics of Microbiomes of Aquatic habitats, Plants, and Soils (MAPS). MAPS mediate disease and productivity of plants, control the quality of water, and moderate edaphic characteristics and greenhouse gas production. We will create an observational and experimental network across the strong precipitation gradient in agriculturally-dominated Kansas, using both agricultural and native sites. The project extends traditional scientific approaches to work at scales ranging from genes to ecosystems, and across habitats (terrestrial to aquatic). Our vision is to use fundamental microbiome research to illuminate how MAPS can be invoked to enhance productivity, mitigate environmental problems in agriculturally-dominated landscapes, and conserve native grasslands and their ecosystem functions. Our goals are to: 1) elucidate MAPS-mediated ecosystem functions useful for predicting ecosystem responses to a variable and changing precipitation regime, 2) develop best practices for promoting MAPS for desired ecosystem services (e.g. plant productivity, soil and water quality), and 3) use MAPS research to enrich educational and outreach opportunities. We have assembled a diverse team of investigators (across institutions, areas of expertise, and career stages) and will supplement it with three faculty hires. This project is based on the generation of a nuanced, mechanistic understanding of the drivers of MAPS structure and function. We specifically aim to quantify how climate and land-use legacies govern MAPS as a means of predicting the resistance and resilience of multiple ecosystem properties to long-term (e.g. contemporary climate change) and punctuated (e.g. extreme climate events, land use changes) perturbations.

Intellectual Merit :

We will create a synergistic interdisciplinary research network with common interests in the role of microbiomes and the combined expertise to tackle these challenging, vitally important issues. We will create databases to link metagenomic data to environmental parameters that will allow us to realize our vision of using MAPS to address productivity and environmental issues in agriculturally-dominated landscapes while conserving native grasslands. While previous efforts have focused on plant, soil and aquatic microbiomes individually, our novel, integrated investigation across these natural biomes can inform best management practices to curb terrestrial resource losses and maximize agricultural productivity, while controlling pollutants. We do this by coupling coordinated sampling and experimental manipulations of terrestrial and aquatic environments across Kansas' precipitation and land-use gradients. These results will be important in Kansas and beyond, informing basic research relevant to myriad agricultural landscapes globally.

Broader Impacts :

The broader impacts of this project include the contributions of the findings as part of a broader societal goal of realizing sustainable food production while protecting soil, water, and human health. MAPS science underpins the function and supply of freshwater ecosystem services that are so crucial to Kansas' water needs. We have developed an integrated group of educational and outreach programs that focus on hypotheses, approaches, and findings that are foundational to our disciplines, and also emphasize the sense of excitement and relevance inherent in scientific problem-solving. Our programs will reach individuals of all ages and levels: K-12 students and teachers; undergraduate and graduate students; faculty members at tribal colleges and four year institutions; and adults interested in broadening their knowledge. These initiatives will not be limited to the classroom, but will fully utilize field stations at the University of Kansas, Kansas State University, and Wichita State University, as well as other venues for outreach. Haskell Indian Nations University (HINU) is an integral part of this project. The proposal supports undergraduate programs at HINU, as well as underrepresented minority students and community colleges across Kansas.

4. Project Description

4.1 Status and Overview

The Challenge. Microbiome science is essential to understanding the Earth's ecosystems and, as such, has been identified as a national priority (Graham et al. 2016). The challenge of simultaneously feeding a growing population, sustaining agriculture, maintaining soil quality, and minimizing greenhouse gases and water contaminants requires new data-driven solutions based in a fundamental understanding of the role and dynamics of Microbiomes of Aquatic habitats, Plants, and Soils (MAPS) (White House OSTP 2016). MAPS mediate disease and productivity of plants, control the quality of water, and moderate greenhouse gas production (Wall and Six 2015, Wall et al. 2015). Significantly, MAPS hold the key to assessing and predicting the effects of global environmental change and mitigating ecosystem degradation (Amundson et al. 2015, Karlen and Rice 2015, Blaser et al. 2016, Graham et al. 2016).

This project transcends traditional microbiological and disciplinary boundaries by harnessing cutting-edge research tools and coupling technologies to rigorous field and experimental environmental approaches while working from genes to ecosystems. The resultant discoveries and enabling technologies will provide critical knowledge to enable scientists, engineers, and policy makers to develop approaches to sustain food production systems while preserving biodiversity and ecosystem services (Blaser et al. 2016). The challenges associated with MAPS are relevant to Kansas, the nation, and the planet. Our research spans five of the eight Grand Challenges outlined by the US National Academies of Science: Biogeochemical Cycles, Biodiversity and Ecosystem Functioning, Climate Variability, Hydrologic Forecasting, and Land Use Dynamics (National Research Council 2001).

Vision, goals, core concepts and implementation mechanisms. Our *vision* is to use fundamental research on MAPS to enhance ecosystem productivity, mitigate environmental degradation in agriculturally-dominated landscapes, and conserve native grasslands. To achieve this vision, our *goals* are to: **1)** elucidate MAPS-mediated ecosystem functions useful for predicting ecosystem responses to a variable and changing precipitation regime; **2)** develop best practices for promoting MAPS for desired ecosystem services (e.g. plant productivity, soil and water quality); and **3)** use our MAPS research to enrich educational and outreach opportunities.

We will merge perspectives of multiple environmental disciplines and powerful genomic approaches to explore how MAPS regulate the environment and are affected by global change. We will explore two *core concepts* (see Section 4.3, for more nuanced hypotheses and sub-hypotheses):

1. *Microbiome structure and function are key drivers of ecosystem responses to climate change and anthropogenic land use.*
2. *Microbiomes exhibit legacies, feedbacks and thresholds such that their response to perturbations depends on land use and climate history.*

Kansas is dominated by agricultural land uses overlaid by a precipitation gradient broadly representative of both current and future precipitation regimes (Fig. 1). It is in these landscapes that society must grapple with food production while sustaining broader environmental health. Thus, Kansas is an ideal living laboratory to explore our core concepts and build future research capacity to improve academic research competitiveness in microbial ecology and environmental science through the following *implementation mechanisms*: 1) *Conduct observational studies and experiments across precipitation and land use gradients to reveal the driving forces governing water quality, soil organic matter and nutrient retention and release, and plant productivity.* This will include a coordinated network of plant, soil and water quality measurements linked to an experimental manipulative mesocosm approach. 2) *Create a base data set on microbiomes of Kansas and their linkages to environmental data that will be used for this RII and future projects.* 3) *Coalesce a nascent team of investigators at diverse career stages, and new faculty hires, across multiple Kansas universities.* We will develop a network of observatories, databases, and studies; our results will extend to other agriculture-intensive areas, both nationally and internationally. This will form a strong collaborative group across universities removing a common barrier to success (see Barriers and Solutions Section).

Our work will produce deliverables in four areas: 1) publicly-available databases describing MAPS and associated environmental variables across the precipitation and land use gradients of Kansas that are directly relevant for understanding how microbiome composition and function vary across space and time; 2) scientific documentation (publications and presentations) of MAPS' capacity to govern ecosystem productivity, soil resource retention (water, nutrients and carbon) and water quality; 3) applied-on-the-ground information about Kansan agroeco-systems, relevant around the world in areas of agricultural dominance, describing how MAPS can be used to promote desired ecosystem services (publications and presentations at local to international venues); 4) enhance teaching and learning capacity at multiple institutions, including those serving underrepresented groups in science by providing support for internships, education programs, and a faculty hire at Haskell Indian Nations University (HINU).

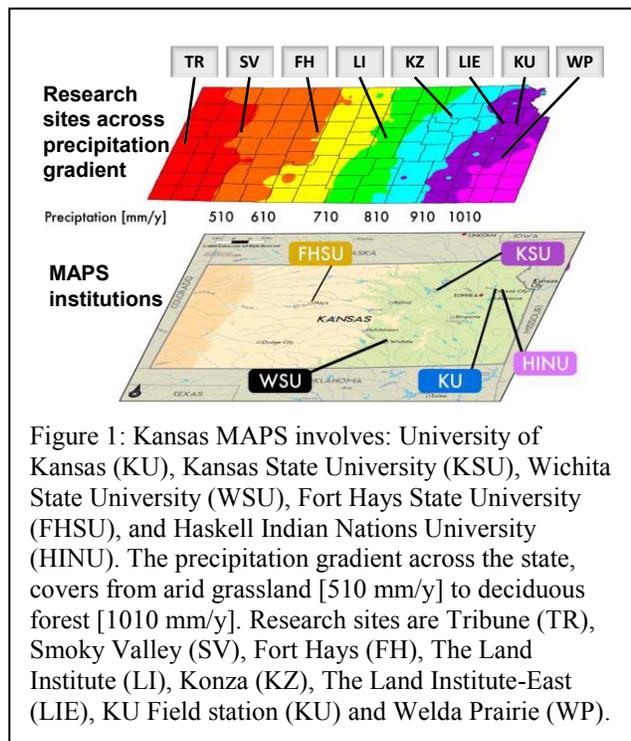


Figure 1: Kansas MAPS involves: University of Kansas (KU), Kansas State University (KSU), Wichita State University (WSU), Fort Hays State University (FHSU), and Haskell Indian Nations University (HINU). The precipitation gradient across the state, covers from arid grassland [510 mm/y] to deciduous forest [1010 mm/y]. Research sites are Tribune (TR), Smoky Valley (SV), Fort Hays (FH), The Land Institute (LI), Konza (KZ), The Land Institute-East (LIE), KU Field station (KU) and Welda Prairie (WP).

Opportunities: Microbial ecology is entering an exciting and productive epoch as powerful new tools are able to identify functional genes and gene expression, promoting a convergence among disparate disciplines towards recognizing microbiomes as functionally significant units across many scales (National Academies of Sciences, Engineering, and Medicine 2015). This view has gained considerable traction within the health-sciences: the positive health benefits of a healthy gut microbiome are just beginning to be understood. The critical role of microbiomes promoting plant production and other services opens the possibility that microbiomes could be managed for environmental benefits in an analogous way as "probiotics" may aid human health. Multiple recent initiatives underscore the relevance of MAPS: 1) the White House announcement of the National Microbiome Initiative which aims to advance microbiome science for our national interests; 2) the international Unified Microbiome Initiative (UMI) seeks to understand the role of microbiomes in all Earth systems; and 3) plant pathologists have created the Phytobiomes Roadmap to increase understanding of interactions among microbes and plants. Our research team will substantially advance understanding of microbiomes and their environmental importance by using the strong precipitation gradient across Kansas as a natural observational network (Fig. 1). We will survey MAPS and associated chemical, biological and physical characteristics at representative locations across Kansas and perform experiments to provide mechanistic understanding to explain observational patterns. We will also integrate educational experiences for K-12 to graduate students into the microbial ecology research activities.

Strengths: The team as human infrastructure. As Kansas NSF EPSCoR (KNE) Project Director, *Kristin Bowman-James* will oversee the project. She is a University Distinguished Professor of Chemistry at the University of Kansas (KU) and has over 10 years of experience leading and facilitating successful large EPSCoR projects. Team members come from a diverse set of institutions including KU, Kansas State University (KSU), Wichita State University (WSU), Fort Hays State University (FHSU), and Haskell Indian Nations University (HINU). Participants are from a spectrum of career stages and disciplines, including terrestrial plant-microbe symbioses, terrestrial and aquatic biogeochemistry, ecohydrology, soil science and education. The MAPS science leads are senior faculty members in the field (*Walter Dodds*, KSU, water quality; *Charles Rice*, KSU, soil carbon and microbial ecology; *James Bever*, KU, plant microbiome interactions; *Sharon Billings*, KU, soil biogeochemistry and microbial ecology). Core team

members have significant expertise working with microbiomes via molecular approaches (*Jumpponen, Zeglin, Whitfield, and Kirk* - KSU; *Bever, Billings, Sikes, and Sturm* - KU). We also have expertise in plant ecology (*Alexander* - KU, *Houseman* – WSU; *Greer* – FHSU; *Nippert* - KSU), water quality and biogeochemistry (*Burgin, Loecke, and Sullivan* – KU; *Dodds* - KSU), microbial bioinformatics (*Liu* - KSU), and theoretical ecology (*Platt* – KSU; *Agusto* and *Reuman* - KU).

We will support an emerging research consortium of theoretical and empirical scientists working on bioinformatics, microbiology, ecosystem, and community ecology. Within the last five years, several new faculty members, who will participate in this project, have been hired. These include *Platt, Zeglin, Liu, Kirk* – KSU; *Reuman, Agusto, Bever, Sikes, Schultz, Sullivan, Burgin, Loecke* – KU; *Greer* - FHSU. This proposal will establish a new collaborative network in the state with a MAPS focus and provide excellent prospects for future success. We will leverage this new expertise using our traditional strengths in grassland and agroecology, plant disease, biodiversity, and ecosystem sciences, and our growing skills in metagenomics (KU Metagenomics Lab, KSU Ecogenomics Center). Faculty hires will be supported to address expertise gaps: 1) bioinformatics faculty at KU and KSU to link large genomic datasets with project objectives and 2) a new environmental sciences faculty member at HINU.

Statewide relevance prepares us for success. MAPS science is also well-aligned with State interests and needs. The Regents' Universities State S&T Plan (*Kansas: Building an Environment of Science and Technology for Innovation*, 2012) clearly identifies agriculture as a “primary driver of its technology economy.” A follow-up, 50-year vision report focusing on Kansas' water needs (*A Long-term Vision for the Future of Water Supply in Kansas*, 2015) serves to emphasize the need for research in areas such as MAPS. The plan preface states, “*Water is a finite resource and without further planning and action we will no longer be able to meet our state's current needs, let alone growth.*” As we will demonstrate, MAPS science underpins the function and supply of freshwater ecosystem services.

Research profile and links with private sector. The three flagship universities in Kansas (KU, KSU, WSU) participate in this proposal, in addition to FHSU and HINU. KU has an enrollment of 28,100 students, KSU has an enrollment of 24,100 students, and WSU has an enrollment of 15,000 students. FHSU had a Spring 2016 enrollment of 12,338 students, and HINU, a Tribal College in Lawrence, Kansas, has an enrollment of 1,000. Kansas ranks 37th in the country in terms of NSF funding. In 2015, NSF awards to Kansas totaled \$33.7 M, or 0.52% of the NSF Research and Related Activities budget.

Established university links to the private sector through partnerships include the Manhattan-KSU Innovation Center, the KSU Institute for Commercialization, the KU Bioscience & Technology Business Center, and WSU's Innovation Campus. KU Cancer Center is now an NIH NCI-designated cancer center, and Manhattan is the future home of the National Bio- and Agro-Defense Facility (NBAF), operated by the Department of Homeland Security (DHS) and the US Department of Agriculture (USDA).

Barriers and solutions: Research universities in Kansas face serious issues: rising start-up costs; multi-user equipment costs and maintenance; lack of research space and critical masses of researchers in high profile fields; and faculty raids.

MAPS is taking an active role in addressing these barriers. We will create a powerful research network in MAPS. This funding will catalyze a thriving, sustained research effort built upon the foundation of core Kansas facilities including the Center for Metagenomic Microbial Community Analysis, the Genomics Center, and the Kansas Applied Remote Sensing Center at KU; and the Bioinformatics Center, the Integrated Genomics Facility, and the Soil Carbon Center at KSU. The MAPS project invests in these facilities to meet the demand for technical expertise and training in microbial genomics and informatics. Our strategy for solutions includes: 1) building a collaborative group of microbial ecologists; 2) attracting and mentoring new faculty to help establish careers in Kansas; 3) creating an open access database on microbiome sequences linked to environmental patterns across Kansas habitats, which will serve as a foundation for MAPS research as well as future research initiatives; 4) purchasing shared infrastructure equipment; and 5) linking STEM programs to our network of researchers to inform and excite the next generation of scientists and citizens while increasing the diversity and skill set of future researchers.

4.2 Results of Prior NSF Support

KNE has a track-record of building research capacity. Two of its seed projects were funded as NSF Centers: an Engineering Research Center (ERC), the Center for Environmentally Beneficial Catalysis (EEC-0310689); and a Science and Technology Center (STC), the Center for Remote Sensing of Ice Sheets (PLR-0424589); the ERC and STC are the direct result of previous NSF EPSCoR investments in the state. The most recent NSF EPSCoR Track-1 project, spanning multiple disciplines and focused on retention of our natural resources, has laid the foundation for this new project by providing baseline data relating climate influences to agricultural changes. This project and three MAPS-related initiatives, with our lead investigators as PIs, are described in the following paragraphs.

RII Track-1: Phase VI: Climate Change and Energy: Basic Science, Impacts and Mitigation, EPS-0903806, 10/2009-09/2016, \$20,000,000. PI: Bowman-James. Intellectual Merit: The Biofuels and Climate Change initiative developed capabilities linking the natural and social sciences by analyzing the complex relationship between environmental and human systems. This information is necessary as agriculture continues to change due to changes in the biofuel economy and climate. A core group of interdisciplinary researchers, including social scientists, was formed across institutions to advance work on tracking agricultural change in the state and beyond. **Broader Impacts.** Novel outreach, including arts-based poster displays, a non-academic book, and video projects resulted from the project. To date, more than 100 researchers and 250 students have been involved in this RII Track-1 project, and researchers have submitted both single investigator and collaborative proposals resulting in additional federal funding totaling > \$38 million, published more than 241 papers in peer-reviewed journals, and filed 19 patent applications. Other impacts included the highly successful *Pathways to STEM Careers* project in partnership with Haskell Indian Nations University. *Pathways* greatly improved the STEM pipeline transition for Native Americans from Tribal Colleges to graduate programs. Each year applications to the program have steadily increased. To date, 53 students have graduated from the program, and 19 have matriculated to graduate programs.

Preferential Allocation of Photosynthate toward Better Mutualists, Spatial Structure, and the Maintenance of the Arbuscular Mycorrhizal Mutualism, DEB-0919434, 8/2009-7/2013, \$468,486. PI: Bever. Intellectual Merit. We found plant preferential allocation to the most effective mycorrhizal mutualists increased with plant need for the resource being exchanged. Theory built on these results predicts environmental dependence of efficiency of resource transfer from the plant microbiome (Bever 2015). **Broader Impacts.** Four postdocs, six PhD students, three technicians and 20 undergraduates were trained. Six restoration experiments on the Indiana University (IU) campus were created while introducing 320 IU undergraduate non-majors to science; three local ecology summer courses for K-12 science educators were taught; and a two-week workshop was initiated on soil microbial biology for high school minority students. Forty papers have been published.

Emerging Trends in Biogeochemical Cycles: Temperature Sensitivity of Substrate Decomposition from Enzymes to Microbial Communities, NSF-0950095, 8/2010-8/2014, \$595,998. PI: Billings. Intellectual Merit. We assessed the temperature sensitivities of decomposition for individual, well-characterized compounds representative of soil organic matter with isolated microorganisms, at varying pH. This work provided baseline values for intrinsic temperature sensitivities of decay, to which investigators can compare relevant results from soils to parse the influence of microbial adaptive responses vs. enzymatic responses to temperature. We quantified how temperature influenced carbon (C) flows through microbes that, up to that point, had only been inferred. We also demonstrated for the first time how isotopic fractionation between microbial biomass and respired CO₂ varies with temperature in a predictable fashion. **Broader Impacts.** Eleven peer-reviewed manuscripts; 17 presentations at national and international conferences (e.g. American Geophysical Union, European Physical Union, and Ecological Society of America); training of five undergraduates, one Ph.D. student, and three postdocs.

Collaborative Research: Stream Consumers and Lotic Ecosystem Rates (SCALER): Scaling from Centimeters to Continents, EF-1065255, 10/2011-9/2017, \$3,304,097. PI: Dodds. Intellectual Merit.

The overarching question being explored is: *How can small-scale ecological experiments be applied to understand operation of entire ecological systems?* Our overall goals are to understand how we can use centimeter- and reach-scale process measurements and consumer manipulation experiments to predict ecosystem characteristics of stream networks, and how do patterns of scaling compare across an array of North American biomes? To date, we have performed a series of continental-scale SCALER experiments. They encompassed five biomes, each of which had six sites with measurements nested at two scales (microhabitat, reach), linked to watershed models. Synoptic sampling at each site was done to characterize watershed scale patterns. Rates of metabolism and nutrient uptake and responses to consumer exclusions were measured at micro (0.1 m) and reach (100 m) scales. We found a threshold of consumer biomass above which ecosystem effects became evident, but this result was context dependent, both within and among biomes. **Broader Impacts.** There are eight institutions involved in this project. To date, KSU has five published manuscripts and 15 conference presentations at national and international conferences. We trained one postdoc, three graduate students, and three undergraduates.

EPSCoR funded faculty on prior awards and EPSCoR hire retention: Thirteen of the 20 faculty hired from NSF EPSCoR awards remain in Kansas. The number of previous NSF EPSCoR-funded faculty who will receive funding from this award (not including the Project Director or the internal evaluator Steven Maynard-Moody) is 11 out of a total of 26 researchers; six were recipients of small awards to provide start-up grants to new Kansas faculty hires (First Awards).

4.3 Research and Education Program

We propose to: **1)** elucidate MAPS-mediated ecosystem functions useful for predicting ecosystem responses to a variable and changing precipitation regime, and **2)** provide information to develop best practices for promoting MAPS for desired ecosystem services (e.g. plant productivity, retention of soil resources, water quality), while providing educational experiences for participants ranging from K-12 to post-docs. First we describe the research background and experimental plans, then our implementation of, and research links with, the education program.

A new scientific revolution is emerging which views plants and animals as inseparable from their microbial symbionts. Microbiomes are communities of microorganisms that are intimately associated with all organisms and habitats on Earth. Microbiomes determine the environmental health and influence the behavior of diverse ecosystems, including effects on human health, climate change, and food security. The Research and Education Program we define here invokes this view, capturing connectivity and non-linear dynamics between and among three microbiomes (Fig. 2): the plant microbiome (PMB), comprised of the phyllosphere (aboveground plant tissues) and the rhizosphere (surrounding plant roots) (Turner et al. 2013); the soil microbiome (SMB), intimately connected to that of the rhizosphere (Dijkstra and Cheng 2007) and linking rhizosphere, topsoil, vadose zone, and permanently saturated groundwater zones via transport (Sposito 2013); and aquatic microbiomes (AMB) of streams and lakes which receive water and materials from the surrounding landscape (Dodds and Whiles 2010).

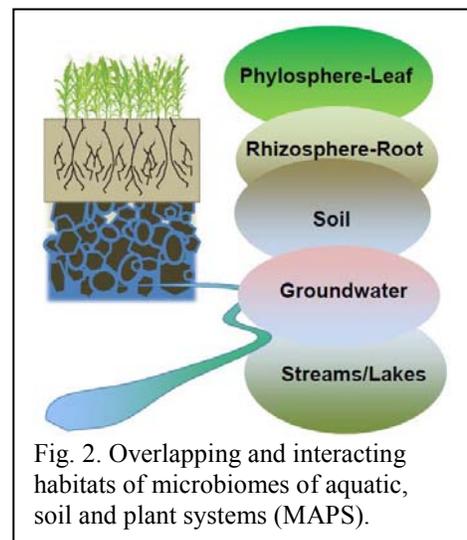


Fig. 2. Overlapping and interacting habitats of microbiomes of aquatic, soil and plant systems (MAPS).

We propose to explore the core concepts (defined in Section 4.1) by developing and testing multiple working hypotheses (detailed below). Implicit in our conceptual framework (Fig. 3) is the idea that microbiomes, like other biological systems, can vary in structure over time and space. The changes in these microbiomes can have important functional consequences. Responses to human perturbations can

be challenging to predict because their behavior can reflect antecedent conditions as well as thresholds and non-linear dynamics. Detailed understanding of MAPS is thus essential to allow prediction of their responses to environmental changes. These provocative ideas have been a source of rich intellectual discussion in the recent past and represent rapidly co-evolving research realms in ecology (Sheffer et al. 2009, Dodds et al. 2010) and microbial ecology (Martiny et al. 2015). Our approach (Fig. 3) is based on the fact that the microbiome of each habitat from the plant to the groundwater acts as a mediator of material transformation and transport as it moves through sub-systems. Cumulative microbiome activities interact and may feed back to maintain or change component response variables. The

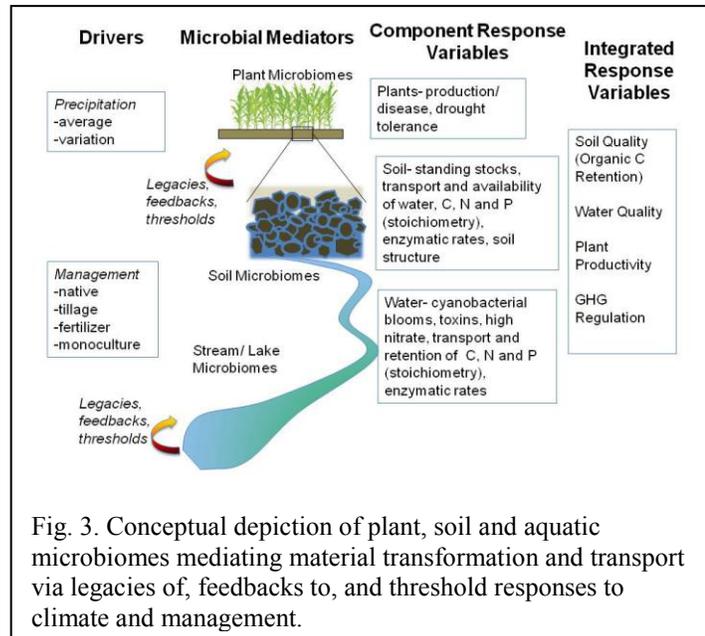


Fig. 3. Conceptual depiction of plant, soil and aquatic microbiomes mediating material transformation and transport via legacies of, feedbacks to, and threshold responses to climate and management.

The component response variables result in integrated response variables, which describe the ecosystem services ultimately mediated by MAPS. Using this approach, we will generate projections of how MAPS-mediated plant productivity, soil health, and water quality will change across the region's precipitation gradient, as well as make recommendations for best management practices to curb plant-soil resource losses, thus maximizing productivity while limiting impairment of water resources.

Guiding Hypotheses and Background

To achieve our goals, we will quantify how climate and land use legacies govern MAPS composition and function as a means of predicting the response of multiple ecosystem properties to long-term (e.g., contemporary climate change) and punctuated (e.g., extreme climate events, land use changes) perturbations. Understanding MAPS is foundational to predicting and mitigating many environmental problems associated with intensive land use and climate disruption. Plant and soil microbiomes moderate terrestrial productivity (e.g., agricultural yields) through soil resource access (e.g., nitrogen, N and phosphorus, P) as well as other mechanisms (Bender et al. 2016). The plant-soil microbiome functions are strongly influenced by external drivers (e.g. climate change) and by land management. Furthermore, land management and climate, together with MAPS activities, control nutrient runoff and emergent water quality (Banner et. al. 2009, Mulholland et al. 2008, Battin et al. 2016). In spite of this knowledge of how MAPS influence ecosystem functions, it remains unclear how plant, soil and aquatic microbiomes and associated ecosystem functions respond to perturbations; both empiricists and theoreticians struggle to understand the drivers of microbiome resistance, resilience, or long-term susceptibility to change (Shade et al. 2012, Guénon and Gros 2013, Hawkes and Keitt 2015, Raaijmakers and Mazzola 2016). Moreover, many specific questions about the relative sensitivity and resilience of plant, soil and aquatic microbiomes to precipitation and land use perturbation effects on ecosystem functions remain. Our hypotheses are unique in that they integrate across the three microbiomes (as illustrated in Fig. 4). Two guiding hypotheses addressing the differential sensitivity of and connectivity among the three microbiomes are:

1. Given the strong regulation of microbiomes by water availability, plant and near surface soil microbiomes will be most sensitive to precipitation changes, whereas deeper soil will be intermediate in sensitivity and the aquatic microbiome least sensitive due to more persistent water connections.
2. Connectivity between plant-soil-aquatic systems is reduced as precipitation decreases, particularly between terrestrial and aquatic microbiomes, and thus management

approaches will more directly link among microbiomes when and where precipitation is greater.

We test these and other cross-microbiome ideas, and specific mechanistic hypotheses within plant, soil and aquatic microbiomes (described in their respective research sections below, and represented by the arrows in Fig. 4), with integrative experiments and observational approaches.

First, we present coordinated efforts across the project where we directly link all three microbiomes – plant, soil, and aquatic (PMB, SMB, AMB) – with a regional-scale observational network exploiting Kansas as a natural laboratory of precipitation and land use gradients. This work will provide the basis for

a living growing database of microbiomes found in diverse habitats across Kansas, to be preserved, managed, and expanded for contemporary and future research efforts. **Second**, we will implement habitat-specific experiments to test mechanisms relevant to focus areas (e.g. mechanisms of plant disease (PMB), or factors influencing toxic cyanobacterial blooms (AMB), recognizing and emphasizing the connections and potential overlaps amongst those habitats. **Third**, we will test linked conceptual models (Fig. 4), which will guide statistical structural equation modeling and allow for testing individual model components and generation of alternative hypotheses if our current working model is not supported statistically. Our data will also be used to inform theoretical models of pathogens and mutualists in the plant microbiome, nitrogen (N)- and carbon (C)-transformations in soil and aquatic sediments, and algal dynamics in reservoirs. The knowledge gaps discussed below reveal the basis for and importance of the integrative and specific mechanistic hypotheses.

Ecological concepts, microbial dynamics, biogeography, and historical legacies

Some ecosystems can resist changes driven by perturbations (resistance) or exhibit resilience and recover quickly from perturbations (Harrison 1979). State variables (measured ecosystem properties; in this proposal microbial community structure and activity) are used to assess ecosystem status. A change in state variables moves the system to an alternate state. Alternate states can be stable, or if the system is resilient, they can be transient (Walker et al. 2004). Rising variance sometimes can indicate if a system is about to change to a new state (Carpenter and Brock 2006, Scheffer et al. 2009). Cumulative temporal effects MAPS state variables can result in environmental legacies that can be rapidly disrupted by human activities, leading to alternate states and potentially compromising valuable ecosystem services.

We highlight terrestrial and aquatic examples to illustrate MAPS-mediated ecosystem resistance, resilience, and alternate states. Native plant productivity and microbial activities of grassland soils over centuries have produced fertile soils across much of North America’s Midwest and accrued considerable organic C under variable climate regimes. In contrast, agricultural soils tend to have lower soil C as well as a less diverse and significantly modified microbial community relative to native grassland soils; following tillage, soils move to an alternative state relatively rapidly (Fierer et al. 2013). Recovery of soil C is slow when grassland plants are re-introduced, suggesting that legacies of abiotic factors and, potentially, of microbiomes are important drivers influencing the maintenance of an alternate state or resilience (assessed here as recovery of soil C). In lakes, eutrophication leads to alternate states, wherein increased P is connected to intensive cyanobacterial (harmful algal) blooms. As algae die and sink to the

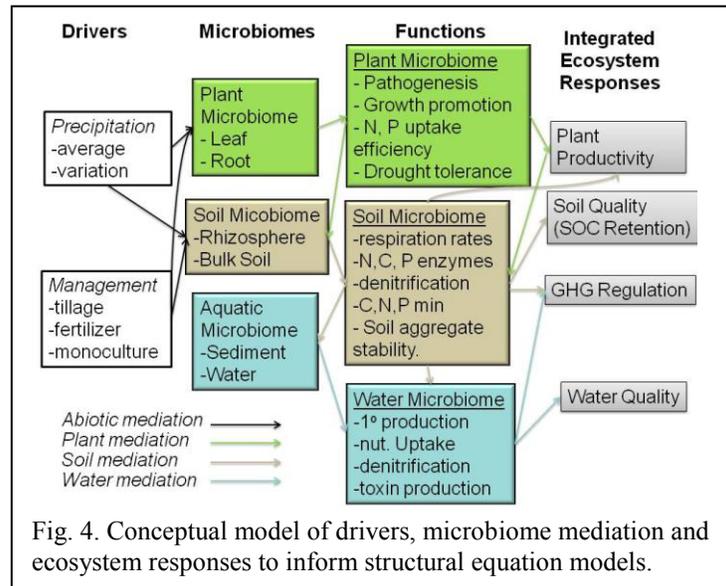


Fig. 4. Conceptual model of drivers, microbiome mediation and ecosystem responses to inform structural equation models.

bottom of the lake, lake microbes consume O₂. Once bottom water becomes anoxic, phosphate bonding to iron is less prevalent, and phosphate is re-mixed into the water column. This feedback further fertilizes surface algae and exacerbates the problem. This positive feedback loop makes it extremely difficult for the lake to return to its original state (Dodds and Whiles 2010). In these two examples, the alternate states are driven by physical as well as microbiological processes and, at present, the extent to which the microbiome is a driver or passenger in the ecosystem dynamic is unknown.

Controversial hypotheses have emerged depicting potential mechanisms driving microbiome structural and functional responses to perturbation. A concept favored by some microbial ecologists is “everything is everywhere, but the environment selects,” because many microbes can disperse widely, so the local environment will determine the local microbial composition. In this view, microbial biogeography and microbiome legacy do not constrain local microbial composition or function. Some microbes disperse widely, but many exhibit limited dispersal coincident with functional specialization (Barberán et al. 2014), which generates substantial variation in local microbial biogeography and creates the possibility of microbial legacies with consequences for microbiome function. This possibility can be illustrated in a third example of a human-induced alternate state (Housman et al. 2008). Post-agricultural habitat is often dominated by non-native plant communities, which are resistant to re-establishment of original native plant species. Part of this resistance appears to be due to altered communities of mycorrhizal fungi. Successful re-establishment of native plants requires inoculation of native mycorrhizal fungi in addition to re-introduction of the plants (Middleton et al. 2015, Koziol and Bever 2015).

Dynamics, functional significance, and knowledge gaps of the plant microbiome

Microorganisms that negatively (pathogens) or positively (mutualists) affect plant survival, growth, and/or reproduction are influential components of the plant microbiome. They directly affect terrestrial plant productivity and plant uptake of soil resources. Microbial pathogens may be facultative or obligate, and have broad taxonomic breadth, including fungi, bacteria, viruses and water molds (Oomycetes). The plant microbiome also includes microbes that protect plants from pathogens and nutritional mutualists such as mycorrhizal fungi and N-fixing bacteria that increase plant access to resources.

The composition and function of the plant microbiome is responsive to both climate and land use history. While we have ideas of how specific components respond to drivers, we have less understanding of how plant microbiome components interact. For example, many plant pathogens, such as Oomycetes, are expected to increase in abundance and virulence with precipitation (Raymond et al. 2012, Bever et al. 2012). However, pathogen accumulation may be countered by the co-evolutionary response of the defensive mutualists in the microbiome (Platt et al. 2012) and host plant co-evolution. As a result, plant microbiomes in mesic environments could both have more pathogens and greater ability to resist these pathogens (hence potentially greater resilience), while microbiomes in drier environments could have lower pathogen pressure, but be more vulnerable to pathogen outbreak when exposed to increased precipitation. Pathogen density and virulence are also expected to be greater in monoculture plantings in agriculture and less with mixed plantings (Bever et al. 2015).

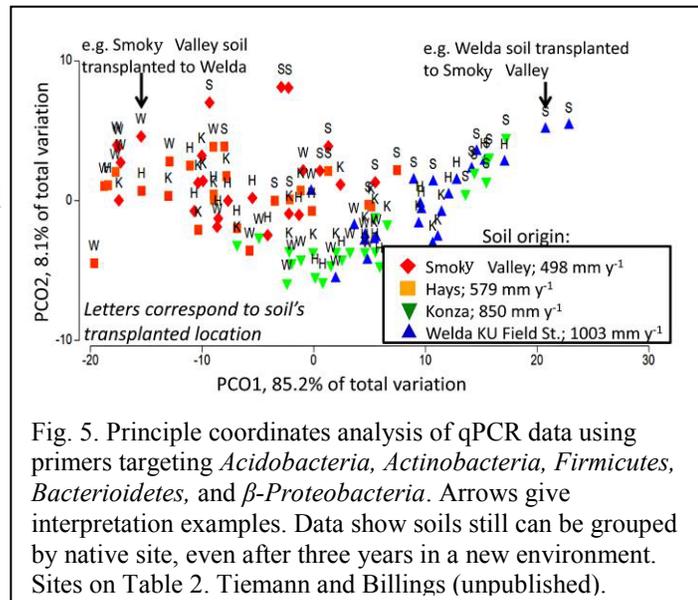
The density and efficiency of nutritional mutualists such as mycorrhizal fungi are expected to increase as below-ground resources become increasingly scarce (Zhang et al. 2015, Bever 2015). Therefore, fertilization changes the mycorrhizal fungal composition toward resource uptake efficiency (Johnson 1993, Corkidi et al. 2002), while drought-prone climates can change mycorrhizal fungal composition toward more efficient water uptake (Stahl and Smith 1984, Giauque and Hawkes 2016). From this assumption, we would expect plant microbiomes to become more beneficial in drier environments (Stachowicz 2001). Alternatively, given that microbial mutualists facilitate uptake of multiple resources, plants can be adapted to their local soil and resource environment (Lambert et al 1980, Johnson et al 2010). The individual and interactive effects of climate and land use histories on the microbiome-mediated efficiency of plant resource uptake are unknown. However, with degradation of the microbiome due to intensification of land use, we might expect to lose the microbiome signature of climate history. In this case, the extent to which the microbiome differentiation facilitated plant functions would indicate the potential and context-dependence of benefits of reintroduction of native microbes.

Dynamics, functional significance, and knowledge gaps of the soil microbiome

The soil microbiome mediates the transport and transformation of resources within and through the soil profile. Much research has focused on the role of the soil microbiome in the global C cycle, given soils can serve as a sink or source of greenhouse gases (GHG) (Tarnocai et al. 2009, Chapin et al. 2006, Paustian et al. 2016). These microbial functions are strongly influenced by microbial resource limitations and available moisture (Billings and Ballantyne 2013, Manzoni et al. 2014). Microbes also influence soil structure (Six et al. 2002, Fabrizzi et al. 2009, Wilson et al. 2009). Soil aggregation mediates soil chemical, physical, and biological properties and improves soil quality and sustainability (Six et al. 2000; Lado et al. 2004). The stability of soil aggregates is critical to our understanding of soil degradation and resilience (Blanco-Canqui et al. 2009, Blanco-Moure et al. 2012, Mikha et al. 2013) and is sensitive to land use history (Duchicela et al. 2012). The interactions between soil microbiome and soil water that mediate biogeochemical cycling are still not well understood, though we know microbiome structure can explain some ecosystem processes (Graham et al. 2016). The role of soil moisture regime as a master variable dictating both resource supplies to microbes within the soil matrix and, ultimately, the loss of soil resources is not clear. For example, a multi-year manipulation that decreased precipitation frequency in tallgrass prairie promoted greater microbial biomass via greater microbial C use efficiency (Zeglin et al. 2013). However, when soil microbiomes from mesic grasslands are subjected to drought via increased intervals between precipitation events, C use efficiency decreases, and microbial CO₂ efflux increases (Tiemann and Billings 2011a). Clearly, more knowledge is needed on soil microbiome responses to climate. Ordination analyses reveal how legacies of past climate regime can be maintained across three growing seasons in a new climate (point distribution across component axes, Fig. 5), suggesting microbiome resistance to perturbation (Cruz-Martinez et al. 2009). These analyses also reveal that novel environmental conditions influence that structure (dominance of “S” labels high on PCO2 in Fig. 5). Understanding temporal changes in soil microbiome structural and functional responses is a major knowledge gap hampering our ability to predict how ecosystem functions respond to changing climate.

Identifying drivers of the long-term persistence of soil organic C (SOC) compounds (Trumbore 2009) represents another challenge for projecting future interactions between soil properties, water, and soil microbial behavior. Deep SOC originates either from deep roots and microbes, or from material transported downward via soil water through surface horizons. The SOC stoichiometry can determine its recalcitrance to microbial consumption. At Konza, a comparison of an agricultural field and grassland found cultivation changed C and biological activity to a depth of 8-10 m (Dodds et al. 1996). In this study, organic C and C:N ratio increased with depth (to 8 m) in the cultivated soil relative to the prairie soil suggesting preferential movement of C relative to N.

Surface activities can be coupled to the subsurface microbiome. In agricultural soils where the C:N of microbial substrates is typically lower than that in native systems (Riggs and Hobbie 2016), microbial communities can become relatively C limited, even where the absolute availability of C is high (Tiemann and Billings 2011b). Our recent work elucidates how environmental conditions govern the propensity of soil microbes to retain C or release it as CO₂ (Lehmeier et al. 2016), and reveals that a lower C:N of available resources can counter-intuitively move microbes to greater mass-specific respiratory C loss.



Apparently energy expenditures linked to substrate transport enzymes decrease efficiency (Min and Billings et. al in prep.). These studies document the primacy of environmental conditions and resource C:N in driving microbial C allocation and associated soil profile C fate. Given the general decline in C:N with depth found in most soil profiles (Rumpel and Kogel-Knabner 2011) and the large variation in substrate C:N observed among native and managed systems (Tiemann and Billings 2011b), characterizing the fundamental responses of soil microbes to resource stoichiometry is an important pathway to understanding deep SOC losses and persistence.

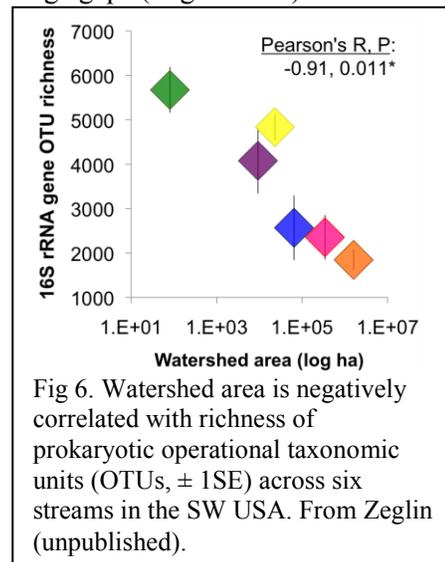
Dynamics, functional significance and knowledge gaps of the aquatic microbiome

As water enters a stream network, it carries microbial cells and nutrients derived from the surrounding watershed soil (Bell et al. 1982). Synoptic (throughout a watershed) surveys of aquatic microbiota collected from headwater streams to large order rivers suggest that headwater stream microbiomes contain a higher diversity of more terrestrial-derived microbial taxa. While terrestrial signals are clear along a continuum from soil to streams and rivers (Ruiz-Gonzalez et al. 2015), this terrestrial signature attenuates as water flows downstream (Crump et al. 2007, Crump et al. 2012; Besemer et al. 2013). For example, the diversity of the aquatic prokaryotic community as represented by prokaryotic 16S rRNA gene richness in six streams reaches from the southwestern USA decreased significantly with watershed size (Fig. 6), suggesting that a terrestrial-derived microbiome signature might be lost as water moves downstream. Lower water retention time drainage networks are expected to lead to a microbiome more influenced by terrestrial habitats (Lindstrom & Bergstrom 2004, Van Rossum et al. 2015). However, reservoirs may reverse this pattern (Meziti et al. 2016), reflecting the direct connection between hydrology (precipitation, flow regulation) and sources of stream nutrient and microbiome loads. Studies on this topic are still few and the interactive effects of natural hydrology and human land- and water-use on aquatic microbiome structure and function are definite knowledge gaps (Zeglin 2015).

Increased transport of nutrients to aquatic habitats causes substantial changes in aquatic ecosystem structure and function. Among the most important of these is the increased formation of toxic cyanobacterial blooms (Smith and Schindler 2009, Paerl et al. 2011) and biodiversity loss attributable to N pollution (Hernández et al. 2016). Increased cyanobacterial blooms are associated with shifts in nutrient loads and stoichiometry (Yuan et al. 2014, Van de Waal et al. 2014, Davis et al. 2015), land use patterns (Beaver et al. 2014), and climate change. Increased water temperatures and more nutrients increase the probability of these blooms. As such, drinking supply and recreational supply waters are more commonly being shut down because of blooms of potentially toxic algae.

Nitrogen pollution and agricultural land use are strong predictors of microcystin concentrations, particularly in the Midwestern US (Beaver et al. 2014, Yuan et al. 2014). These trends are particularly worrisome given increasing evidence linking liver and colorectal cancers diseases to toxin exposure (Zhang et al. 2015). Understanding when these blooms occur and their toxicity requires an understanding of N and P supply rates and their stoichiometry (Paerl et al. 2011). The influence of microbiome interactions on the probability of bloom formation is complex and not well understood.

The majority of work aimed at predicting the controls on microcystin expression and associated microbiome shifts has focused on the role of nutrients or environmental factors. Increasingly, metagenomic approaches are connected to environmental data, but, to date, much of this work has centered on the Great Lakes region (Michalak et al. 2013, Beversdorf et al. 2015a, 2015b, Davis et al. 2015, Harke et al. 2015). This region is distinct from the Central Plains in terms of climate, land use and hydrology (Beaver et al. 2014). While the presence of toxin-producing genes is not necessarily linked to



toxin production (Beverdorf et al. 2015a), integrating metagenomic approaches with environmental data can be useful for predicting microbiome shifts (Harke et al. 2015).

Intellectual Merit and Broader Impacts

The **intellectual merit** of this project is a mechanistic understanding of the drivers of MAPS structure and function. We will provide unique and cutting edge scientific information by quantifying how climate and land use legacies govern MAPS composition and functioning, as a means of predicting the resistance and resilience of multiple ecosystem properties to long-term (e.g. contemporary climate change) and punctuated (e.g. extreme climate events, land use changes) perturbations. While the functional importance of plant, soil and aquatic microbiomes has often been studied individually, researchers rarely work across habitats, and little is known on their relative sensitivities to precipitation and land use perturbations, or their potential interactive effects. We couple coordinated sampling and experimental manipulations of terrestrial and aquatic environments across Kansas' precipitation and land use gradients to provide a basis for a long-term coordinated research network and guide best management practices to curb plant-soil resource losses, thus maximizing productivity while limiting water pollution and greenhouse gas production.

The **broader impacts** of this project include informing potential management applications that realize low-cost, energy-efficient and sustainable food production while protecting soil, water, and human health; these results have from local to international importance. As we will demonstrate, MAPS science underpins the function and supply of freshwater ecosystem services that are so crucial to Kansas' water needs (*A Long-term Vision for the Future of Water Supply in Kansas*, 2015). Two of the project leaders are Kansas Agricultural Experiment Station faculty and participate in outreach activities that disseminate project outcomes to extension agents and producers. We have developed educational and outreach programs that focus on the hypotheses, approaches, and findings that are foundational to our disciplines, and that emphasize the sense of excitement and relevance inherent in problem-solving in science and engineering. Our programs will use MAPS to reach individuals of all ages and levels: K-12 students and teachers; undergraduate and graduate students; faculty members at community colleges, tribal colleges, and four year institutions; and adults interested in broadening their knowledge. Underrepresented groups in STEM will be accessed through a program at a tribal college (Haskell Environmental Research Studies Institute at HINU) and minority students in community colleges (LSAMP through KSU). These initiatives will not be limited to the classroom; project researchers participate in educational and outreach activities associated with field stations at KU, KSU, and WSU, as well as other venues for outreach. In addition to its potential for global impact, MAPS research in Kansas will especially address needs of agricultural and land use management stakeholders in Kansas, including those in tribal communities.

PROPOSED RESEARCH PLAN

The sections below are organized by field surveys, and then experiments. Each area presents activities by the plant microbiome (PMB), soil microbiome (SMB), and aquatic microbiome (AMB) groups. We introduce the research team and describe research sites, then the individual experiments. We finish with details on microbiome methods, statistics, and synthesis and theoretical approaches. Outcomes from this research and education initiative include: 1) publicly-available databases describing MAPS and associated environmental variables across the precipitation and land use gradients of Kansas; 2) scientific documentation (in the form of publications and presentations) of MAPS' capacity to govern ecosystem productivity, soil resource retention and water quality; 3) applied-on-the-ground information about Kansan agroeco-systems; and 4) enhanced teaching and learning capacity at multiple institutions, including those serving underrepresented groups in science.

4.3.a. Research team, facilities, and importance of EPSCoR funding for increasing competitiveness in NSF funding. The proposed collaborative research will strengthen ties both within and across universities, allow researchers to share skills, and create scientific synergies to increase productivity and creativity, improve efficiency of training students, and eliminate redundancy (e.g., through equipment and expertise sharing). Thus, EPSCoR funding will increase competitiveness for future NSF funding (see Section 4.7 *Sustainability Plan*). We will maintain a strong mentoring environment by implementing a

horizontal project structure that values input from all team members (from graduate students to professors). This approach has sound support from social science research on how to form productive collaborative groups (Goring et al. 2014, LINX collaborators 2014).

Table 1. Senior level participants for MAPS.

Project Leaders	Title	Affiliation	Expertise	Roles*
Kristin Bowman-James	University Dist. Prof.	KU	Project Director	PI
Walter Dodds	University Dist. Prof.	KSU	Water quality, aquatic ecology	Co-PI, AMB
James Bever*	Fdn. Dist. Prof.	KU	Plant-microbe evolutionary ecology	Co-PI, PMB
Sharon Billings	Professor	KU	Ecosystem ecology, biogeochemistry	Co-PI, SMB
Charles Rice	University Dist. Prof.	KSU	Soil microbial ecology	Co-PI, SMB
Group Leaders	Title	Affiliation	Expertise	Roles*
Terry Loecke*	Assistant Professor	KU	Plant soil microbe interactions	SMB
Ben Sikes*	Assistant Professor	KU	Microbial community ecology	PMB
Amy Burgin*	Associate Professor	KU	Aquatic biogeochemistry	AMB
Anna Whitfield	Professor	KSU	Plant disease, ecological genomics	PMB
Lydia Zeglin*	Assistant Professor	KSU	Soil and water microbial ecology	AMB
Matthew Kirk*	Assistant Professor	KSU	Subsurface biogeochemistry	SMB
Research Team	Title	Affiliation	Expertise	Roles*
Ari Jumpponen	Professor	KSU	Fungal ecology	PMB
Belinda Sturm	Associate Professor	KU	Wastewater microbial ecology	AMB
Dan Reuman*	Associate Professor	KU	Theoretical ecology	PMB
Fola Augusto*	Assistant Professor	KU	Epidemiology	PMB
Greg Housman	Associate Professor	WSU	Plant community ecology	PMB
Helen Alexander	Professor	KU	Plant Ecology	PMB
Jen Roberts	Associate Professor	KU	Microbial geochemistry	SMB
Jesse Nippert	Associate Professor	KSU	Plant physiology water relations	PMB
Mitch Greer*	Assistant Professor	FHSU	Grassland ecology	SMP
Pamela Sullivan*	Assistant Professor	KU	Ecohydrology, hydrogeochemistry	S/AMB
Sanzhen Liu*	Assistant Professor	KSU	Pathology and microbial genomics	PMB
Thomas Platt*	Assistant Professor	KSU	Microbial ecology/ disease ecology	PMB
New Faculty Hire	Assistant Professor	KSU	Environmental metagenomics	P/S/AMB
New Faculty Hire	Assistant Professor	KU	Environmental metagenomics	P/S/AMB
Education Team	Title	Affiliation	Expertise	Roles*
Peggy Schultz*	Associate Specialist	KU	Soil microbial ecology, Edu. liaison	PMB
Jay Johnson	Professor	KU	Environmental sci., HINU liaison	HERS
Joseph Brewer*	Assistant Professor	KU	Environmental sci., HINU liaison	HERS
Melinda Crow	Dean	HINU	Environmental sci., HINU liaison	HERS
New Faculty Hire	Assistant Professor	HINU	Environmental science	HERS

Project roles: PI – Project Director KNE, co-PI - Lead Research Principal Investigator, GL- Research Group Leader (one for each group from each university), AMB- Aquatic microbiome, PMB- Plant microbiome, SMB- soil microbiome, S/AMB – SMB + AMB, etc., HERS – Haskell Environmental Research Studies Institute. *recent hires.

The specific research administrative structure will include two co-PIs at KSU (*Rice* and *Dodds*) and two at KU (*Billings* and *Bever*; Table 1) in addition to oversight of the entire research and education program by Bowman-James (See Section 4.8 *Management, Evaluation and Assessment Plan*). The four co-PIs will serve as the points of interaction with the KNE office and the science advisory team, as well as oversee the information manager and project assistant. They will administer the AMB, PMB and SMB research groups and mentor junior faculty group leaders. Each of three focus areas will have one group leader at each university (see Table 1) and will coordinate activities of individual groups. Groups and group leaders will meet directly or via videoconference bi-monthly, and the science team will have annual meetings in conjunction with the State EPSCoR meeting. Dr. *Peggy Schultz* has training in microbial ecology and education, and will serve as a liaison between the science and education sections of our projects. Three faculty members will be recruited in the first year of the award to start in year two,

including a much-needed faculty hire in the environmental science at HINU. (See Section 4.8 *Management, Evaluation and Assessment* for more detail about administrative structure.)

The project will also support three postdoctoral researchers, 14 graduate students per year, 10 undergraduate research assistants, and six summer REU students. Undergraduate researchers, REUs and graduate students will be recruited in part through the Haskell Environmental Research Studies (HERS) Institute and the NSF-funded Louis Stokes Alliance for Minority Participation (LSAMP) education internships (supported in part by this proposal and described under workforce development) to promote underrepresented groups researching MAPS topics.

4.3.b. Field surveys across sites with varying climate and land use histories.

While the functional role of microbiomes in individual systems is increasingly appreciated (e.g. Goodrich et al. 2016), important ecosystem properties such as terrestrial productivity, greenhouse gas production and water quality most certainly depend upon the cumulative and potentially interactive effects of MAPS. Studying these interconnections requires integrated sampling of plant-soil-water microbiome composition and function across gradients of precipitation and land use history, with each terrestrial sampling campaign linked to aquatic sampling efforts within a watershed. By coordinating sampling of microbiome and microbial functions, we can identify patterns in process and structure, and test the relative importance of MAPS in mediating ecosystem functions using statistical approaches such as path analysis/structural equation modeling (as represented in Fig. 4). We specifically hypothesize the mechanisms that might drive such patterns in subsequent sections.

4.3.b.1 Field Site Selection. Kansas is a natural laboratory for the study of interactions between moisture and land use effects on microbiome functions. There is a steep precipitation gradient across Kansas (Fig. 1). Across this precipitation gradient we work at several well-established experimental sites valuable for the proposed work (Table 2). Three of these sites will be intensively sampled: 1) Smoky Valley Ranch (west); 2) Konza Prairie Biological Station (central); and 3) KU Field Station (east). Each of these sites has been the subject of research exploring microbial composition and function; some have past experimental manipulations now available to us as research platforms. We will supplement these core sites with additional sites that have independent land use and climate histories to augment our ability to implement structural equation modeling (see below).

Table 2. List and brief description of proposed sites for observation and manipulative studies.

Site	Ann. precip. mm	Typical soil series	Latitude/longitude	Native plant community
Welda Prairie (TNC/KUFS)	1045	Olpe-Kenoma	38.2/-92.3	Tallgrass
KU Field Station (KUFS)	1000	Kennebec, Grundy	39.0/-95.2	Tallgrass
Land Institute – East	1000	Kennebec	39.0/-95.3	Tallgrass
Konza LTER, KSU Agric. Experimental Station	865	Dwight-Irwin, Chase, Kennebec	39.1/-96.6	Tallgrass
Land Institute	760	New Cambria, Cass	38.8/-97.6	Tall/mixed grass
Hays Experimental Range	533	Harney	38.9/-99.3	Mixed grass
Smoky Valley Ranch (TNC)	489	Keith, Ulysses	39.1/-101.0	Mixed/short grass
Tribune Ag. Expt. Station	422	Richfield	38.3/-101.4	Mixed/short grass

We have selected sites where co-locating plant, soil, and aquatic microbiome sampling across the precipitation and land use gradients is feasible. We will take advantage of the strong network of agricultural research stations (Kansas State Research and Extension, (KSRE)) and well-established sites for environmental work (Konza Prairie Biological Station (KSU) and University of Kansas Field Station (KU)). Each site will pair three land uses (grain agriculture, post-ag grasslands, and native prairie) and couple individual terrestrial sites with adjacent surface water features. To maximize cross-site climate variation and minimize within site hydrologic variation, sites will transverse Kansas from east to west surrounding the 39th parallel along the precipitation gradient, be located within the Kansas or greater Missouri River basins in a similar landscape position (e.g., toeslope or floodslope terrace), and have similar edaphic characteristics (e.g., mineralogy). Where sites contain ephemeral surface waters,

groundwater sampling and monitoring will be used to bridge gaps in aquatic system understanding. We chose sites that are owned by KU or KSU or private holdings with a history of cooperative relationships with these universities (e.g. The Nature Conservancy and The Land Institute). The sites span the regional breadth of climate and land use histories, and have supported a history of research on microbial composition and function. We supplement these terrestrial centric sites with targeting sampling of six reservoirs distributed along the rainfall gradient. Each reservoir integrates a unique distribution of land use histories.

4.3.b.2 General Sampling Strategy. Initial intensive sampling at core sites in year one will link the structure and function of plant-soil-water microbiomes and establish sampling properties for broader terrestrial and aquatic surveys in subsequent years. Plant, soil, and aquatic microbiomes will be intensively sampled at the three core sites (Smoky Valley Ranch, Konza, and KU Field Station). At each core site, we will coordinate plant, terrestrial and aquatic sampling, then use this data to guide subsequent sampling at core and additional sites. Terrestrial sampling will involve establishment of 4 replicate, 1 m² permanent plots per land use per site combination (4*3*3=36 plots). Plant community composition, plant microbiome sampling, and soil cores will be collected during spring, summer and fall of the first year. Within plots, plant samples will come from 3 individuals (see details below) with above and belowground tissue separated. Three replicate soil cores per plot will be separated into four depth categories (to ~3m based on site-specific auger refusal depth) and composited to generate representative samples, by depth, of the plot-level biotic communities and abiotic properties. Stream's benthos near each plot will be sampled as well as the downstream reservoir's epilimnion once per season (4). The reservoirs integrate multiple land uses while providing an integrated aquatic network perspective of microbiome composition and function. Molecular analyses in years 2-4 will include sampling across five additional terrestrial and nearby aquatic sites (4 plots X 8 sites X 3 land use histories = 96 plots) and nearby reservoirs. Using data from year 1 at core sites, broader sampling in subsequent years will be scaled back to a single season and a single soil depth. Amplicon sequencing will be done on all samples, but metagenomic analyses will be limited to select samples and experiments. General molecular methods and statistical approaches follow the sections on individual experiments.

4.3.b.3 Plant Microbiome (PMB) Hypotheses and Measurements, Field Surveys (Alexander, Bever, Schultz, Sikes KU; Greer FHSU; Housman WSU; Jumpponen, Liu, Nippert, Platt, Whitfield KSU)

PMB Hypothesis a. *Plant-microbiome assisted resource acquisition will increase with environmental stress.* We will test whether plant microbiomes in drier climates increase plant growth promotion potential and the potential to alleviate drought stress compared to those in wetter climates.

PMB Hypothesis b. *Pathogens build up in mesic environments and in monocultures.* We will test whether pathogen density and virulence increase with rainfall and decrease with plant diversity.

PMB Hypothesis c. *Pathogen inhibition potential of the microbiome will increase with pathogen density and microbial diversity.* Both pathogen density and microbial diversity may increase with rainfall which could generate climatic patterns in the ability of the plant microbiome to inhibit pathogens.

PMB Hypothesis d. *Beneficial plant microbiome functions will degrade with land use intensification.* As mutualistic microbes, such as mycorrhizal fungi, can be sensitive to disturbance, we expect mutualistic functions of the plant microbiome to decline with land use intensification.

We will use two strategies to sample the plant microbiome: one targeting different plant tissues directly and a second representing the microbes in the soils surrounding roots. We will target the microbiome from the roots and shoots of individual plants. We will target big bluestem (dominant native in east), sideoats gamma grass (dominant native in west), smooth brome (commonly planted pasture grass) or sorghum (crop planted across the state) where available or the most abundant alternative where they are not present. Secondly, we will use samples and data from pooled 0-20cm (where most plant-soil interactions are concentrated) soil samples, collected and analyzed jointly with the soil microbiome sampling (below) to represent the below-ground microbiome of the site. We will assess microbiome composition using amplicon sequencing and microbiome function by sequencing functional genes and direct inoculation tests on phytometers (common test plants in common environments).

At a given plot, we will randomly select three individuals of the target plant species. Tissue from shoot, leaf and reproductive tissue will be clipped from above-ground plant portions. These individual plants will be dug up with root systems intact and clippings taken from primary, secondary and tertiary rooting structures to complete the belowground sampling.

4.3.b.3.1 Molecular characterization. Sample storage, nucleic acid extraction, and molecular analyses will be carried out as outlined in the general molecular analysis section. Above- and below-ground tissue will be extracted and analyzed separately. *Amplicon sequencing* will target bacteria, fungi, AM fungi and oomycetes using taxon-specific primers (listed in general section). Known viruses (e.g. yellow dwarf virus) will be detected and quantified using ELISA and qRT-PCR. The great diversity of unknown viruses will be characterized using random primers from ribosomal RNA-depleted samples following the methods of Nagano et al. (2015). Approximately 20g of root tissue sieved from the soil cores will be used to generate a community profile of the “plant community microbiome.” In addition to targeting microbial taxon, plant specific primers will be used to assess the composition of the roots. We will test universal plant barcodes used previously to identify roots (*rbcL*, *matK*, *trnH-psbA* –Hollingsworth et al. 2011, Jones et al. 2011) choosing a single marker for experimental work based on pre-screening with aboveground plant material. *Metagenomic analyses* will identify differences in functional microbiome genes including known a) pathogen virulence genes and host resistance genes directly related to plant productivity hypotheses, and b) genes with biogeochemical relevance which functionally link plant and soil microbiomes (see below). First, genes with these putative functions will be identified by matching to databases (Guttman et al. 2014, Bulgarelli et al 2015). Second, computational analyses will be used to predict which genes may be unidentified pathogen or plant defensive genes (Guttman et al. 2014, Knief et al. 2012). The DESeq2 (Love et al. 2014) and edgeR (Robinson et al 2010) packages available through Bioconductor in R will be used to identify genes showing abundance patterns consistent with the survey gradient and/or correlated with known pathogen/plant defensive genes (McMurdie and Holmes 2014).

4.3.b.3.2 Functional assays using plant phytometers. We will directly assess the effect of the soil and plant microbiome on plant growth promotion using soils and the root and leaf material as inocula on common plant hosts in a common environment. Plant response to soil microbiome inoculation in plant-soil feedback assays has been found to yield results highly relevant to ecological function (Bever et al. 2015, van der Putten et al. 2013). We have recently demonstrated a similar inoculation approach can be used to assess the effect of leaf microbiomes on hosts (Bever, unpublished). Here, we use big bluestem (native dominant in Eastern grasslands), sideoats grama (native dominant in western grasslands) and sorghum (domesticated crop planted across range) as phytometers. Sterile replicate seedlings of these three species will be inoculated with 1) soil and 2) with root and leaf material from each site and grown in a common greenhouse at KSU. Prior to treatment initiation, we will develop allometric relationships for the 3 species to infer changes in above- and below-ground biomass every 3-5 days without requiring destructive harvests. We will assess the role of the microbiome on species drought tolerance by measuring leaf water potential - stomatal conductance curves. These curves provide insight into the limitation of gas exchange as a function of increasing xylem tension (Tucker et al. 2011, Craine et al. 2013). To quantify photochemical responses to progressive drought, we will conduct chlorophyll fluorescence light response curves (Rascher et al. 2000). Separate inoculation assays will test resistance to common pathogens (e.g. *Fusarium oxysporum*) (e.g. Sikes et al. 2009).

Data from microbiomes, metagenomes and phytometer assays will be used to test hypotheses relating plant microbiome structure and function with precipitation and land use gradients. Data will be analyzed using linear models and results integrated with microbial composition using structural equation modeling (Section 4.3.f.1). *Houseman, Greer, Bever* and *Sikes* will conduct field sampling. *Jumpponnen, Sikes, Liu Whitfield, Bever,* and *Platt* will process samples and analyze the sequence data. *Bever, Nippert, Whitfield* and *Alexander* will conduct phytometer assays. All faculty will participate in statistical analyses.

4.3.b.4. Soil Microbiome (SMB) Hypotheses and Measurements Field Surveys (*Billings, Loecke, Sullivan, Roberts KU; Kirk, Rice KSU*)

SMB Hypothesis a. Land uses permitting development of relatively deep rooting systems promote greater

organic C and N inputs in deeper horizons; retention of these pools is controlled by interactions between them and inputs derived from surface soil organic C and N percolating down, the deep soil microbiome, and mineral surfaces. In deeper layers of agricultural soils, the largest source of organic matter is water-transported material derived from surface horizon. Disturbance due to tillage and climate will alter the microbiome and SOC and soil organic N (SON) dynamics, and the composition of organic compounds percolating to deeper soil layers. In contrast, SOC and SON in deeper layers of native soils are derived from both percolation of surficial OM and inputs from deep roots. In drier systems, C and N inputs to deep horizons via percolation decrease. Thus, we hypothesize total C and N inputs to deep horizons are most limited in semi-arid agricultural systems and greatest in semi-humid native systems, and less diverse microbial communities in drier climates. Linkages between fungal activity and soil aggregate formation further suggest that fungi, reduced at depth but relatively enhanced in native mesic systems, promote the retention of SOC and SON via necromass and aggregate formation. These processes will interact with each profile's mineralogy to drive retention of SOC and SON.

SMB Hypothesis b. *Moisture regime and land use interact to govern stoichiometric controls on soil microbiome mediation of SOC and SON stocks.* Based on laboratory results, we expect that in agricultural surface soils, low C:N of microbial substrates promotes relatively greater allocation of microbial C to mass-specific respiratory CO₂ losses than in higher C:N native systems (lower C use efficiency), a counterintuitive phenomenon apparently linked to the respiratory costs of microbial foraging (Min et al. in prep). These results suggest less C will be allocated to microbial biomass, particularly bacterial groups linked to the uptake of relatively labile substrates. These effects should penetrate deeper within soil profiles where higher precipitation and low C:N substrates can percolate; we will measure respiratory losses per unit microbial biomass from soils at multiple depths to test for the precipitation effect. This approach assumes that measured microbial biomass is a proxy for active microbial cells. This work has the potential to elucidate one mechanism driving the fate of both surficial and deep SOC and SON stocks.

4.3.b.4.1 Approach: During the first year, three land uses at the three core sites (3*3*4 replicates = 36 plots) will be intensively sampled in three seasons (spring, summer, fall) at 4 depths: ~0-20 cm, ~50-60 cm, ~1 m, and ~3 m. These depths may change to accommodate diagnostic horizon locations. DNA from the 0-20 cm samples will be shared with the plant-microbiome for their soil portion (see above). Microbiomes will be assessed via amplicon sequencing, and microbial functions will be assessed via a combination of sequencing functional genes and performing biogeochemical assays. Analysis of seasonal and depth variation in year 1 by mixed effect linear models will determine the combination of four treatments to be used in years 2-4. Soil moisture and time since precipitation will be noted at time of sampling. This sampling strategy permits us to assess microbiome structural changes over seasonal and yearly timescales, helping to link shorter timescale microbial changes – known to occur in these soils (Billings and Tiemann 2014) – to biogeochemical fluxes across the longer timescales relevant for projections of yearly elemental cycles.

All samples will be characterized according to protocols described in the general molecular section. *Amplicon sequencing* will target bacterial and archaeal communities, and fungi. Past experience suggests that several grams of soil will be sufficient from each sample to generate the needed quantities of DNA. *Metagenomic analyses* will be used to identify genes of known biogeochemical relevance (e.g. nir, nor, and nos, all linked to production of enzymes critical for the denitrification process, or laccase genes linked to the degradation of the plant residue lignin). As with the plant microbiome, we will use computational approaches (e.g., Multivariate Hierarchical Bayesian) to identify genes exhibiting spatial patterns of abundance that vary across the precipitation gradient, and with depth. We also will attempt to identify genes that correlate with well-characterized genes already linked to biogeochemical functions.

We will subject all soil samples to multiple biogeochemical assays to characterize their function in a common environment including 1) denitrification assays (Tiedje 1994); 2) aerobic CO₂ production and N mineralization at -0.033 MPa (e.g. Bandyopadhyay and Lal 2014); 3) exo-enzyme assays linked to microbial acquisition of labile C, N, and P (β -glucosidase, N-acetylglucosaminidase, and acid or alkaline phosphatase, depending on soil pH), as well as the decay of slower-turnover substrates (phenol oxidase;

Lehmeier et al. 2013); 4) aggregate structure and stability (Mikha and Rice 2004, Wilson et al. 2009) to draw linkages between microbiome composition and aggregate formation; 5) mineral nutrient concentrations (N, P) and organic C and N concentrations; and 6) soil bulk density to compute nutrient mass of C and N. Stable isotopic signatures (^{13}C , ^{15}N) of soil organic C and N relative to that of the plant inputs at each site will permit characterization of C and N cycling, including the relative degree of microbial processing that each soil has supported (Billings and Richter 2006). We will install lysimeters at our three core locations (KU Field Station (KUFS), Konza, Smoky Valley Ranch), along with CO_2 and O_2 and soil moisture sensors. The sensors will be placed in contrasting land uses, at three depths, and logged continually. Lysimeters will be sampled ~ biweekly for the first year of the project to assess optimal sampling frequency for the remainder of the project. Combined, these assays will permit comparison of key soil biogeochemical properties across the precipitation gradient and variation with depth, and will be used as components of path analyses to elucidate causal relationships (see Section 4.3.f.1). *Loecke, Sullivan, Kirk, Roberts, Rice and Billings* will conduct field sampling, with *Sullivan and Kirk* managing lysimeters. *Roberts and Billings* will perform sequencing, *Rice and Loecke* will generate soil physical data, *Billings* will perform exo-enzyme assays and stable isotopic analyses, and both *Loecke* and *Billings* will perform incubation-related tasks. All SMB faculty members will participate in statistical analyses.

4.3.b.5 Aquatic Microbiome (AMB) Specific Hypotheses and Measurements (Burgin, Sturm, Sullivan KU; Dodds, Zeglin KSU)

AMB Hypothesis a. *Hydrologic factors will influence stream microbiome composition (and nutrient loads) via differences in the level of connectivity of stream networks with the terrestrial landscape and upstream land use, so that microbiome composition in drier regions or areas downstream from reservoirs will have a reduced signature of terrestrial inputs.*

AMB Hypothesis b. *Contrasting land management practices and levels of terrestrial-aquatic connectivity will create different amounts of nutrient loading that will drive rates of microbial biogeochemical processes, such that both aquatic nutrient loading and removal is higher in mesic regions.*

We will assess how the stream microbiome and water quality varies across the Kansas precipitation gradient, and how land use influences this pattern. If differences in connectivity, precipitation regime or land use select for different microbial types, this contrast will be reflected as differences in headwater stream microbiomes. If hydrological regulation and nutrient loading affect aquatic microbial structure and function, this will be reflected as differences in microbiomes above, below and within reservoirs across the state.

We will monitor the water chemistry and microbiome composition in stream waters and dominant bed substratum from drainage networks of the western, middle and eastern portions of Kansas, directly downstream of sites associated with plant and terrestrial arms of the project, and all feeding into the main Smoky Hill River - Kansas River corridor. We will measure nutrient loads and changes in stream microbiome (targeting both the 16S rRNA gene for prokaryotes and 18S rRNA gene for algae) with periodic grab sampling from streams draining terrestrial study sites at base flow conditions coincident with terrestrial sampling times. In addition, our sampling network will include points above and below reservoirs to evaluate the influence of these potential sources and sinks on microbiome and nutrient loads. Finally, we will conduct higher resolution temporal sampling at core sites, and evaluate the genetic potential for links between microbiome structure and function by producing metagenomic data during select sampling periods. *Sullivan* will monitor physical stream characteristics, *Dodds* and *Burgin* will be responsible for water quality sampling and analyses, and *Sturm* and *Zeglin* will oversee microbial sampling and analyses.

AMB Hypothesis c. *Aquatic microbial communities from locations with a history of greater nutrient loading (more connectivity with the terrestrial landscape) will process nutrients faster than communities from locations with lower nutrient loading.* Support for this hypothesis ("local" microbiome operating better in "local" conditions) has been fairly commonly observed as a "home-field advantage" for microbiomes better adapted to grow on locally abundant organic matter substrates (Judd et al. 2006,

Strickland et al. 2009). Yet, it is important that the home-field advantage hypothesis be better evaluated in the context of aquatic nutrient removal. While organic matter availability often impacts both nutrient removal rates and microbiome structure, there is less evidence that contrasting levels of nutrient loading select for unique microbiomes in stream ecosystems (e.g. Baxter et al. 2012, Zeglin 2015). In fact, nutrient processing functions may be among the least phylogenetically-conserved microbial traits, and thus least related to microbiome structure (Martiny et al. 2015). Defining the strength of feedbacks between different microbiomes and nutrient loads is essential for understanding how the microbiome mediates water quality under shifting climatic and land management scenarios. These studies are especially important to agricultural and land use management issues throughout Kansas. For example, *Johnson* (KU), *Brewer* (KU), and *Crow* (HINU) have been meeting with environmental management staff from four Kansas tribes (Potawatomi, Kickapoo, Iowa, Sac & Fox) about integrating the land use management needs of the tribes with environmental science and GIS education at HINU and KU to provide not only a synergistic and grounded educational experience but much needed research outcomes for the tribes.

Drivers of nutrient release and retention will be further tested experimentally in a smaller scale laboratory study on near-surface stream sediments (0-5 cm) from sites that experience a range of nutrient loads, due to the contrasting hydrology and land use across our sampling network. In the lab, we will expose all sediments to the same defined levels and ratios of N and P, to simulate a range of nutrient loading scenarios. Microbiome structural and functional responses will be measured via targeted 16S rRNA gene and *nosZ* gene and transcript quantitation and sequencing (Phillipot et al. 2013). We will measure N and P processing rates and N removal potential (denitrification enzyme potentials) of all sediments with methods similar to those used for terrestrial sediments. *Dodds* will direct nutrient sampling, *Zeglin* and *Sturm* will oversee molecular sampling, *Burgin* will supervise enzymatic measurements.

4.3.c. Manipulative Tests of Coupled Terrestrial and Aquatic Mesocosms (CTAM)

While the survey approach establishes patterns, these patterns are fundamentally correlational and vulnerable to spurious patterns generated by covariation in predictors. Land use intensification, for example, involves tillage, fertilization, irrigation and reduction of plant diversity, each of which individually could be important drivers of microbiome function. Even strongly supported causal paths would need to be validated by experimentation. As such, we will use a series of coupled mesocosms to: 1) test ecosystem resilience; 2) manipulate and test properties of specific microbiomes; and 3) measure multiple characteristics of soil water effluent. Effluents and estimates of erodibility via soil aggregate stability will be used to inform selection of compound mixtures to be used as inputs to a series of aquatic mesocosms that test stream and reservoir microbial functions, and critically, provide integrative linkages among plant, soil, and aquatic microbiomes.

4.3.c.1. Measurement of Climate Adaptation and Resilience of Net Terrestrial Function. The field survey is unable to measure resilience of ecosystem functions. As such, we propose to test resilience of critical functions in terrestrial ecosystems, such as resource retention, within manipulated mesocosms. By exposing mesocosms to drought followed by intense precipitation, we can assess change (resistance) and recovery (resilience) in ecosystem functions. Five hypotheses relevant to the plant and the soil microbiomes are outlined below:

CTAM Hypothesis a. *The plant-soil microbiome is most resilient in its native climate as expected from local adaptation of beneficial microbes (Fig 7).* We will test if terrestrial resource retention is maximized in native climates.

CTAM Hypothesis b. *Legacies of past drought increases resilience of the plant microbiome to future drought (Fig. 7).* We will test for maximum resource retention in terrestrial systems from drought prone environments.

CTAM Hypothesis c. *Microbiomes with legacies of heavy pathogen loads will confer greater microbial mediated resistance to pathogens, and thereby confer greater system resilience.* As pathogens are

expected to increase with precipitation, western microbiomes may be more vulnerable to plant pathogen responses to precipitation and therefore more vulnerable to resource loss (Fig. 7).

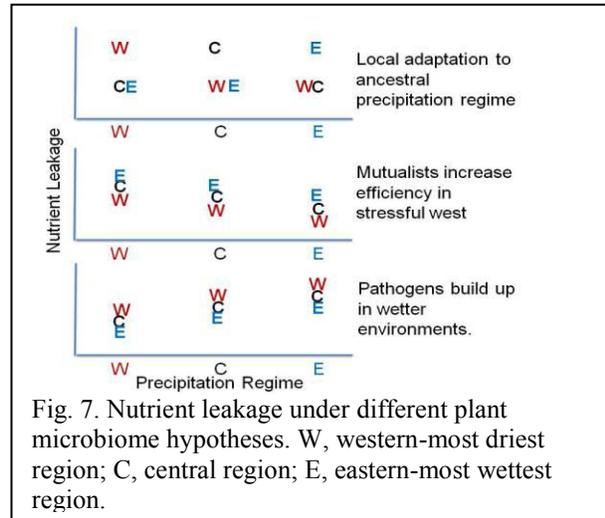
CTAM Hypothesis d. *Land use intensification reduces microbiome resilience.*

CTAM Hypothesis e. *Legacies of past climate and land use govern contemporary responses of soil microbiomes to perturbation.* Soil microbiomes subjected to perturbations in precipitation regime and modified land use over time exhibit potentials for nutrient losses progressively more similar to those mediated by microbiomes native to the perturbing regime, but retain some features of their native structural and biogeochemical functional signal for years post-perturbation due to the persistence of microbial “seedbanks.”

4.3.c.1.1 Experimental Plan (All Plant and Soil Microbiome Faculty). We propose to manipulate climate by excavating plant-soil monoliths and placing them intact into "pots" (60 cm in diameter by 50 cm in depth) designed to capture hydrologic flow-through (Loecke and Robertson 2009). We will obtain these monolith mesocosms from three land use histories (native remnant grassland, post-agriculture grassland, conventional grain monocrop) from each of three climate histories represented by our core sites (Smoky Valley, Konza, KUFS). Replicated monolith mesocosms will be housed in a common greenhouse and exposed to three climate regimes by watering once every 2nd, 5th, or 8th day. These frequencies are proportional to the mean number of days per year (1981-2010) required to accumulate 90th percentile of the annual precipitation at each site across the precipitation gradient – an integrated measure of precipitation frequency and intensity. Climate adaptation and alternative hypotheses (see hypotheses in Fig.7) will be tested from measures of resource leakage. Resilience of terrestrial functions will be measured by exposing monoliths to "weather whiplash" (periods of drought followed by intense precipitation episodes) events. These events will be scheduled during Spring, Summer, and Fall. Resource leakage will be measured as N, P, and C export in flow-through water, gaseous N fluxes, and measures of erodibility of soil from soil aggregate stability. We will sample the plant and soil microbiomes and functions each year for three years to monitor their acclimation to changing climate vs. legacies of their past circumstances. Plant and soil microbiomes will be measured as in Section 4.3.b.3.1. The resilience and resistance of microbial community composition and function to these manipulations will be assessed with repeated measures and “before-after-control-impact” comparisons within a mixed effects modeling framework. Mesocosms will be maintained within the KUFS greenhouse by *Loecke* and *Sikes*.

These frequencies are proportional to the mean number of days per year (1981-2010) required to accumulate 90th percentile of the annual precipitation at each site across the precipitation gradient – an integrated measure of precipitation frequency and intensity. Climate adaptation and alternative hypotheses (see hypotheses in Fig.7) will be tested from measures of resource leakage. Resilience of terrestrial functions will be measured by exposing monoliths to "weather whiplash" (periods of drought followed by intense precipitation episodes) events. These events will be scheduled during Spring, Summer, and Fall. Resource leakage will be measured as N, P, and C export in flow-through water, gaseous N fluxes, and measures of erodibility of soil from soil aggregate stability. We will sample the plant and soil microbiomes and functions each year for three years to monitor their acclimation to changing climate vs. legacies of their past circumstances. Plant and soil microbiomes will be measured as in Section 4.3.b.3.1. The resilience and resistance of microbial community composition and function to these manipulations will be assessed with repeated measures and “before-after-control-impact” comparisons within a mixed effects modeling framework. Mesocosms will be maintained within the KUFS greenhouse by *Loecke* and *Sikes*.

4.3.c.1.2 Measurement of Climate Adaptation and Resilience of Terrestrial Microbiome (All Plant and Soil Microbiome Faculty). While the first experiment tests net ecosystem properties of the terrestrial system, it does not isolate the effects of the microbiomes from that of plants or soil. In this study, we will directly test microbial legacy effects of climate and land use histories on net terrestrial ecosystem functions. We will use soils from the same eight sites as in Section 4.3.b.1 to inoculate mesocosms filled with sterilized background soil and planted with a common plant community. The effectiveness of soil microbe inoculation will be assessed using amplicon sequencing. These mesocosms will be planted into one of two plant communities: a mixture of native plants from eastern and western prairies or a monoculture of sorghum and then exposed to the same precipitation manipulations as described in Section 4.3.c.1.1. The experiment (10 inoculation treatments (9 inocula+control)*2 plant communities*3 precipitation treatments) will be replicated 5 times for a total of 300 mesocosms. The mesocosms will be



allowed two months to establish and then resilience and microbiome composition and functions will be monitored as described in Sections 4.3.b.3.2 and 4.3.b.4.1. Statistical interference for this experiment will be similar to the previous experiment. Mesocosms will be initiated by all PMB and SMB faculty and maintained within the KUFS greenhouse by *Bever* and *Billings*.

4.3.c.2 Independent and interactive effects of root and leaf microbiome (RLM) on plant function (Plant Microbiome Faculty).

RLM Hypothesis.a. *Root and shoot microbiomes act synergistically on plant functions.* Evidence remains inconclusive whether shoot and root microbiomes act additively or synergistically (Larimer et al. 2010).

4.3.c.2.1 Experimental Plan: Microbial communities residing within the phyllosphere and the rhizosphere are distinct and may differentially influence plant function. In Section 4.3.b.3, we assessed the joint effects of root and leaf microbiomes on plant functional response. Here, we disentangle these effects by inoculating the two microbiomes independently. We propose an inoculation experiment measuring the independent and interactive impacts of water availability and microbiome origin to predict vulnerability to drought using functional traits such as plant growth rate, photosynthetic capacity, nutrient leakage and drought tolerance (as proposed in Section 4.3.b.3.2, and similar to Skeleton et al. 2015). For this experiment we will use big bluestem and sideoats grama from the three native locations described in Section 4.3.c.1. We expect to have big bluestem in the eastern and central sites and sideoats grama in the central and western sites, giving four microbial inocula sources. Individual plants will be uninoculated, inoculated with rhizosphere only, phyllosphere only, or rhizosphere and phyllosphere microbiomes collected from these three locations. Each of these microbial manipulations will be watered according to one of three watering regimes as described for Section 4.3.c.1.1 in a full factorial design involving 320 pots per plant species. In all mesocosm experiments, seeds will be surface sterilized and germinated in sterile soil prior to treatment group assignment. Rhizosphere inoculum prepared from a mix of conspecific plant roots and associated soils will be seeded into sterile background soil, while phyllosphere inoculum prepared from macerated aboveground conspecific tissue will be sprayed onto the plant shoot. Mesocosms will be initiated by all PMB faculty and maintained within the KSU greenhouse by Whitfield, Platt and Nippert. Samples will be processed and tested as described in Section 4.3.b.3.2 and 4.3.b.4.1.

4.3.c.3 Aquatic Mesocosm Experiments (AMC) (Burgin, KU; Dodds, KSU; Sturm KU; Zelgin KSU)
A key question is, “How will changes in N, P and C loading from altered precipitation and management affect lake microbiome susceptibility to shift towards nuisance species and toxin production?” We predict that extant lake microbiome will interact with climate and land use factors that alter nutrient loads, which in turn will affect the lake’s susceptibility towards nuisance species domination and toxin production.

AMC Hypothesis a. *Microbiomes from lakes already susceptible to toxin production and nuisance species will be more likely to shift into a “bloom” state under higher nutrient loads derived from the interaction of climate and land use change.*

4.3.c.3.1 Approach. We will use 10,000L mesocosms located at the KU Field station filled with lake water from Cross Reservoir (non-susceptible lake due to its intact, forested watershed) or Milford Reservoir (susceptible to, and regularly closed because of, excessive algal blooms) to conduct a replicated field experiment. Results from the soil mesocosms will be used to set the high and low range and stoichiometry of C, N, and P loads to reflect different land management and precipitation impacts. We will then treat the aquatic mesocosms with appropriate N, P, and C loads. Our design employs a total of 32 mesocosms effluent with lake microbiome (16 non-susceptible and 16 susceptible) and under four nutrient loads (control, low, median and high) with four different stoichiometric ratios of N and P. We will measure time series of nutrients, toxin levels, and microbiome composition (with algal microbiome measures verified with direct cell counts) to assess how altered climate and land use will affect nutrient loading to lakes, and how extant lake microbiome will react to altered loads. *Burgin* will oversee mesocosm operation and algal toxin assays, *Sturm* molecular sample processing, *Dodds* nutrient analyses.

4.3.d. Focused Experiments on Mechanisms Necessary to Link Microbiomes (All Faculty)

We will take an *adaptive approach* to the research over the five years of this grant and reserve funds

for additional experiments in years 3-5. We will use the results of observational sampling and mesocosm manipulations to inform experiments targeting specific mechanisms, sub-hypotheses or microbes. Potential examples include: 1) field sampling and mesocosm manipulations to address the long-term and short-term consequences of climate on microbial composition and function, effects of perturbations of intermediate length, which will be investigated through sampling microbiomes in long-term rainout experiments, fertilization manipulations, and tillage/rotation/CRP experiments that are already in place at Konza, KUFS, and the Hays and Tribune Ag Experimental Stations; 2) virulence trials targeting particular groups of culturable plant pathogens including *Macrophomina* (Ascomycota) and *Pythium* (Oomycota) isolated from our precipitation and land use gradient. 3) microbiome inoculation trials, which will be based on our experience with native AM fungi, where we found that mixtures of native microbes can work as well as the best individual microbe and significantly better than commercially available cultivars (Vogelsang et al. 2006, Middleton et al 2015); 4) metatranscriptomic studies targeting temporal shifts in biogeochemical functions of soil or aquatic systems; 5) manipulative experiments of nutrient loading to soil cores to inform downstream aquatic effects and enhance the integration among the terrestrial and aquatic components of our studies; 6) more detailed sampling for cyanobacterial blooms based on results of aquatic mesocosm studies. These projects are prime targets for seed funding (see Section 4.3.2).

4.3.e. General Molecular Analyses

Next generation sequencing has made microbial ecology one of the most rapidly expanding areas in biology. This technology uses massively-parallel sequencing of DNA isolated from complex environmental samples to assess microbial community and functional gene composition. Kansas researchers have expertise in advanced methods to measure microbial function and in sequencing of microbial communities, in plants, soils, and water (Kansas Metagenomics Center, Ecological Genomics Center). Our proposal unites these groups and provides the necessary infrastructure to drive novel and synthetic discoveries about the importance of microbiomes in the targeted environments that will promote the research trajectories of researchers at all career stages in the region across our universities.

Our methods include: 1) *amplicon sequencing* to assess diversity and composition of microbiomes, 2) *metagenomic analyses* to identify differential abundance of functional genes and examine DNA viruses, and 3) *metatranscriptomic analyses* to identify RNA viruses. Sample numbers and which particular analyses are conducted will depend on the specific experiments. Multiple group members, in collaboration with the KSU Integrated Genomics Facility (IGF) and the KU Center for Metagenomics (CMMCA), have well-established procedures for these analyses (e.g. Bengtsson-Palme et al. 2013, Brown and Jumpponen 2014, Powell and Sikes 2014, Oliver et al. 2015, Sikes et al. 2016).

4.3.e.1 Sample collection and nucleic acid extraction: For DNA analyses, samples will be collected using sterile methods, placed on ice, and frozen at -80°C within 6 hours. For RNA analyses, collected samples will be placed directly in RNALater (Qiagen, Germantown, MD, USA) and frozen at -20°C within 6 hours. DNA and RNA extractions will be carried out in 96-well formats using MoBio PowerPlant, PowerSoil, or PowerWater DNA or RNA Isolation Kits depending on sample type and target molecule (MoBio, Carlsbad, CA, USA). Ribo-depleted RNA will be treated with DNase (Ambion, Austin, TX, USA) for removal of contaminant DNA.

4.3.e.2 Community analyses using amplicon sequencing: Amplicon sequencing will use taxon-specific primers to assess diversity and composition of different broad groups that comprise the microbiome. The general workflow is shown in Fig. 8. Specific groups and primer targets differ based on the sample type, presence of non-target DNA, and hypothesized links being explored between specific groups and microbiome functions. Taxa and sample-specific PCRs will be carried out separately. For plants, bacteria-

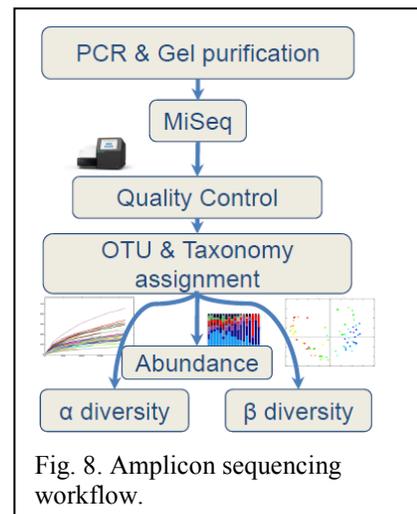


Fig. 8. Amplicon sequencing workflow.

specific primers will be used to target the 16S V5, V6 and V7 hypervariable rDNA regions which exclude chloroplast amplification (Bulgarelli et al. 2012, Copeland et al 2015), fungus-specific primers targeting the ITS2 region (Ihrmark et al. 2012), AM-fungal specific primers targeting the LSU (House et al. 2016) and Oomycota-specific (water-molds) primers targeting the *cox2* gene (Choi et al 2015). Fungi will be categorized as pathogenic, saprophytic or mutualistic with plants based on the FunGuild application (Nguyen et al. 2016). Soils will use bacteria-specific primers targeting the standard 16S V3 and V5 rDNA region (Klindworth et al. 2013) or fungus-specific primers used above. Aquatic samples will use the same bacterial/archaeal primers as soil and primers to target V4 18S rDNA region to identify eukaryotic algae (Mangot et al. 2013). Unique barcodes are attached to PCR products from individual samples (for bioinformatic separation), and samples then pooled for sequencing (“multiplexing”). Final pooled libraries will contain the smaller of either 384 samples or all samples from a single experiment. Sequences will be generated using Illumina Mi-seq (Illumina, San Diego, USA) at the KSU (IGF) or the KU Genome Sequencing Core (GSC). Bioinformatic processing will use either Qiime v.1.9.0 (Caporaso *et al.* 2010) or Mothur 1.36 (Schloss et al. 2009) and taxon specific reference databases (e.g. greengenes, SILVA, and UNITE). Diversity metrics and compositional differences will be assessed in R using the vegan and phyloseq packages.

4.3.e.3 Metagenome analyses of functional genes: Metagenomic sequencing will be used to assess changes in the diversity of functional genes in microbiomes. Metagenomic libraries will be created using the “tagmentation” procedure (Picella et al. 2014), which simultaneously fragments DNA in a sample and inserts a unique barcode. For sequencing, the number of samples combined in a single library (e.g. multiplexing as above) depends directly on the metagenome size and diversity, which will vary among sample types. Previous work at the CMMCA and literature reviews show average coverage of 5k megabases/metagenome. This target will be used as a starting place in year 1 to determine sequencing depth of plants, soils and water samples to get ~5X coverage for each sample. Based on this estimate we can multiplex ~40 samples on a single run. Metagenomic sequences will be generated using Illumina HiSeq (Illumina, San Diego, USA) at the KU GSC. We will work directly with the KU Genome Sequencing Core to optimize coverage across sample types. Bioinformatic processing of metagenomes will consist of quality control, contig assembly, and annotation using functional gene databases. As with amplicon sequencing, the CMMCA and co-PI *Billings* have established bioinformatics protocols for metagenomics (Gomez et al. 2016, Billings et al. unpublished data). Multivariate statistical comparisons will be carried out in R (using Bioconductor Tools) in order to determine microbial genes correlated with specific parameters of interest (e.g. organic carbon concentration).

4.3.e.4 Analyses of Plant Associated viruses (DNA and RNA): To capture the diversity of viruses found in plants, RNA will be the molecule of choice for assessing virus population richness and abundance. DNA and RNA viruses both have RNA products during replication and/or transcription (Nagano et al., 2015) and this commonality can be in conjunction with RNA-Seq and informatics to identify viruses in plant tissues. Ribosomal RNA will be depleted using standard procedures (i.e., RiboMinus plant kit, ThermoFisher). For library preparation, total RNA will be reverse transcribed using random hexamers (creating cDNA), with no mRNA enrichment step due to interest in concurrent eukaryotic and prokaryotic gene expression. Double stranded cDNA will be digested, purified, and sequenced (Illumina HiSeq). For identification of viruses, reads will be assessed for quality and then compared to the NCBI/GenBank virus genome database using bowtie 2 and read counts calculated using RSEM (Nagano et al., 2015). Customized and optimized pipelines for informatics analysis will be created by *Liu* as needed to streamline data analysis.

4.3.f. Synthesizing results among sub-projects

Mechanistic hypotheses targeting individual microbiomes will be tested with field surveys and targeted experiments will be analyzed using linear models and multivariate statistical methods (e.g. perMANOVAs). However, assessment of our overall goals and integrative hypotheses requires integration across microbiomes and across experiments. Outcomes could be self-evident, as would be the case if one microbiome (e.g. Aquatic) did not vary in function or composition with climate history, while

another microbiome did (e.g. the Soil). However, it is likely that plant, soil and water microbiome compositions and functions will vary to some extent across climate and land use histories. In this case, assessing the sensitivity of water quality, for example, to changes in plant, soil, and aquatic microbiomes will require more sophisticated approaches. Below, we outline a statistical and a modeling approach to integrating and testing across Section 4.3.

4.3.f.1 Structural Equation Modeling (All Faculty). The potential causal paths represented in Figure 4 will be tested using Structural Equation Modeling (SEM), also known as Path Analysis. This method allows initial testing of models and subsequent refinement based on explanatory strength of various pathways of influence through chains of linked variables, and will be used to test the influence of plant, soil and aquatic microbiome composition on ecosystem functions. Analyses testing hypotheses represented in Fig. 4 and alternatives will be conducted iteratively in conversation with all faculty, with alternative models being evaluated using information criteria (e.g. AICc, Bever et al. 2013).

4.3.f.2 Theoretical Integration (Agusto, Reuman, Billings, Bever, Sturm KU; Dodds, Platt KSU). We will complement this statistical effort by constructing analytical and simulation models of individual subcomponents, including pathogens and mutualists in the plant microbiome, C and N transformations in soil and sediments, and algal dynamics in reservoirs. For example, we plan to project pathogen burden across the precipitation gradient by parameterizing general models of host-pathogen interactions (e.g. Agusto and Gumel 2013), using data from Sections 4.3. Pathogen burden will also depend upon plant diversity and microbiome composition, which we include with additional submodels (e.g. Platt et al. 2012). We will extend and parameterize these and additional models (both formalized and conceptual) addressing mutualists (e.g. Bever 2015), N transformations (Billings and Tiemann 2014) and algal dynamics (Carpenter et al 2008) with data collected in Sections 4.3 so that individual submodels will be used to test connections of land use and climate history to microbial and ecosystem functions (as represented in path models). We will build on current efforts to model SOC dynamics in a changing climate, modifying them to apply models of SOC pool size to the scenarios imposed by our research platform. Specifically, we will assess how variation in microbial substrate availability with land use and precipitation influences SOC pools via models such as Eqn. 1:

$$\frac{dB}{dt} = E\mu_a B \frac{C_a}{C_a + K_m} - \mu_{mb} B \quad \text{Eqn. 1}$$

where B is microbial biomass C, E is microbial C use efficiency (described in Section 4.3.b.4, SMB Hypothesis b), C_a is assimilable C, and μ_a and μ_{mb} are rate constants for assimilable C and microbial biomass generation (Wutzler and Reichstein 2013; Wang et al. 2016). This approach, and related equations, allows us to project how different SOC pools (our example here is just one, microbial biomass) will change as C_a and other SOC pools are modified across land use and precipitation gradients, and depth. Our parameterization of SOC pool sizes will be enabled by our empirical data. We will aim to integrate our diverse submodels by coupling them to test their relative and interactive importance on net ecosystem properties (e.g. plant productivity, greenhouse gas flux, SOC pool sizes, water quality). These results will be compared to experimental results as a means of testing model accuracy, and the models themselves will be used to explore conditions for thresholds between alternative states.

4.3.g. Cyberinfrastructure

We have excellent expertise in many areas of microbial ecology; supporting additional environmental metagenomics faculty members, however, will help advance the work of these research groups and keep them at the cutting edge. Accordingly, we will hire a new faculty member at KU in bioinformatics to complement the recent hire at KSU (*Sanzhen Liu*) to enhance and integrate this expertise across campuses using cyberinfrastructure. We will also use funding to improve our hardware cyberinfrastructure. At KU, we propose: 1) three to six computing nodes and storage for the Center for Research Computing, 2) 50TB of backed-up storage at Advanced Computing Facility (ACF), with storage for at least 10 years, 3) a RAID drive for local back up, and 4) 3 dedicated desktop computers to interface with ACF. At KSU, we will obtain access to resources through the Institute for Computational Research in Engineering and Science including a storage node (88TB) and 20 archival storage drives to be shared via Globus for

efficient, scalable data transfer. Use of the Beocat cluster is available to all KSU faculty and students and collaborators (e.g. WSU, KU). We will enhance high speed networking (10 GbE switches and fiber optic connection) in individual labs. Increased speed of networking is increasingly important for moving data among institutions and to take advantage of the new 100 gigabit-per-second HyperCore network slated to come online this summer, replacing the 10 gigabit-per second backbone that connects KSU, KU, KU Medical Center, and WSU. The HyperCore also provides connection points to regional, national and international networks that constitute the high-performance research and education internet so researchers can share data with collaborators around the world.

4.3.h. Project timeline (4 quarters per year a - summer, b - fall, c - winter and d - spring).

Task (deliverable)	2017				2018				2019				2020				2021			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
Buy equipment	■	■	■	■																
Hire project personnel			■	■	■	■														
Faculty hires					■	■	■	■												
Annual meeting				■				■				■				■				■
Review (report and response)				■				■				■				■				■
Field surveys (electronic database)		■	■			■	■			■	■			■	■			■	■	
Initial experiments (data anal. done; in Focused experiments						■	■	■		■	■	■		■	■	■		■	■	■
Attend national meetings (> 50 presentations plus posters including 15-20 Prepare publications (~ 150-200 total)						■	■	■		■	■	■		■	■	■		■	■	■
Write res. and edu. proposals (5-10 major)						■	■	■		■	■	■		■	■	■		■	■	■
HERS coordinated into MAPS				■		■	■	■		■	■	■		■	■	■		■	■	■
LSAMP coordinated into MAPS				■		■	■	■		■	■	■		■	■	■		■	■	■
MAPS REUs				■		■	■	■		■	■	■		■	■	■		■	■	■
KS Ecosystems Institute for Secondary				■		■	■	■		■	■	■		■	■	■		■	■	■
Train postdocs/ grad students		■	■	■		■	■	■		■	■	■		■	■	■		■	■	■
Mentor junior faculty (tenure, awards, other)	■	■	■	■																

4.3.1 Workforce Development

The MAPS project seeks to build a diverse and competitive workforce in related STEM fields by pursuing four objectives as outlined below. The timelines are provided in Section 4.3.h.

Workforce Objective 1: Implement a program of faculty professional development.

Rationale: Recent faculty recruitment of strong researchers in ecology, geology, plant pathology, and microbial biogeochemistry, are steps forward in building MAPS research capacity. Nonetheless, Kansas still lacks a systematized model for ensuring that diverse and junior faculty members build successful careers. KNE has acknowledged a strategic need to improve various axes of diversity in its senior and, particularly STEM-focused, faculty representation, an approach that starts with a necessary focus on faculty tenure success, research competitiveness, and thus long-term retention.

Early Career Mentoring. As part of their career development, focus will be on providing an incubator-like setting for early career faculty members. Junior faculty will be cross-mentored by senior MAPS investigators, who among other activities will 1) provide constructive reviews of drafts of papers and research proposals; 2) support professional skills training; and 3) advise on professional growth and career advancement plans. As part of this process, we will involve junior faculty in leadership positions and mentor them in leading collaborative groups of researchers. Within this project, 10 of the 26 tenure track faculty researchers are assistant professors. **Metrics: Base, 0 faculty; Yrs 1-5, 6 faculty/yr.**

First Awards. Five to seven First Awards up to \$100,000 per award will be funded yearly based on NSF-formatted proposals written by Assistant Professors in areas related to MAPS. First Awards are for junior faculty to help in the start of their independent academic career. Proposal submission will be in response to a yearly call for proposals. Awardees will be selected based on out-of-state reviews and review by the Chief Research Officers of the participating institutions. This continues a successful ongoing KNE initiative. **Metrics: Base**, 6 faculty/year; **Yrs 1-5**, 5-7 faculty/yr.

Research Merit Awards: At the end of Year 4, five meritorious MAPS researchers will be selected, based on their research achievements during the course of the award, to receive Research Merit Awards of \$50,000 each. Preference will be given to junior faculty, and thus will promote ongoing career development. **Metrics: Base**, 0 faculty; **Yr 5**, 5 faculty.

Workforce Objective 2: Train the next generation of MAPS scientists by fostering an active MAPS research environment for undergraduate and graduate students.

Rationale: KNE recognizes that the breadth and scope of the proposed initiatives will result in an expanded and highly capable future STEM workforce. Goals are to create new pipelines to students from the wide-ranging cultural, ethnic, and geographical landscape of Kansas by introducing them to the cutting edge research of this project, as well as mentorship of students and early career faculty.

Research Experiences for Undergraduates. There are NSF-funded Ecology and Evolutionary Biology programs at both KU (DBI-1262795) and KSU (DBI-1460802). These initiatives will be leveraged by providing stipends for six students each year, participating either in the REU programs or working with WSU researchers. We will also use the LSAMP and HERS programs (described below), to recruit underrepresented groups into our REU slots. In addition, the KSU Konza REU program has committed to supporting at least one student per year affiliated with this project, as well as hosting other KSU REU students within their project that are funded by us to help synergize both projects. KNE will support six REU MAPS students who will interact through biweekly teleconferences with student/faculty presentations. There will also be trips to KU, KSU, and WSU Field Stations. **Metrics: Base**, 0 participants from this project, **Yrs 1-5**, 6 annually.

Collaboration with Kansas LSAMP Program. The Kansas State Louis Stokes Alliances for Minority Participation (KS-LSAMP, HRD-1305059) program has created an alliance between KSU and four minority-serving Kansas community colleges (Dodge City Community College (Dodge City), Garden City Community College (Garden City), Seward County Community College (Liberal) and Donnelly College (Kansas City, KS), emphasizing transfer of students to four-year institutions and recruitment and retention of STEM students at the community colleges and at KSU. During this program, students funded by KNE will be paired with a KSU faculty member and introduced into a research environment. The transfer rate of KS-LSAMP summer research students into STEM majors at 4-year institutions is currently 68%. This KNE initiative will support 3 LSAMP students. **Metrics: Base**, 0 LSAMP participants; **Yrs 1-5**, 3 participants/yr.

Haskell Environmental Research Studies (HERS) Institute. This project will prepare future Native American STEM professionals to understand the myriad impacts of environmental change and develop strategies for sustainable responses that combine Indigenous ecological knowledge with scientific approaches. Prof. *Jay Johnson* in the Department of Geography at KU, with co-PIs, Prof. *Joseph Brewer*, a new faculty hire in Geography at KU, and *Melinda Crow*, currently Chair of Environmental Science at HINU will oversee the program. Once the new hire at HINU is made he or she will also help to oversee HERS Institute. The eight-week summer internship will provide instruction and field research experiences in wetland, prairie, and mountain ecosystems. Goals are to: 1) recruit American Indian, Alaska Native, Native Hawaiian and Pacific Island students into STEM disciplines, and 2) provide professional development and mentoring for Native American graduates to pursue graduate educational opportunities. The curriculum includes research methods and food, energy, and water systems science integrating Indigenous knowledge and scientific data. MAPS researchers will also present talks to the interns, and interns will visit MAPS research labs during the course of the program, for example KSU researchers' labs during a trip to the Konza Prairie. Recruitment plans include working with tribal colleges and

backbone organizations such as the American Indians Science & Engineering Society to identify strong candidates. Interns will receive seven weeks of classroom and field instruction including time at the Konza Prairie Biological Station and the University Corporation for Atmospheric Research (UCAR) in Boulder, CO to provide research tools on ecosystem sustainability in Indigenous communities (e.g., field ecology, GIS, air and water quality monitoring). The interns will choose individual research topics at the intersection of Indigenous and sustainability sciences that are aligned with the project. Interns will finalize a research paper based on their projects in their tribal community during week eight. Three Native American graduate students at KU will serve as mentors. The program director and the co-leaders will document and publish details of this program's design and curriculum so that other tribal and minority serving colleges can learn from our successes and challenges. Interns will interact with researchers via mentoring and discussions of research. Year 1 will be used for planning for the new Institute and program development. **Metrics: Base**, 53 students; **Yrs 2-5**, 15 interns; 3 GRAs/yr.

Undergraduate and Graduate Student Travel Awards. Up to ten travel awards of \$500 per award will be funded yearly based on an open application process for students working in areas related to MAPS. Awards will be granted based on merit review by project researchers, and will focus on students from underrepresented groups. **Metrics: Base**, 0; **Yrs 1-5**, 10 students annually.

Workforce Objective 3: Create new pipelines for K-12 students and teachers from diverse backgrounds that integrate them into research.

Rationale: Education and workforce development initiatives will span the range from K-12 and beyond to colleges and universities. Lesson development and research opportunities constitute just two of the initiative venues. During the strategic planning retreat required by NSF at the start of the project, each of the researchers will volunteer for education and workforce development initiatives. There will be the opportunity to change these choices during the course of the project. This organizational structure will allow all the researchers to participate in a variety of initiatives and will result in interweaving the education and science initiatives so that both benefit from the close relationship. For classroom guest lectures, schools targeted will be rural schools or inner city schools with large percentages of economically disadvantaged or minority students. Since KU, KSU, and WSU have all had NSF GK-12 grants, we already have established fruitful relationships with such schools, and those resources will be leveraged under these activities.

Elementary School Students. Dr. *Peggy Schultz*, KU Environmental Studies and the Kansas Biological Survey, will lead researchers, who will develop and implement inquiry-based K-5 science curricula that enhance student performance on the Kansas Next Generation Science Standard assessments. Experimentally-driven lessons will focus on elements of the ecosystems of Kansas including native plant communities, agricultural areas and aquatic systems. Project research will be integrated into grade-level curricula through discussion with school district science coordinators and master teachers. MAPS affiliated faculty and graduate and undergraduate students will provide support in the classrooms (e.g., discussions, lessons). The program will be initiated in rural, urban and Title 1 schools in Lawrence, Kansas City, Topeka and surrounding school districts with the expectation that the program will expand to Manhattan and Wichita from either KNE Research and Education Innovation Awards or external funding. Pre- and post-assessments of student learning will be utilized. Dr. *Schultz* has over five years of experience running similar programs at Indiana University and will serve as coordinator. Past outreach experiences by faculty members provide a strong foundation for this work. Examples include involvement with KSU's Agronomy Kids Field Day (*Rice*), sharing basic economics with elementary students (Earnhart), and Girl Scout involvement (*Sturm*, activities for "Wonders of Water" badge). **Metrics: Base**, 0 participants, **Yrs 1 - 5**: 140 - 200 students.

Secondary Teachers and Students. A Kansas Ecosystems Summer Institute for Secondary Teachers led by Dr. *Schultz* will facilitate the integration of ongoing research of the PIs' with the Kansas secondary school Next Generation Science Standards. Dr. *Schultz* led two similar successful summer institutes at Indiana University. With the aid of the Science Consultant for the Kansas Department of Education, science teachers from across Kansas will be invited to apply and 10 will be selected annually to learn

about participating faculty members' research and to work with individual faculty members to develop innovative, transferable exercises and experiments focused teacher and student needs. Project faculty members have already participated in secondary teacher initiatives. For example, Alexander co-led an institute at the KU Field Station that led to the creation of a 0.57 ha ecology experiment on the Free State High School campus, Lawrence, KS. *Alexander, Sikes, and Schultz* have studied plant and microbes at this site with students from Free State and JC Harmon High School (an urban Kansas City school). *Sturm* has also been involved with NSF RET programs. Assessment of model lessons will occur in years 2-5 by an advisory panel selected with input from the Kansas State Department Science Consultant. Curricula will be shared with teachers the Kansas Association of Biology Teachers websites. **Metrics: Base, 0** participants, **Yrs 1, 6** teachers, **Yrs 2 - 5: 10** teachers/yr with potential to impact 1800 students/year.

Workforce Objective 4: Connect to Community Needs

Rationale: MAPS will be a nexus for research alignment with Kansas' communities to encourage participation across STEM fields. Team leaders will meet with community stakeholders at least twice a year in planned meetings and at informal forums in addition meeting with private company CEOs and project managers, including those serving on the EPSCoR Steering Committee. Overall goals for these interactions will be to: 1) identify applications for the research conducted and users for the knowledge embedded in MAPS; and 2) provide internships and other industry-based training opportunities.

The Konza Environmental Education Program (KEEP). The Konza Environmental Education Program's objective is to teach the process of science through hands-on ecological research at the Konza Prairie Biological Station. Docent volunteers lead informative nature hikes to 4500 visitors/year, 75% of which are USD (Unified School District) 475 school district students (50% minority and underserved groups). A week-long summer workshop provides 3-10 K-12 teachers the opportunity to learn about the prairie, construct tailored lessons for their class, and plan future classroom visits. Researchers will present talks in the docent training program and at teacher workshops (3/year). Fact sheets for microbiomes in prairie will be provided to be used as educational materials (1 per year). Several of our researchers have an active working relationship with KEEP (*Zeglin, Dodds, and Jumpponen*). **Metrics: Base, 0** participants, **Yrs 1-5, 10** docent trainees and 3 - 10 teachers annually.

4.3.2 Seed Funding and Emerging Areas

Seed funding will be provided through several mechanisms, First Awards, Research Merit Awards, and Research and Education Innovation Awards (Section 4.3.1). First awards, as noted, are for junior faculty at the Assistant Professor level to help in the start of their independent academic career. Research Merit Awards are designated for highly productive researchers (through the course of this initiative) to continue in new expanded directions as broadly related to MAPS. Research and Education Innovation (REI) Awards are for small projects that will either allow for networking and planning or for the immediate pursuit of larger projects for developing new transformational concepts. These are awarded for both research *and* education, given the close relationship of research and education. The maximum award will be \$50 K for one year. These awards are in the same spirit as and share goals with NSF EAGER (Early Concept Grants for Exploratory Research) awards, for high risk/high gain research ideas. An open call for proposals will exist, with submissions being evaluated by a subcommittee of the Council of Chief Research Officers (CoCRO) of the Regents Universities as several times each year. Funding decisions will be based both on NSF merit review criteria and additional review criteria to promote collaborations across institutions and disciplines.

4.4 Diversity Plan

Rationale: The importance of increasing the STEM workforce, especially expanding the participation of women and minorities, is well documented (Changing the Equation 2016, Carnevale et al. 2011, Olson et al. 2012, Cozzens 2008, Jackson 2001). The number of STEM college degrees and certificates in Kansas fell 13% in the last decade (Changing the Equation). As Robin Kimmerer noted, "*Native Americans are the most underrepresented group in the American scientific community.*" A decade later, in 2012, only 13 of the 102 doctorates awarded to American Indians and Alaska Natives were in the physical sciences and

engineering, demonstrating a significant decline from 0.5% to 0.3% of total doctorates awarded (Kimmerer, 2002). Additionally, women and persons of color accounted for 28% and less than 10% of 4-year STEM degrees in 2009, compared to the national percentage of 30 and 18%, respectively (Changing the Equation 2016). KNE is a strong advocate for and is dedicated to supporting innovative solutions, such as out-of-school programs related to STEM careers, and improvement of STEM content and pedagogy in classes, to increase the percentages of underrepresented groups in STEM areas.

The MAPS project currently includes 27 faculty/research scientists, including 12 women and four underrepresented minorities. MAPS will provide an inclusive environment for STEM research in Kansas. Our goal is to increase the participation of women, persons of color (particularly Native Americans), first generation college graduates, and researchers from rural Kansas backgrounds by significant percentages (> 30%) within each of the research initiatives. Three objectives for successful accomplishment of this goal are described below.

Diversity Objective 1. Create new programs for K-12 students and teachers from diverse Kansas areas that introduce them to MAPS science.

K-12 Students and Secondary School Teachers: Three of the initiatives described in earlier involve these students and teachers (see Section 4.3.1 *Workforce Development*, Workforce Objectives 3 and 4 for details), including a significant number of inner city and rural area schools. These are the schools that have significant populations of minority, disadvantaged, and first generation college students. These programs are anticipated to reach up to 200 elementary school students and 20 teachers each year.

Diversity Objective 2. Continue to build momentum in a Native American internship program initiated in the previous funded Track 1.

HERS Institute. The leaders of the *Pathways to STEM Careers* summer intern program, made possible by NSF EPSCoR funding six years ago, have successfully started an expanded ongoing program with KNE funding during the academic year. Current collaborations (and outcomes of the prior funded project) include dual-campus undergraduate courses and a KU-based Professional Science MS degree in Environmental Assessment. From its start with 10 students in the first year, the summer internship now has a critical mass of 15 interns per year. A new HINU faculty member, who will become a member of the MAPS team, will be recruited in environmental science and GIS. In six years, the program has graduated 53 interns, with 19 currently or recently completing graduate degrees at U.S. universities (almost 40%). Interns who have graduated have received NSF Graduate Research Fellowships, IGERT Fellowships, the Udall scholarship at Dartmouth, and a Sloan Fellowship (Cornell). The internship program will be continued with expanded nation-wide recruitment and year-round professional development activities for former interns and other HINU students. Crow, co-lead of the internship, who has an M.S. degree in environmental science, will start her Ph.D. degree at KU with release time provided by this funding. Our goal is 50% or more HERS graduates attending graduate school. We also plan to build upon existing collaborations between the Environmental Sciences/Studies programs at HINU and KU. The leaders of this project expect to obtain sustainable funding by year five of the project (see Workforce Objective 2, Section 4.3.1 *Workforce Development*).

Diversity Objective 3. Leverage already established programs with additional funds to target minority populations.

LSAMP: MAPS will partner with and leverage the number of students recruited as part of the KS-LSAMP initiative at KSU (See Workforce Objective 2, Section 4.3.1 *Workforce Development*).

REUs: Minority and other underrepresented students will be sought for these programs from mechanisms that span from dissemination of REU opportunities to minority serving institutions, PUIs, and community colleges, particularly in rural areas of Kansas. Additionally, students in the LSAMP and HERS internships will be strongly encouraged to apply for the REU programs at both KU and KSU that are being leveraged by MAPS funding (see Workforce Objective 2, Section 4.3.1 *Workforce Development*).

4.5 Partnerships and Collaborations

MAPS will expand its current partnerships and collaborations (Microsoft, National Ecological Observatory Network, Critical Zone Observatory Network, Novozymes, Monsanto, The Nature

Conservancy, and National Corn Growers), and begin new partnerships/collaborations (National Supercomputer Centers, MycoBloom) to provide opportunities for commercialization of research and education products and to pave the way for economic development in Kansas. As the program grows (years 2 – 5), new partnerships (five or more) will be identified and solidified.

Cyberinfrastructure Collaborations. *Microsoft:* Researchers at KU (Sykes from this project) are collaborating with Internet2, Microsoft Research, and the Microsoft Azure cloud service to produce comparative analyses of bioinformatic pathways using Azure. *National Supercomputer Centers* such as XSEDE (Extreme Science and Engineering Discovery Environment) and NCSA (National Center for Supercomputing Applications) will provide supercomputing platform for projects that require high computational power (Liu).

Foundation Funding. *Earth Microbiome Project:* Earth Microbiome Project is a major multidisciplinary effort to characterize and study microbial communities across the earth from a taxonomic and functional perspective. Individuals and groups of researchers in the MAPS project will collaborate with this organization by submitting sequence data, as well as integrating their work with the large microbiome databases at EMP. EMP is currently funded by the John Templeton Foundation (Convergent Evolution of the Vertebrate Microbiome Award #44000) and the W. M. Keck Foundation. Previously, the project was supported by Argonne National Laboratory (ANL, U.S. Dept. of Energy Contract DE-AC02-06CH11357).

Federal Funding. *NEON: The National Ecological Observatory Network* consist of US sites that are representative of distinct landforms, vegetation, climate, and ecosystem dynamics. While there has been a recent change in management in NEON, two of our core research sites (e.g., Konza Prairie Biological Station, University of Kansas Field Station) are terrestrial NEON sites, and one (Konza) is an aquatic site. The sampling units have been installed and the sites are already partially operational. The NEON program collects and has an open data policy on environmental parameters including land use, land cover, biogeochemistry, and ecohydrology as well as sequencing microbiomes. These data will ultimately place ours in a longer-term context, allow for KNE researchers to synergize their efforts with others involved in using NEON data, and provide independent verification of our results. Co-PI *Dodds* has advised NEON on aquatic sampling and Group Leader *Zeglin* provides feedback on NEON microbiome protocols as a current member of the NEON Microbial Technical Working Group. **CZO network:** NSF's Critical Zone Observatories investigate Earth processes from vegetation canopies down into groundwater systems. This original geologically-focused network is increasingly populated with biologists, as the importance of understanding ecosystem dynamics in a changing environment becomes more appreciated (Richter and Billings 2015). Datasets revealing interactions among each site's geology, hydrology, ecology, and microbiomes are increasingly made available via the CZO web interface and genomics-focused web platforms, and some researchers who are part of the MAPS team work at multiple CZOs and are active contributors to the development of a CZO proposal for the Konza Prairie. Co-PI *Billings* and colleagues' CZO work was recently featured as a NSF "Science 360" headline (Dialynis et al. 2016), and *Billings* contributes to efforts hosted by ANL to coordinate NEON, CZO, and Long-Term Ecological Research microbiome sampling. *Sullivan, Dodds, Rice, Billings* and *Kirk* are working to obtain a CZO site in Kansas when the next solicitation is released.

Other Partnerships/Collaborations (including a new start-up). Microbial cultures that are found to benefit plant production, soil health or water quality will be made available to start-ups. For example, native AM fungi isolated and characterized in the lab of *Bever* (co-PI) and *Schultz* (Education Lead) have been found to improve establishment, growth and restoration success of prairie plant species (Middleton et al. 2015). These cultures have been distributed to restoration practitioners and land managers through the microbial inoculant start-up company MycoBloom. MycoBloom is moving to Kansas and is interested in distributing AM fungi found to be beneficial within Kansas' climatic regions. Co-PI *Rice* has been in conversation with Novozymes and Monsanto on microbial assessments and is involved in a partnership with The Nature Conservancy, National Corn Growers, and Monsanto to assess soil health.

4.6 Communication and Dissemination Plan

The goal of the Communication and Dissemination Plan is to reach a broad audience of researchers, educators, students, stakeholders and the general public via the platforms below to promote MAPS activities highlighted in the research and education programs. Communication and dissemination will include the collection, preparation, and dissemination of project news via various outlets. These efforts will be led by the KNE Education and Outreach Coordinator (*Rosemary Blum*) with assistance from other KNE staff and researchers. Specific activities are briefly described below:

Research. MAPS scientific results will be disseminated via a number of different venues. These include publications, some of which may be chosen to be highlighted/publicized by the journals as journal covers or via other press releases. Researchers will publish their work in journals and other high impact venues (e.g., book chapters), and will attend national and international meetings to network and present posters or talks. A website will be created for the research project where information regarding highlights, events, and other items of public and professional interest will be posted.

Education Outreach. To inform the K-12 science curricula needs of the State, the KNE office will consult with the Science Consultant for the State of Kansas to integrate MAPS curricula with the Kansas K-12 Next Generation Science Standards, train teachers to connect the research to their lessons, and create peer reviewed activities to share on the Kansas Association of Biology Teachers' website.

Museum Outreach. In the current Track-1 project, KNE started a program of museum outreach that spanned across an art museum (Spencer Art Museum at KU), a Natural History Museum (KU), and the newly-opened Flint Hills Discovery Center in Manhattan. The exhibit at the Discovery Center is now traveling throughout Kansas courtesy of the Kauffman Museum Foundation. These museum exhibits elicited such positive responses that we will build upon and expand them to MAPS research as results are obtained, including both the original museums and those in more remote (western, southern and northern) areas of Kansas. Projects will be chosen by consultation between museum staff, researchers and KNE office.

Statewide Meeting. An Annual Statewide Meeting will be held, its location rotating among campuses. Results and impacts of the project's activities will be presented to an audience of faculty, students, educators and stakeholders as well as the Science and Education Advisory Committee. Panels and breakout sessions will afford interaction and audience feedback. Panelists will include Kansas stakeholders (CEOs and Program managers of Kansas-based companies) as well as scientists relevant to MAPS research (e.g., NEON and CZO leaders).

Social Media. KNE maintains broad public outreach and communication through several methods: YouTube Channel, Facebook, Twitter, a Blog, as well as providing an active social media presence posting the latest EPSCoR content as it develops. Capitalizing on the social media connection, Twitter will also serve as one of the recruitment strategies for advertising the summer REU opportunities, Graduate Student Travel Grant opportunities, and EPSCoR sponsored middle school and high school programs. Capitalizing on the NSF EPSCoR's Communicating Science Workshops, short videos (one to three minutes) will be created based on Highlights collected for the NSF reports.

4.7 Sustainability Plan

4.7.1 Education and Human Resources Development

The goal of MAPS is to use this new multi-institutional, multi-disciplinary project, already consisting of a significant number of women and minorities, to leverage the research capacity in Kansas through strategic investments of personnel plus new research infrastructure and education initiatives. Implementation of these initiatives will have significant impact not only on Kansas universities, but will reach throughout the State to many Kansas stakeholders. Milestones and timelines for these initiatives are provided in Table 1. MAPS will provide a supportive, collegial environment within which women and other underrepresented minority scholars will thrive. See Section 4.7.2 for some of the extramural funding anticipated to enhance education and human resources.

Three new faculty hires will also have significant impact on education and human resources. A critical need for this project as well as for the entire research enterprise in Kansas is a strong foundation in bioinformatics. KSU recently hired research team member *Sanzhen Liu* in microbial bioinformatics. Three additional faculty hires will be made, one each at KU, KSU, and HINU. For KSU and KU the hires will be in bioinformatics with expertise in environmental genomics. The ability to deal quantitatively with large amounts of genomic data coupled with environmental data will increase the scientific depth of our MAPS group and increase probability for future funding. The new hire at HINU will be in environmental sciences and will be instrumental in helping to build the HERS Institute.

4.7.2. Post RII Track -1 Extramural Funding

The goal of MAPS is to use new research findings in microbiome science to submit compelling proposals for additional federal funding from a variety of sources. The recognized need for understanding the role of microbiomes throughout ecological systems has led to a number of targeted federal initiatives. The MAPS project positions Kansas researchers to be more competitive for these opportunities, especially focusing on the microbiomes of the critical zone in agricultural landscapes. Opportunities include INFEWS (Innovations at the Nexus of Food, Energy and Water Systems), CZO (Critical Zone Observatories) and standard NSF Department of Environmental Biology grant opportunities. Synergies with other investments in Kansas, such as the Konza Long-Term Ecological Research site, will provide other mechanisms for sustainability. DOE, NSF, NIH, and USDA all have opportunities for funding. At DOE, most of the opportunities come out of the Joint Genomic Institute. USDA's initiatives are more aligned with MAPS. In particular, a joint NSF/USDA initiative for technology development for microbiomes science has put out a call for EAGER proposals, NSF 16-058, to "Develop and Enable Breakthrough Technologies for Animal and Plant Phenomics and Microbiomes." Other targeted programs are: NSF-NIH Ecology and Evolution of Infectious Disease, NSF Dynamics of Coupled Natural and Human Systems, and NSF Dimensions of Biodiversity Program. Given that EAGER awards are meant for exploratory work in its early stages, offshoots from MAPS as early as Year 1 would be a possibility. Timelines for most submissions would be in years 2-5, or for specific limited calls, within the timeline given.

Educational initiatives to be pursued include an NSF NRT (NSF Research Traineeship) midway through the project on microbiome feedbacks. The project will provide a nucleus for the advancement of women in engineering and science that could be developed as an NSF ADVANCE (Increasing the Participation and Advancement of Women in Academic Science and Engineering careers, NSF 14-573) or INCLUDES (Inclusion across the Nation of Communities of Learners of Underrepresented Discoverers in Engineering and Science, NSF 16-544) program across the STEM fields. In addition to single investigator initiatives, several multi-investigator projects will be planned and submitted to the NSF or other federal agencies. It is also noteworthy that many faculty members on this project already are involved in STEM outreach programs for K-12 students and teachers that will impact the next generation of scholars. It is anticipated that other external funding will be in hand by year five to sustain the HERS Institute, such as INCLUDES or TCUP (Tribal Colleges and Universities Program, NSF 16-531) funding.

MAPS will facilitate new collaborations within and among universities, as a result of the constant conversation among researchers in the three research areas of plant, soil and water microbiomes. While we work on different microbial functions, we all draw upon similar molecular infrastructure and informatics tools, which could be part of larger shared instrumentation and computational infrastructure building proposals, e.g., NSF MRI (Major Research Instrumentation, NSF 15-504) to be submitted early in the project (year 1 or 2).

4.8 Management, Evaluation and Assessment Plan.

4.8.1 Project Management Team

The **management structure** is provided in Scheme 1. The Kansas Board of Regents (KBOR) provides matching funds for the project, and hence provides State oversight of KNE. KBOR appoints members to the EPSCoR Steering Committee (seven members) that evaluates projects going forward to NSF and

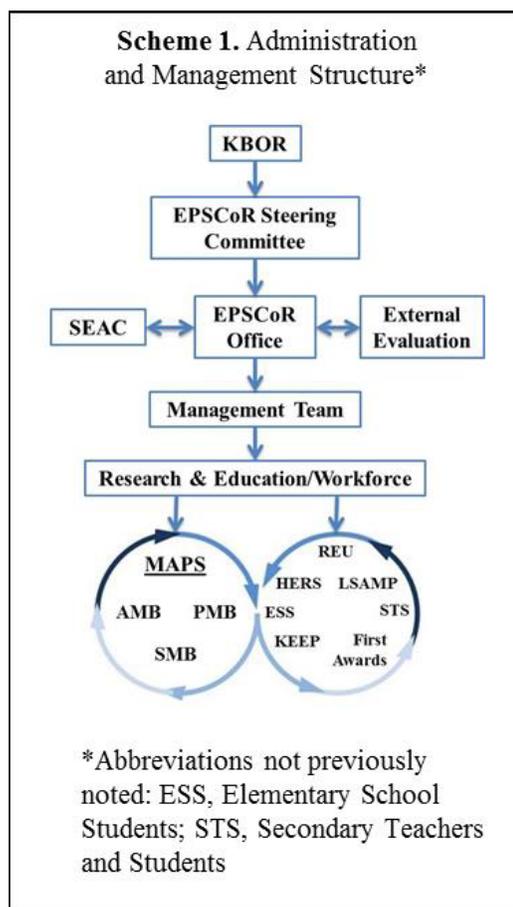
decides on matching funds. The Chair, Stan Ahlerich, is a member of the Kansas Department of Commerce and is Executive Director of the Governor’s Council of Economic Advisors. The Committee is made up of business managers, two CEOs, one academic (Dean), and a government official. The Project Director also consults with the Council of Chief Research Officers (CoCRO) of the Regents Universities several times per year. Members of these advisory committees are also invited to the KNE Yearly Symposium.

The **KNE office** is responsible for effective and efficient operation and supervision of EPSCoR projects. The Project Director (PD)/PI (*Bowman-James*) administrates all KNE operations and external relations (including institutional leaders and KBOR constituencies); provides vision and leadership; and oversees report preparation, proposal writing, and strategic planning for current and future initiatives (the latter with input from the Steering Committee). The office staff consists of an Assistant Director (PA, *Doug Byers*), a Business Manager (*Candi Wilbur*), and an Education and Outreach Coordinator (*Rosemary Blum*). The PA oversees project management including such as data collection for reports, interacting with other jurisdiction PAs to determine Best Practices. The Business Manager is in charge of fiscal oversight for the project and budget preparation. The Education and Outreach Coordinator oversees and monitors all external communication of research and education initiatives (K-12, Field Stations, museums, etc.).

The **Management Team** will consist of the four co-PIs for the research project, *Dodds* (KSU), *Rice* (KSU), *Billings* (KU), *Bever* (KU); the lead investigator for HERS, *Johnson* (KU); and the KNE PD, PA and the Education and Outreach Coordinator. They will meet on a monthly basis. The team leaders of the research initiatives will meet on a biweekly basis for day-to-day management of the project. The team leaders, researchers and students will also hold biweekly research meetings for each of the research areas: plant, aquatic, and soil microbiomes, with larger inter-group meetings every two months. Additional detail about the management structure within the research initiative is described in Section 4.3.a.

The **Science and Education Advisory Committee** (SEAC) will provide objective scientific and education input and guidance. This committee will be informed by a yearly external evaluation (see Section 4.8.2) and will provide input to KNE on a) progress toward goals and scientific objectives, b) necessary adjustments as the

project progresses, and c) feedback on NSF reactions after reverse site visits. The SEAC meets once a year during the yearly statewide conference. The four members confirmed (two women, two men, of whom one is a minority) are: Dr. *Ed Galindo*, Professor of Soil, Water and Environmental Science, Director, Natural Resources Tribal Cooperative of the Aquaculture Research Institute, University of Idaho; Dr. *Katharine Suding*, Associate Professor of Ecology and Evolutionary Biology, University of Colorado; Dr. *Laura G. Leff*, Professor and Chair Biological Sciences, Kent State University; and Dr. *Jim Tiedje*, University Distinguished Professor and Director of the Center for Microbial Ecology, Michigan State University. One more committee member will be invited. The close collaboration among the oversight groups, the SEAC and the External Evaluation Team will allow for mid-stream modifications and quick responses to important new opportunities to maximize project impact.



Succession plans for the KNE Project Director include appointment of an Associate Director in the first two years of the project, who will become familiar with the running of the program. Several faculty members have been contacted and are under consideration. Project Directors are chosen by the Vice Chancellor for Research at KU and ratified by the CoCRO. Newly hired Foundation University Professors are under consideration because of excellent research portfolios and new ideas. The Associate Director will have a strong record of obtaining external funding and in working across disciplines.

4.8.2 Evaluation and Assessment Plan

An evaluation support and feedback system will assist KNE to attain the full range of proposed goals and objectives set out in the MAPS project. Evaluation and assessment of KNE will integrate formative and summative components that include a) ongoing tracking of performance metrics, b) review and guidance by the SEAC, and c) an annual external multi-method evaluation, all of which are designed with a focus on assessing capacity development for research, innovation, inclusion of diverse populations, and workforce development within KNE institutions and the State.

Ongoing tracking of performance metrics. To track progress toward goals, the KNE project team will develop and implement a performance tracking process designed around NSF annual report metrics and external evaluation needs. An online reporting system will enable reporting to NSF. Table 3 provides an illustration of examples of activities and benchmark targets.

Table 3. Example Performance/Process Benchmark Targets.

Strategic Activity	Year 1	Years 2-5
Produce new knowledge	5 journal articles	Average 1.5/year/researcher
Hire new faculty	None	KSU, KU and HINU faculty hires
Recruit graduate students	14 students hired	Recruitment as needed
Submit proposals	4 single investigator	8 per year
Support student research	3 students present at conferences	10 presentations/year
Expand HERS Institute	Integrate new curriculum	15 Native American interns/year

The **external evaluation** will provide formative input and summative assessment to complement the ongoing collection and reporting of performance metrics. This effort will be led by Dr. *Eric Welch*, Professor of Public Affairs and Director of the Center for Science, Technology and Environment Policy Studies at Arizona State University. Dr. *Steven Maynard Moody*, Director of the Institute of Policy and Social Research at the University of Kansas, will serve as the internal liaison to the evaluation and focus on its qualitative and formative aspects. The evaluation team will be in regular communication with the KNE Director. Once the project strategic plan is in place, the evaluation team, in collaboration with the project leaders, will develop a logic model to provide the theoretical connection between goals and outcomes/impacts. Evaluation design and data collection address the following topics (Table 4): 1) production and knowledge impacts of scientific discovery and development of research capacity; 2) cross-project collaboration and learning; 3) interdisciplinary and cross-institutional collaborations across academic disciplines that affect program outcomes; 4) skill development, career progression, STEM interests, and other student impacts; 5) diversity initiatives and outcomes; 6) development and quality of outreach plans; and 7) overall functioning and management. The evaluation design and data collection effort will be guided by evaluation questions such as the following:

- How is the KNE Project functioning and developing overall? To what extent does management facilitate the research?
- What have been the scientific and knowledge development outcomes? How interdisciplinary are the academic outputs (publications and grant proposals)? What impacts does MAPS research have on the academic research community?
- What are the barriers and facilitators to collaboration across institutions, disciplines, and between students and faculty? How are early career researchers (postdocs, junior faculty) integrated?
- To what extent has KNE developed interactions with external stakeholders that advance project goals?

- How are students being integrated into the research? What opportunities and benefits are students realizing through their KNE experience?
- How have underrepresented minority students and teachers in partner institutions benefited from workforce development initiatives?

Table 4. Indicators and Metrics.

Broad Impact	Impact Category	Indicators/Metrics	Evaluation Methods
Capacity development through research collaboration, integration and production	Research Production and Impact	Academic production (# papers, presentations, inventions); journal impact factor; # citations; # grant submissions and awards; awarded %; national/international visibility and reputation (# awards, invitations, nominations)	Leadership team interviews; project document review (annual)
	Cross Institutional Capacity Development	Cross institutional production (#articles, conferences, grants); # cross-institutional ties, change in ties and tie structure over time; cross-project learning; knowledge exchange, collaboration, production	Faculty survey including collaboration networks (annual)
	Cross Disciplinary Capacity Development	Interdisciplinary academic production (# papers, presentations, publications); new collaborative teams/partnerships developed; evidence of interdisciplinary knowledge development (new journals; journal subjects)	
Workforce development through hiring, junior faculty and student impacts, and outreach	Junior faculty; new hires; postdocs	# new hires; evidence that postdocs, junior and new faculty integrated in research: collaboration structure, articles, conferences, grants, inventions	Key faculty interviews (annual)
	Student activity, interests and impacts	# student products (posters, presentations, publications, co-authorships, interdisciplinary interaction); changes in student interest in STEM fields/career direction; student learning outcomes (technical information, research process); dissertation activity; job/graduate school placement	Partner and stakeholder interviews (Y2, Y4)
	Outreach activities and impacts	establishment outreach program to govt./industry stakeholders; use of findings for practice/decision making; evidence effectiveness of workforce development activities	
State-wide capacity: partner campus / stakeholder integration/impacts	Stakeholder integration and impacts	Evidence of partners/stakeholders communication; perceptions and interest; student internships; perceived value of research for decision making, planning, strategy development.	Bibliometric analysis (Y3-Y5)
	Cross-campus integration and impacts	Cross-campus collaboration and production; data sharing and integration; evidence of changed methods/outputs	Online performance metrics data (annual)
Outreach to and integration of diverse populations	Project-wide diversity	% representation of gender, race, first generation, compared to campus levels; # of new Hispanic and low-income students in rural community college partners	Mini-Cases (Y2-Y4)
	HERS activities and impacts	# Native Americans in HERS; and; # HERS interns pursuing STEM degree, graduate programs, careers; participating in conferences (posters, presentations); evidence of coordination across campuses on Native American education; # students trained as URM mentors; perceived quality of mentors	Student network surveys (Y2-Y5) Student focus groups (Y2-Y5)
Project Implementing & management	Human resources, workshops, meetings, online data	Human resources hires (students, faculty, postdocs); # workshops held; annual meetings attendance, learning and effect on research; cross-project interaction and knowledge exchange; evidence of effectiveness of all mentoring programs	

The **external evaluation methodology** will involve a combination of quantitative and qualitative data collection, including *semi-structured interviews*, *survey data collection*, *bibliometric data*, and *mini-case studies*. Impacts and related indicators are presented in Table 4. Interviews, review of documentation, and surveys will be used to gather a rich set of data that address institutional development and management. Interviews will be conducted annually with KNE leadership and selected faculty.

Interviews or focus groups with students, industry/outreach partners and stakeholders will occur in years two through five. Surveys of faculty and students (years 2-4) will capture collaboration, productivity, workforce development, and outputs and outcomes. Survey data will also capture collaboration, productivity, workforce development, diversity and stakeholder activity, outputs and outcomes. Bibliometric analysis will be used to examine a) academic production, b) collaborative

development as evidenced by co-authorship patterns, c) interdisciplinary production (Porter and Rafols, 2009), and d) research impacts from citation data. Bibliometric data collection/analysis (years 3-5), will be used to examine a) academic production, b) collaborative interdisciplinary development, and c) research impacts from citation data. The evaluation will incorporate network data collection and analysis to demonstrate structural and relational dimensions of capacity development of the project. Network analysis will capture early cross-campus and interdisciplinary collaboration, as well as identify isolates, cliques, potential barriers to information and resource flows, and opportunities for greater inclusion. Network analysis will also be applied to map co-author networks. The evaluation will also conduct mini-case studies to examine key topical issues of importance to the project.

The annual evaluation report will examine progress toward accomplishments of goals and objectives, measure capacity relevant outputs and outcomes, collect evidence indicating post-award sustainability and provide recommendations designed to help the project better align activities with project goals. The project team will carefully consider the external evaluator’s report and will prepare a written response outlining changes or answering questions raised.

4.8.2 Summary Tables of Requested NSF Support

Budget Table A. Summary of Requested NSF Support by Institution (\$ K)^a

Awardee	Yr 1, \$K	Yr 2, \$K	Yr 3, \$K	Yr 4, \$K	Yr 5, \$K	5-Yr Total	%
KU-Lead	3,123	3,139	3,321	3,323	3,429	16,335	81.68%
KSU	280	606	627	645	346	2,504	12.52%
WSU	73	69	73	72	71	358	1.79%
FHSU	55	55	55	55	55	275	1.38%
Haskell	151	115	86	87	89	528	2.64%
Total	3,682	3,984	4,162	4,182	3,990	20,000	100.00%

^aDollars reported in the KU budget include those necessary to maintain the KNE office located on the KU campus, and includes a significant percentage of funds not yet allocated (via First Awards, etc., to other campuses).

Budget Table B. Summary of Requested NSF Support by Project Activity (\$K)

Activity	Yr 1, \$K	Yr 2, \$K	Yr 3, \$K	Yr 4, \$K	Yr 5, \$K	Tot, \$K	% of Total
MAPS –Equip. in Matching Funds	512	723	712	706	410	3,063	15.32%
Education	102	94	94	94	94	478	2.39%
Workforce Development	68	74	75	75	76	368	1.84%
Cyberinfrastructure	37	124	144	148	154	607	3.04%
Seed Funding	746	644	717	712	596	3,415	17.08%
Diversity Plan	360	314	316	306	306	1,602	8.01%
Partnerships and Collaborations	2	2	2	2	2	10	0.05%
Communications & Outreach Plan	107	111	115	119	124	576	2.88%
Sustainability Plan	30	30	30	30	280	400	2.00%
Management	381	399	417	438	454	2,089	10.45%
Evaluation and Assessment	140	142	147	153	159	741	3.71%
Indirect Cost	1,197	1,327	1,393	1,399	1,335	6,651	33.26%
Total	3,682	3,984	4,162	4,182	3,990	20,000	100.00%

5. References Cited

- A long-term vision for the future of water supply in Kansas.
http://www.kwo.org/50_year_vision/rpt_Kansas_Water_Vision_Final_Draft_012815.pdf
- Agusto, F.B., and A.B. Gumel. 2013. Qualitative dynamics of lowly- and highly-pathogenic avian influenza strains. *Mathematical Biosciences*. 243:147-162.
- Amundson, R., A.A. Berhe, J.W. Hopman, C. Olson, A.E. Stein, and D.L. Sparks. 2015. Soil and human health in the 21st century. *Science*. 348: doi: 10.1126/science.1261071.
- Bandyopadhyay, K.K., and R. Lal. 2014. Effect of land use management on greenhouse gas emissions from water stable aggregates. *Geoderma*. doi:10.1016/j.geoderma.2014.05.025.
- Banner, E., A. Stahl, and W. Dodds. 2009. Stream discharge and riparian land use influence in-stream concentrations and loads of phosphorus from Central Plains watersheds. *Environmental Management* 44:552-565.
- Barberán, A., K.S. Ramirez, J.W. Leff, M.A. Bradford, D.H. Wall, and N. Fierer. 2014. Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. *Ecology Letters*, 17: 794-802.
- Battin, T.J., K. Besemer, M.M. Bengtsson, A.M. Romani, and A.I. Packmann. 2016. The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology*. 14: 251-263.
- Beaver, J.R., E.E. Manis, K.A. Loftin, J.L. Graham, A.I. Pollard, and R.M. Mitchell. 2014. Land use patterns, ecoregion, and microcystin relationships in U.S. lakes and reservoirs: A preliminary evaluation. *Harmful Algae*. 36: 57-62.
- Bell, C.R., M.A. Holderfranklin, and M. Franklin. 1982. Correlations between predominant heterotrophic bacterial and physicochemical water-quality parameters in 2 Canadian rivers. *Applied and Environmental Microbiology*. 43: 269-283.
- Bender, S.F., C. Wagg, and M.G. van der Heijden. 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology & Evolution*. 31: 440-452.
- Bengtsson-Palme, J., M. Ryberg, M. Hartmann, S. Branco, Z. Wang, A. Godhe, P. Wit, M. Sánchez-García, I. Ebersberger, F. Sousa, and A. Amend. 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution*. 4: 914-919.
- Besemer, K., G. Singer, C. Quince, E. Bertuzzo, W. Sloan, and T.J. Battin. 2013. Headwaters are critical reservoirs of microbial diversity for fluvial networks. *Proceedings of the Royal Society B: Biological Sciences*. 280: 1760.
- Bever, J. D. 2015. Preferential allocation, physio-evolutionary feedbacks, and the stability and environmental patterns of mutualism between plants and their root symbionts. *New Phytologist*. 205: 1503-1514.
- Bever, J.D., T.G. Platt, and E.R. Morton. 2012. Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annual Review of Microbiology*. 66:265-83
- Bever, J.D., L.M. Broadhurst, and P.H. Thrall. 2013. Microbial phylotype composition and diversity predicts ecological function and plant-soil feedbacks. *Ecology Letters*. 16: 167-174.
- Bever, J.D., S. Mangan, and H. Alexander. 2015. Pathogens maintain plant diversity. *Annual Review of Ecology and Systematics*. 46: 305-325.

- Beversdorf, L.J., S.D. Chaston, T.R. Miller, and K.D. McMahon. 2015a. Microcystin *mcyA* and *mcyE* Gene Abundances Are Not Appropriate Indicators of Microcystin Concentrations in Lakes. *PLoS ONE*. doi:10.1371/journal.pone.0125353.
- Beversdorf, L.J., T.R. Miller, and K.D. McMahon. 2015b. Long-term monitoring reveals carbon–nitrogen metabolism key to microcystin production in eutrophic lakes. *Frontiers in Microbiology*. 6:456
- Billings, S.A., and F. Ballantyne IV. 2013. How interactions between microbial resource demands, soil organic matter stoichiometry, and substrate reactivity determine the direction and magnitude of soil respiratory responses to warming. *Global Change Biology*. doi:10.1111/gcb.12029.
- Billings, S.A., and D.D. Richter. 2006. Changes in stable isotopic signatures of soil nitrogen and carbon during forty years of forest development. *Oecologia*. 148: 325–333.
- Billings, S.A., and L.K. Tiemann. 2014. Warming-induced enhancement of soil N₂O efflux linked to distinct response times of genes driving N₂O production and consumption. *Biogeochemistry*. 119: 371-386.
- Blanco-Canqui, H., M.M. Mikha, J.G. Benjamin, L.R. Stone, A.J. Schlegel, D.J. Lyon, M.F. Vigil, and P.W. Stahlman. 2009. Regional study of no-till impacts on near-surface aggregate properties that influence soil erodibility. *Soil Sci. Soc. Am. J.* 73: 1361-1368.
- Blanco-Moure, N., D. Moret-Fernandez, and M.V. Lopez. 2012. Dynamics of aggregate destabilization by water in soil under long-term conservation tillage in semiarid Spain. *Gatena*. 22: 34-41.
- Blaser, M.J., Z.G. Cardon, M.K. Cho, J.L. Dangi, T.J. Donohue, J.L. Green, R. Knight, M.E. Maxon, T.R. Northen, K.S. Pollard, and E.L. Brodie. 2016. Toward a predictive understanding of earth's microbiomes to address 21st century challenges. *mBio*. doi: 10.1128/mBio.00714-16
- Brown, S.P., and A. Jumpponen. 2014. Contrasting primary successional trajectories of fungi and bacteria in retreating glacier soils. *Molecular Ecology*. 23:481-97.
- Bulgarelli, D., M. Rott, K. Schlaeppi, E. Ver Loren van Themaat, N. Ahmadinejad, F. Assenza, P. Rauf, B. Huettel, R. Reinhardt, E. Schmelzer, J. Peplies, F.O. Gloeckner, R. Amann, T. Eickhorst, and P. Schulze-Lefert. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*. 488: 91-95.
- Bulgarelli, D., R. Garrido-Oter, P.C. Münch, A. Weiman, J. Dröge, Y. Pan, A.C. McHardy, and P. Schulze-Lefert. 2015. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell host & microbe*. 17: 392-403.
- 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. *ISME Journal*. 6: 1621-1624.
- Caporaso, J.G., J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Peña, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B.D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature methods*. 7: 335-336.
- Carnevale, A.P., N. Smith, and M. Melton. 2011. STEM: Science Technology Engineering Mathematics. *Georgetown University Center on Education and the Workforce*.
- Carpenter, S., and W. Brock. 2006. Rising variance: a leading indicator of ecological transition. *Ecology Letters*. 9:311-318.

- Carpenter, S.R., W.A. Brock, J.J. Cole, J.F. Kitchell, and M.L. Pace. 2008. Leading indicators of trophic cascades. *Ecology Letters*. 11: 128-138.
- Change the Equation. <http://changetheequation.org/stemstatistics-facts-figures>
- Chapin, F.S., G.M. Woodwell, J.T. Randerson, E.B. Rastetter, G.M. Lovett, D.D. Baldocchi, D.A. Clark, M.E. Harmon, D.S. Schimel, R. Valentini, C. Wirth, J.D. Alber, J.J. Cole, M.L. Goulden, J.W. Harden, M. Heimann, R.W. Howarth, P.A. Matson, A.D. McGuire, J.M. Melillo, H.A. Mooney, J.C. Neff, R.A. Houghton, M.L. Pace, M.G. Ryan, S.W. Running, O.E. Sala, W.H. Schlesinger, and E.D. Schulze. 2006. Reconciling carbon-cycle concepts, terminology, and methods. *Ecosystems*. 9: 1041-1050.
- Choi, Y.J., G. Beakes, S. Glockling, J. Kruse, B. Nam, L. Nigrelli, S. Ploch, H.D. Shin, R.G. Shivas, S. Telle, H. Voglmayr, and M. Thines. 2015. Towards a universal barcode of oomycetes—a comparison of the *cox1* and *cox2* loci. *Molecular ecology resources* 15: 1275-1288.
- Copeland, J.K., L. Yuan, M. Layeghifard, P.W. Wang, and D.S. Guttman. 2015. Seasonal community succession of the phyllosphere microbiome." *Molecular Plant-Microbe Interactions*. 28: 274-285.
- Corkidi, L., D.L. Rowland, N.C. Johnson, and E.B. Allen. 2002. Nitrogen fertilization alters the functioning of arbuscular mycorrhizae at two semiarid grasslands. *Plant and Soil*. 240: 299–310.
- Cozzens, M.B. 2008. *Increasing the Quantity and Quality of the Mathematical Sciences Workforce Through Vertical Integration and Cultural Change*. NSF.
- Craine, J.M., T.W. Ocheltree, J.B. Nippert, E.G. Towne, A.M. Skibbe, S.W. Kembel, and J.E. Fargione. 2013. Global diversity of drought tolerance and grassland climate-change resilience. *Nature Climate Change*. 3:63-67.
- Crump, B.C., H.E. Adams, J.E. Hobbie, and G.W. Kling. 2007. Biogeography of bacterioplankton in lakes and streams of an arctic tundra catchment. *Ecology*. 88: 1365-1378.
- Crump, B.C., L.A. Amaral-Zettler, and G.W. Kling. 2012. Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *ISME Journal*. 6: 1629-1639
- Cruz-Martínez, K., K.B. Suttle, E.L. Brodie, M.E. Power, G.L. Andersen, and J.F. Banfield. 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. *The ISME journal*. 3: 738-744.
- Davis, T.W., G.S. Bullerjahn, T. Tuttle, R.M. McKay, and S.B. Watson. 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during planktothrix blooms in sandusky bay, Lake Erie. *Environmental Science and Technology*. 49:7197–7207
- Dialynas, Y.G., S. Bastola, R.L. Bras, S.A. Billings, D. Markewitz, and D.D. Richter. 2016. Topographic variability and the influence of soil erosion on the carbon cycle. *Global Biogeochemical Cycles*. 30: 644–660.
- Dijkstra, F.A., and W. Cheng. 2007. Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecology Letters*. 10:1046-1053.
- Dodds, W.K., and M.R. Whiles. 2010. *Freshwater Ecology: Concepts and Environmental Applications of Limnology*. 2nd edition. Academic Press, Burlington.
- Dodds, W.K., W. Clements, K. Gido, R. Hilderbrand, and R. King. 2010. Thresholds, breakpoints, and nonlinearity in freshwaters as related to management. *Journal of the North American Benthological Society*. 29:988-997.

- Dodds, W.K., M.K. Banks, C.S. Clennan, C.W. Rice, D. Sotomayor, E. Strauss, and W. Yu. 1996. Biological properties of soil and subsurface sediments under abandoned pasture and cropland. *Soil Biol. Biochem.* 28:837-846
- Duchicela, J., K.M. Vogelsang, W. Kaonongbua, E. Middleton, P.A. Schultz, and J.D. Bever. 2012. Non-native plants and soil microbes contribute to reduced soil aggregate stability in disturbed N. American grasslands. *New Phytologist.* 196: 212–222.
- Fabrizzi K.P., C.W. Rice, T.J.C. Amado, J. Fiorin, P. Barbagelata, and R. Melchiori. 2009. Protection of soil organic C and N in temperate and tropical. *Biogeochemistry.* 90: 129-143.
- Fierer, N., J. Ladau, J.C. Clemente, J.W. Leff, S.M. Owens, K.S. Pollard, R. Knight, J. Gilbert, and R. McCulley. 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. *Science.* 342:621-624.
- Fierer, N., J.P. Schimel, J.P., and P.A. Holden. 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biol. Biochem.* 35: 167-176.
- Andersen, G.L., and J.F. Banfield. 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. *The ISME Journal.* 3: 738–744.
- House, G.L., S. Ekanayake, Y. Ruan, U.M.E. Schütte, W. Kaonongbua, G. Fox, Y. Ye, and J.D. Bever. 2016. Phylogenetically structured differences in rRNA gene sequence variation among species of arbuscular mycorrhizal fungi and their implications for sequence clustering. *Appl. Environ. Microbiol.* doi:10.1128/AEM.00816-16
- Giauque, H., and C.V. Hawkes. 2016. Historical and current climate drive spatial and temporal patterns in fungal endophyte diversity. *Fungal Ecology.* 20:108–114.
- Gomez, L., A. Jumpponen, M. Herman, and K.A. Garrett. 2016. Soil Metagenomics to identify indicators of soil degradation in the Bolivian Highlands. *In prep.*
- Goodrich, J.K., E.R. Davenport, J.L. Waters, A.G. Clark, and R.E. Ley. 2016. Cross-species comparisons of host genetic associations with the microbiome. *Science.* 352:532-535.
- Goring, S., K.C. Weathers, W.K. Dodds, P.A. Soranno, L.C. Sweet, K.S. Cheruvilil, J.S. Kominoski, J. Rüegg, A.M. Thorn, and R.M. Utz. 2014. Improving the culture of interdisciplinary collaboration in ecology by expanding measures of success. *Frontiers in Ecology and the Environment* 12: 39–47.
- Grabherr, M.G., B.J. Haas, M. Yassour, J.Z. Levin, D.A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, and Q. Zeng. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology.* 29: 644-652.
- Graham, E.B., J.E. Knelman, A. Schindlbacher, S. Siciliano, M. Breulmann, A. Yannarell, J.M. Beman, G. Abell, L. Philippot, J. Prosser, A. Foulquier, J.C. Yuste, H.C. Glanville, D.L. Jones, R. Angel, J. Salminen, R.J. Newton, H. Bürgmann, L.J. Ingram, U. Hamer, H.M.P. Siljanen, K. Peltoniemi, K. Potthast, L. Bañeras, M. Hartmann, S. Banerjee, R-Q. Yu, G. Nogaro, A. Richter, M. Koranda, S.C. Castle, M. Goberna, B. Song, A. Chatterjee, O.C. Nunes, A.R. Lopes, Y. Cao, A. Kaisermann, S. Hallin, M.S. Strickland, J. Garcia-Pausas, J. Barba, H. Kang, K. Isobe, S. Papaspyrou, R. Pastorelli, A. Lagomarsino, E.S. Lindström, N. Basiliko, and D.R. Nemergut. 2016. Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? *Front. Microbiol.* 7:214. doi: 10.3389/fmicb.2016.00214
- Guenon, R., and R. Gros. 2013. Frequent wildfires with shortened time-since-fire affect soil microbial functional stability to drying and rewetting events. *Soil Biology and Biochemistry.* 57:663-674.

- Guttman, D.S., A.C. McHardy, and P. Schulze-Lefert. 2014. Microbial genome-enabled insights into plant-microorganism interactions. *Nature Reviews Genetics*. 15: 797-813.
- Harke, M.J., T.W. Davis, S.B. Watson, and C.J. Gobler. 2015. Nutrient-controlled niche differentiation of western Lake Erie cyanobacterial populations revealed via metatranscriptomic surveys. *Environmental Science & Technology*. acs.est.5b03931
- Harrison, G.W. 1979. Stability under environmental stress: resistance, resilience, persistence, and variability. *American Naturalist*. 113:659-669.
- Hawkes, C.V. and T.H. Keitt. 2015. Resilience vs. historical contingency in microbial responses to environmental change. *Ecology Letters*. doi: 10.1111/ele.12451
- Hernández, D.L., D.M. Vallano, E.S. Zavaleta, Z.Tzankova, J.R. Pasari, S.Weiss, P.C. Selmants, and C. Morozumi. 2016. Nitrogen pollution Is linked to US listed species declines. *Bioscience*. doi: 10.1093/biosci/biw003.
- Hollingsworth, P.M., S.W. Graham, and D.P. Little. 2011. Choosing and using a plant DNA barcode. *PLoS One*. doi:10.1371/journal.pone.0019254.
- Houseman, G.R., G.G. Mittelbach, H.L. Reynolds, and K.L. Gross. 2008. Perturbations alter community convergence, divergence, and formation of multiple community states. *Ecology*. 89:2172-2180.
- Ihrmark, K., I.T.M. Bödeker, K. Cruz-Martinez, H. Friberg, A. Kubartova, J. Schenck, Y. Strid, J. Stenlid, M. Brandström-Durling, K.E. Clemmensen, and B.D. Lindahl. 2012. New primers to amplify the fungal ITS2 region—evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* 82: 666-677.
- Jackson, S.A. 2003. *Envisioning A 21st Century Science and Engineering Workforce for the United States: Tasks for University, Industry, and Government*. National Academies Press.
- Johnson, N.C., G.W.T. Wilson, M.A. Bowker, J.A. Wilson, and R.M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences, USA*. 107: 2093–2098.
- Johnson, N.C. 1993. Can fertilization of soil select for less mutualistic mycorrhizae? *Ecological Applications*. 3: 749–757.
- Jones, F.A., D.L. Erickson, M.A. Bernal, E. Bermingham, W.J. Kress, E.A. Herre, H.C. Muller-Landau, and B.L. Turner. 2011. The roots of diversity: below ground species richness and rooting distributions in a tropical forest revealed by DNA barcodes and inverse modeling. *PLoS One*. 6: e24506.
- Judd, K.E., B.C. Crump, and G.W. Kling. 2006. Variation in dissolved organic matter controls bacterial production and community composition. *Ecology*. 87: 2068-2079.
- Kansas: Building an Environment for Science and Technology, 2012.
http://www.nsfepscor.ku.edu/pdf/KansasBEST_10-28-12_HR.pdf
- Karlen, D.L. and C.W Rice. 2015. Soil degradation: will humankind ever learn? *Sustainability*. 7:12490-12501.
- Klindworth, A., E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn, and F.O. Glöckner. 2012. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic acids research*. 41:gks808.
- Knief, C., N. Delmotte, S. Chaffron, M. Stark, G. Innerebner, R. Wassmann, C.V. Mering, and J.A. Vorholt. 2012. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *The ISME journal* 6: 1378-1390.

- Koziol, E., and J.D. Bever. 2015. Mycorrhizal response trades off with plant growth rate and increases with plant successional status. *Ecology*. 96:1478–1484.
- Lado, M., A. Paz, and M. Ben-Hur. 2004. Organic matter and aggregate-size interactions in saturated hydraulic conductivity. *Soil Sci. Soc. Am. J.* 68: 234-242
- Lambert, D.H., H. Cole, and D.E. Baker. 1980. Adaption of vesicular-arbuscular mycorrhizae to edaphic factors. *New Phytologist*. 85: 513-520.
- Larimer, A.L., J.D. Bever, and K. Clay. 2010. Meta-analysis of the interactive effects of plant microbial symbionts. *Symbiosis*. 51: 139-148.
- Lehmeier, C.A., F. Ballantyne IV, K. Min, and S.A. Billings. 2016. Temperature-mediated changes in microbial carbon use efficiency and ¹³C discrimination. *Biogeosciences*. 13: 3319-3329.
- Lehmeier, C.A., K. Min, N.D. Niehues, F. Ballantyne IV, and S.A. Billings. 2013. Temperature-mediated changes of exoenzyme-substrate reaction rates and their consequences for the carbon to nitrogen flow ratio of liberated resources. *Soil Biology and Biochemistry*. 57:374-382.
- Lindström, E.S., and A.K. Bergström. 2004. Influence of inlet bacteria on bacterioplankton assemblage composition in lakes of different hydraulic retention time. *Limnology and Oceanography*. 49: 125-136.
- LINX collaborators: Dodds, W.K., J.R. Webster, C.L. Crenshaw, A.M. Helton, J.M. O'Brien, E. Martí, A.E. Hershey, J.L. Tank, A.J. Burgin, N.B. Grimm, S.K. Hamilton, D.J. Sobota, G.C. Poole, J.J. Beaulieu, L.T. Johnson, L.R. Ashkenas, R.O. Hall Jr., S.L. Johnson, W.M. Wollheim, and W.B. Bowden. 2014. The Lotic Intersite Nitrogen Experiments: an example of successful ecological research collaboration. *Freshwater Science*. 33:700-710.
- Loecke, T. D., and G.P. Robertson. 2009. Soil resource heterogeneity in terms of litter aggregation promotes nitrous oxide fluxes and slows decomposition. *Soil Biology and Biochemistry*. 41: 228–235.
- 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, 1-21.
- Mangot, J.F., I. Domaizon, N. Taib, N. Marouni, E. Duffaud, G. Bronner, and D. Debroas. 2013. Short-term dynamics of diversity patterns: evidence of continual reassembly within lacustrine small eukaryotes. *Environmental Microbiol.* 15:1745-58.
- Manzoni, S., S.M. Schaeffer, G. Katul, A. Porporato, and J.P. Schimel. 2014. A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils, *Soil Biol. Biochem.* 73: 69-83.
- Martiny, J.B.H., S.E. Jones, J.T. Lennon, and A.C. Martiny. 2015. Microbiomes in light of traits: A phylogenetic perspective. *Science*. doi: 10.1126/science.aac9323
- McMurdie, P.J., and S. Holmes. 2014. Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput Biol.* doi:10.1371/journal.pcbi.1003531
- Meziti, A., D. Tsementzi, K.A. Kormas, H. Karayanni, and K.T. Konstantinidis. 2016. Anthropogenic effects on bacterial diversity and function along a river-to-estuary gradient in Northwest Greece revealed by metagenomics. *Environmental Microbiology*. in press.
- Michalak, A.M., E.J. Anderson, D. Beletsky, S. Boland, N.S. Bosch, T.B. Bridgeman, J.D. Chaffin, K. Cho, R. Confesor, I. Daloglu, J.V. Depinto, M.A. Evans, G.L. Fahnenstiel, L. He, J.C. Ho, L. Jenkins, T.H. Johengen, K.C. Kuo, E. Laporte, X. Liu, M.R. McWilliams, M.R. Moore, D.J. Posselt, R.P. Richards, D. Scavia, A.L. Steiner, E. Verhamme, D.M. Wright, and M.A. Zagorski. 2013. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends

- consistent with expected future conditions. *Proceedings of the National Academy of Sciences of the USA*. 110:6448–52.
- Middleton, E., S. Richardson, L. Koziol, C.E. Palmer, Z. Yermakov, J.A. Henning, P.A. Schultz, and J.D. Bever. 2015. Locally-adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. *Ecosphere*. 6:276.
- Mikha, M.M., and C.W. Rice. 2004. Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. *Soil Science Society of American Journal*. 68:809-816.
- Mikha, M.M., M.F. Vigil, and J.G. Benjamin. 2013. Long-term tillage impacts on soil aggregation and carbon dynamics under wheat-fallow in the Central Great Plains. *Soil Science Society of American Journal*. 77: 594-605.
- Min, K., C.A. Lehmeier, F. Ballantyne IV, and S.A. Billings. *In prep*. Temperature responses of heterotrophic microbial respiration, carbon uptake affinity, and ¹³C discrimination change with resource stoichiometry.
- Mulholland, P.J., A.M. Helton, G.C. Poole, R.O. Hall, S.K. Hamilton, B.J. Peterson, J.L. Tank, L.R. Ashkenas, L.W. Cooper, C.N. Dahm, W.K. Dodds, S.E.G. Findlay, S.V. Gregory, N.B. Grimm, S.L. Johnson, W.H. McDowell, J.L. Meyer, H.M. Valett, J.R. Webster, C.P. Arango, J.J. Beaulieu, M.J. Bernot, A.J. Burgin, C.L. Crenshaw, L.T. Johnson, B.R. Niederlehner, J.M. O'Brien, J.D. Potter, R.W. Sheibley, D.J. Sobota, and S.M. Thomaset. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature* 452:202-6.
- Nagano, A.J., M.N. Honjo, M. Mihara, M. Sato, and H. Kudoh. 2015. Detection of plant viruses in natural environments by using RNA-Seq. *Plant Virology Protocols: New Approaches to Detect Viruses and Host Responses*. 1236: 89-98.
- National Academies of Sciences, Engineering, and Medicine. 2015. *Convergence: Facilitating Transdisciplinary Integration of Life Sciences, Physical Sciences, Engineering, and Beyond*. National Academies Press Washington DC.
- National Research Council. 2001. *Grand challenges in environmental sciences*. National Academy Press, Washington, D.C.
- Nguyen, N.H., Z. Song, S.T. Bates, S. Branco, L. Tedersoo, J. Menke, J.S. Schilling, and P.G. Kennedy. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*. 20: 241-248.
- Oliver, J.E., P.A. Cobine, and L. De La Fuente. 2015. Xylella fastidiosa isolates from both subsp. multiplex and fastidiosa cause disease on southern highbush blueberry (Vacciniumsp.) under greenhouse conditions. *Phytopathology*. 105:855-862 .
- Olson, S., and D.G. Riordan. 2012. *Engage to Excel: Producing One Million Additional College Graduates with Degrees in Science, Technology, Engineering, and Mathematics*. Report to the President. *Executive Office of the President*.
- Paerl, H.W., N.S. Hall, and E.S. Calandrino. 2011. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *The Science of the Total Environment*. 409:1739–45.
- Paustian, K., J. Lehman, S. Ogle, D. Reay, G.P. Robertson, and P. Smith 2016. Climate-smart soils. *Nature*. doi:10.1038
- Philippot, L., A. Spor, C. Henault, D. Bru, F. Bizouard, C.M. Jones, A. Sarr, and P.A. Maron. 2013. Loss in microbial diversity affects nitrogen cycling in soil. *ISME Journal*. 7: 1609-1619.

- Picelli, S., Å.K. Björklund, B. Reinius, S. Sagasser, G. Winberg, and R. Sandberg. 2014. Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. *Genome research* 24.12: 2033-2040.
- Platt, T.G., C. Fuqua, and J.D. Bever. 2012. Public goods and resource competition determine the fitness of *Agrobacterium tumefaciens*' virulence plasmid. *Evolution*. 66: 1953–1965
- Powell, J.R., and B.A. Sikes. 2014. Method or Madness: Does OTU delineation bias our perceptions of fungal ecology? *New Phytologist*. 202: 1095-1097.
- Raaijmakers, J.M., and M. Mazzola. 2016. Soil immune responses. *Science* doi: 10.1126/science.aaf3252
- Rascher, U., M. Liebig, and U. Luttge. 2000. Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. *Plant Cell and Environment*. 23: 1397-1405
- Raymond, B., S.A. West, A.S. Griffin, and M.B. Bonsall. 2012. The dynamics of cooperative bacterial virulence in the field. *Science*. 337, 85-88.
- Richter, D.D., and S.A. Billings. 2015. 'One physical system': Tansley's ecosystem as Earth's critical zone. *New Phytologist*. doi: 10.1111/nph.13338.
- Riggs, C.E., and S.E. Hobbie. 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. *Soil Biology and Biochemistry*. 99:54-65.
- Robinson, M.D., D.J. McCarthy, and G.K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 26: 139-140.
- Ruiz-González, C., J.P. Niño-García, and P.A. del Giorgio. 2015. Terrestrial origin of bacterial communities in complex boreal freshwater networks. *Ecology letters*. 18: 1198-1206. doi:10.1111/ele.12499
- Rumpel, C., and I. Kögel-Knabner. 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant and Soil*. 338: 143-158.
- Scheffer, M., J. Bascompte, W.A. Brock, V. Brovkin, S.R. Carpenter, V. Dakos, H. Held, E.H. Van Nes, M. Rietkerk, and G. Sugihara. 2009. Early-warning signals for critical transitions. *Nature*. 461:53-59.
- Schloss, P.D., S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, R.A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robinson, J.W. Sahl, B. Stres, G.G. Thallinger, D.J. Van Horn, and C.F. Weber. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and environmental microbiology*. 75: 7537-7541.
- Seemann, T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*. btu153
- Shade, A., H. Peter, S.D. Allison, D.L. Baho, M. Berga, H. Bürgmann, D.H. Huber, S. Langenheder, J.T. Lennon, J.B.H. Martiny, K.L. Matulich, T.M. Schmidt, and J. Handelsman. 2012. Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology*. doi: 10.3389/fmicb.2012.00417.
- Sikes, B.A., C.V. Hawkes, and T. Fukami. 2016. Plant and root endophyte assembly history: interactive effects on native and exotic plants. *Ecology*. 97: 484-493.
- Six, J., E.T. Elliott, and K. Paustian. 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology Biochemistry*. 32: 2099-2103

- Six, J., C. Feller, K. Deneff, S.M. Ogle, J.C. Moraes Sa, and A. Albrecht. 2002. Soil organic matter, biota and aggregation in temperate and tropical soil-effect of no-tillage. *Agronomie*. 22: 755-775.
- Six, J., and K. Paustian. 2014. Aggregate-associated soil organic matter as an ecosystem property and a measurement. *Soil Biology and Biochemistry*. 68: A4–A9.
- Smith, V.H., and D.W. Schindler. 2009. Eutrophication science: where do we go from here? *Trends in ecology & evolution*. 24:201–7.
- Sposito, G. 2013. Green water and global food security. *Vadose Zone Journal*. 10.2136/vzj2013.02.0041.
- Stachowicz, J.J. 2001. Mutualisms, positive interactions, and the structure of ecological communities. *BioScience*. 51:235-246.
- Stahl, P.D., and W.K. Smith. 1984. Effects of different geographic isolates of *Glomus* on the water relations of *Agropyron smithii*. *Mycologia*. 76: 261–267
- Strickland, M.S., C. Lauber, N. Fierer, and M.A. Bradford. 2009. Testing the functional significance of microbial community composition. *Ecology*. 90: 441-451.
- Tarnocai, C., J.G. Canadell, E.A.G. Schuur, P. Kuhry, G. Mazhitova, and S. Zimov. 2009. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochemistry Cycles*. 23: GB2023.
- Tiedje, J. M. 1994. Denitrifiers. *Methods of Soil Analysis: Part 2—Microbiological and Biochemical Properties*. *Methods of Soil Analysis* 2. 245-267.
- Tiemann, L.K., and S.A. Billings. 2011a. Changes in variability of soil moisture alter microbial community C and N resource use. *Soil Biology and Biochemistry*. 43:1837-1847.
- Tiemann, L.K., and S.A. Billings. 2011b. Indirect effects of nitrogen amendments on organic substrate quality increase enzymatic activity driving decomposition in a mesic grassland. *Ecosystems*. 14:234-247.
- Trumbore, S. 2009. Radiocarbon and Soil Carbon Dynamics. *Annual Review Earth Planetary Science*. 37: 47-66.
- Tucker, S.S., J.M. Craine, and J.B. Nippert. 2011. Physiological drought tolerance and the structuring of tallgrass prairie assemblages. *Ecosphere*. 2:art48. doi:10.1890/ES11-00023.1
- Turner, T.R., E.K. James, and P.S. Poole. 2013. The plant microbiome. *Genome Biology*. 14:10.1186.
- Van de Waal, D.B., V.H. Smith, S.A.J. Declerck, E.C.M. Stam, and J.J. Elser. 2014. Stoichiometric regulation of phytoplankton toxins. *Ecology Letters*. 12: 1326-1335
- Van der Putten, W.H., R.D. Bardgett, J.D. Bever, T.M. Bezemer, B.B. Casper, T. Fukami, P. Kardol, J.N. Klironomos, A. Kulmatiski, J.A. Schweitzer, K.N. Suding, T.F.J. Van de Voorde, and D.A. Wardle. 2013. Plant-soil feedback: the past, the present and future challenges. *Journal of Ecology*. 101: 265-276.
- Van Oost, K., G. Govers, and P. Desmet. 2000. Evaluating the effects of changes in landscape structure on soil erosion by water and tillage. *Landscape Ecology*. 15:577-589.
- Van Rossum, T., M.A. Peabody, M.I. Uyaguari-Diaz, K.I. Cronin, M. Chan, J.R. Slobodan, M.J. Nesbitt, C.A. Suttle, W.W. Hsiao, P.K. Tang, N.A. Prystajek, and F.S. Brinkman. 2015. Year-long metagenomic study of river microbiomes across land use and water quality. *Frontiers in Microbiology*. 6: 1-15.

- Vogelsang, K.M., H.L. Reynolds, and J.D. Bever. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of the tallgrass prairie system. *New Phytologist*. 172: 554–562.
- Walker, B., C.S. Holling, S.R. Carpenter, and A. Kinzig. 2004. Resilience, adaptability and transformability in social–ecological systems. *Ecology and Society*. 9:5.
- Wall, D.H., and J. Six. 2015. Give soils their due. *Science*. 34: 695.
- Wall, D.H., U.F. Nielsen and J. Six. 2015. Soil biodiversity and human health. *Nature*. 528: 69-76.
- Wang, Y. P., J. Jiang, B. Chen-Charpentier, F.B. Agosto, A. Hastings, F. Hoffman, M. Rasmussen, M.J. Smith, K. Todd-Brown, Y. Wang, X. Xu, and Y.Q. Luo. 2016. Responses of two nonlinear microbial models to warming and increased carbon input. *Biogeosciences*. 13: 887–902.
- Whitehouse OSTP. 2016. <https://www.whitehouse.gov/blog/2016/05/13/announcing-national-microbiome-initiative>.
- Wilson, G.W.T., C.W. Rice, M.C. Rillig, A. Springer, and D.C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecology Letters*. 12: 452-461
- Wu, S., Z. Zhu, L. Fu, B. Niu, and W. Li. 2011. WebMGA: a customizable web server for fast metagenomic sequence analysis. *BMC genomics*. 12: 1.
- Wutzler, T., and M. Reichstein. 2008. Colimitation of decomposition by substrate and decomposers – a comparison of model formulations. *Biogeosciences*. 8: 749–759.
- Yuan, L.L., A.I. Pollard, S. Pather, J.L. Oliver, and L.D. Anglada. 2014. Managing microcystin: Identifying national-scale thresholds for total nitrogen and chlorophyll a. *Freshwater Biology*. 59: 1970-1981.
- Zeglin, L.H., B. Wang, F. Rainey, C. Waythomas, 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.
- Zeglin, L.H. 2015. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Frontiers in Microbiology*. 6: 454.
- Zeglin, L., F. Rainey, B. Wang, C. Waythomas, and S. Talbot. 2013. Soil microbial structure and function post-volcanic eruption on Kasatochi Island and regional controls on microbial heterogeneity. *AGU Fall Meeting Abstracts*. 1: 0322.
- Zhang, F., J. Lee, S. Liang, and C.K. Shum. 2015. Cyanobacteria blooms and non-alcoholic liver disease: evidence from a county level ecological study in the United States. *Environ. Health*. 14: 41.
- Zheng, C., B. Ji, J. Zhang, F. Zhang, and J.D. Bever. 2015. Shading decreases plant carbon preferential allocation toward most effective mycorrhizal mutualist. *New Phytologist*. 205: 361-368