Improving Soil Sampling Methodologies for the Evaluation of Spatial and Temporal Dynamics of Soil Microbial Communities in Managed Agroecosystems

Introduction

The long-term goal of this research is ***to assess the role that soil microbial community play in ecosystem services in managed croplands, pastures and grasslands.*** **These include** site productivity, biodiversity, biogeochemical and energy cycles all of which are necessary for air and water quality, and sustained food security in a rapidly changing climate. The aim of this proposal is twofold. First is to quantify the spatial and temporal variability in microbial communities as impacted by the abiotic and biotic environment and site management practices in agro-ecosystems. Second is to evaluate existing and to develop much needed field sampling protocol to better capture the seasonality and spatial variability in microbial communities. Results from this study will serve as the foundation for hypothesis development targeted to address the long-term goal of this study and to provide critical preliminary results for the 2018 National Institute of Food and Agriculture (USDA-NIFA) ecosystem studies research grant proposal.

Background/Rationale

In order to meet growing food demands from the world's expanding populations which are projected to reach 9.6 billion people by 2050 (Grafton 2016); resilient and productive agricultural systems will increasingly depend and rely on healthy soil ecosystems (Scloter et al. 2003). The health of any soil refers to its quality and its capacity to continually provide nutrition, energy, water to sustain plant, animal, and human life (Doran and Zeiss 2000, Bardgett and van der Putten 2014). The soil ecosystem constitutes of living organisms such as plant roots, microbes (e.g. fungi, bacteria) and earthworms, all interacting in their physical environment to provide ecosystem services. These ecosystem services include: improving site productivity, biodiversity, nutrient cycling, water quality and quantity, carbon sequestration, decomposition and detoxification of both organic and inorganic pollutants and pest control (Schulz et al. 2013, Bardgett and van der Putten, 2014).

Soil microbial abundance and diversity serve as an indicator for soil quality and have been shown to impact productivity (Bossio, 1998, Singh et al. 2011, Lal 2015). Healthy soils are resilient and can better withstand anthropogenic management and the adverse impacts of climate variability and extremes (e.g. soil erosion as a result of high intensity rain evens; Lal 2015). Soil microbes are well known to facilitate the development of healthy soil structure. Moreover, an abundance of soil microbes working in consortium may suppress soil pathogens and improve productivity (Singh et al. 2011, Weller et al. 2002).

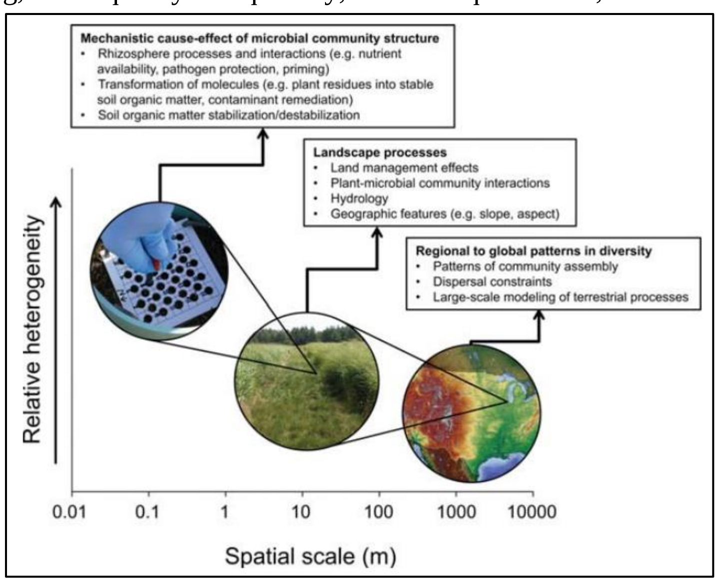

Fig 1: Conceptual Figure illustrating relative heterogeneity of soils over various scales (Source: O'Brien et al. 2016).

By understanding the factors driving spatial soil microbial ecology we expect to be able to select best management practices that will maintain and regulate soil biodiversity and enhance microbial-soil ecosystem functions. However, measuring microbial abundance and diversity has been very challenging over a range of spatial and temporal scales (Nakajima et al. 2016, O'Brien et al. 2016) Fig. 1 illustrates how heterogeneous and diverse soil microbial populations are, even when taken at 10 cm intervals (O'Brien et al. 2016). Researchers use a variety of strategies when conducting spatial studies (Wang et al.

2012). One is the randomized sampling strategy that is very common among scientists. Another is the application of transects across the site of study; guided by physical variables such slope or water gradients (Wang et al. 2012). Each strategy has its shortcomings especially where soils are spatially variable. Many scientists however concur that soil characteristics are the main driver of observed high spatial variability in microbial populations (e.g., water, structure, and temperature; Naveed et al. 2016, O'Brien et al. 2016). For instance, Naveed et al. (2016) determined that bacterial richness and biodiversity was highly correlated to the ratio of clay to organic carbon. Soil water characteristics, in their study site were shown to influence both fungal and bacterial diversities (Naveed et al. 2016). Soil apparent electrical conductivity (EC) measures the amount of electric current that soil can conduct (Peralta and Costa 2013). This property relates to soil texture because soils with high clay content exhibit higher EC values (Triantafilis and Lesch 2005). The percentage of clay particles in soils influences its physical texture. Additional soil properties such as salinity, water, organic matter content, and cation exchange capacity (CEC) are directly correlated with EC. Large commercial producers apply and recommend EC for delineating homogeneous zones in fertilizer application and other management practices (e.g. Peralta et al. 2013). We view EC as a potential proxy of soil properties and as a very useful tool to delineate zones in the field exhibiting similarities in microbial richness and abundance. We ***hypothesize*** that delineating field plots by the measure of EC will assist in developing procedure in stratified soil sampling. Robust sampling strategies based on soil properties like EC are expected to fill knowledge gaps in soil microbial richness and abundance, and in ecosystem functioning at the finer spatial scales that account for soil variability.

Objectives

Our research efforts are tailored to encompass the soil ecosystem (living organisms in their physical environment). We will examine the interconnectedness of soil microbial biodiversity and abundance and their role in biogeochemical nutrient recycling with soil properties. We will evaluate whether stratified (clustered) sampling based on soil EC is more informative than random sampling and/or the application of transects across fields for studies related to soil microbial diversity and abundance. We will characterize the microbial community in designated Long-term Agro-ecosystem Network located NE (LTAR) sites separated by EC clusters/groups in order to better understand their role in biogeochemical transformations in croplands and grazing pastures.

The objectives of this project are to:

1) Determine if EC is a good proxy for delimiting soil microbial diversity and abundance.
2) Identify the spatial and temporal distribution of microbes in grazing and cropland sites.
3) Identify key environmental factors that influence microbial distribution in grazing and cropland sites.
4) Determine how the heterogeneous soil structure and water availability influence the microbial community.

Proposed Plan

The **objective** of this project is to determine whether the application of EC **as a proxy for soil properties** will be useful in delimiting zones of similar soil microbial abundance and diversity. The study will be conducted on sites in the LTAR network of NE. The NE LTAR was established in 2014, and is one of 18 sites that are distributed across the USA with the aim to maintain sustainability and profitability of agroecosystems in the face of climate variability and change. The Network of NE is a partnership between the Agriculture Research Service (ARS in USDA) and UNL. We will test the EC clustered-based approach sampling strategy against both random and transect-based sampling strategies. With the funding from this grant, we will select **two contrasting sites** for our preliminary study: one pasture site and one continuously irrigated corn. We expect that the results from these two dissimilar sites will help determine whether an EC-based stratified sampling procedure encompasses the spatial variability of microbial richness and diversity in our sites and whether it is appropriate when making generalized inferences about soil microbial population distribution at the LTAR sites. Five sampling activities will be conducted over

2017: 1) the winter, frozen soils, 2) early spring, in March, at the beginning of the growing season after snow melt, 3) mid-season during wet periods of May-June, 4) in August after a dry period and 5) in the fall after soil moisture recovery.

Prior to taking soil samples, the pasture site will be characterized for its forage species richness and diversity (led by J. Okalebo and T Awada). T. Franz will lead the effort in providing both EC and soil water content maps for the two sites. He will employ the novel **cosmic-ray neutron rover technology that measures soil water content integrated over the top 20 cm of depth** (Franz et al. 2015). Fig. 2 illustrates soil spatial variability as depicted by an EC map developed for our newly established pasture site. The field data was collected on 17 March, 2016. Jane Okalebo, Trenton Franz, and Andy Suyker will select sub-plots and sampling points in the cropland sites as guided by the EC and soil water content maps. We will use EC as a way to pick 20 landscape positions with 7 repeated samples in time to investigate our hypothesis in time and space. We will then apply EC to upscale those patterns using multivariate statistics (Empirical Orthogonal Function/Principal Component Analysis). Repeated sampling in time and landscape position has been applied to upscale soil physical properties and we expect that this method will suffice for microbial populations. Galen Erickson, who leads the livestock pasture grazing experimental research, will also guide us in selecting plots based on past historical data. Following the three sampling procedures: 1) randomized 2) transect across field and 3) EC-stratified, soil samples will be collected at depths of 0-15 cm. The soils will be transported and processed in the laboratory before being tested for their richness and diversity using the Phospholipid Fatty Acid Analysis (PLFA) test. Scientists worldwide have also employed the PLFA analysis to estimate the total biomass changes in the community of soil microbes (Bossio et al. 1998). Analysis will be conducted in Brian Wienhold's soil biology laboratory and will be completed late Fall of 2017. Microbial populations will be analyzed for their biodiversity as measured using ecology indices such as the Shannon Diversity Index (Ma et al. 2016). Data will also be analyzed to determine any sampling effects using relevant statistical models. Through this project an undergraduate student will be trained in field and laboratory skills. The student's summer stipend will originate from the funding that we obtain from this grant application.

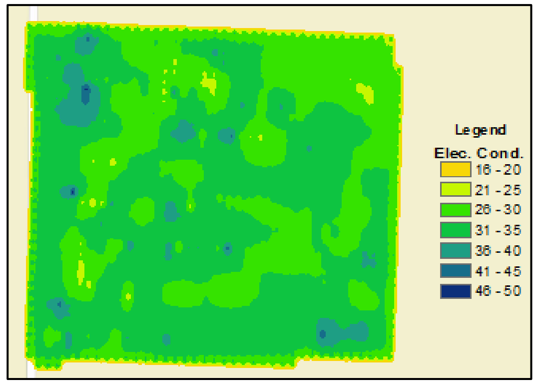

Fig 2: EC map of PR-HPA pasture site. (3/17/2016.)

Expected Outcomes: Our understanding of soil physical and chemical properties and processes is way ahead of our understanding of biological processes. We expect that we can show the usefulness of EC measurements; which are faster and less expensive to obtain at the field-scale; in guiding and informing our soil sampling procedures thereby enabling us ***to assess the role that soil microbial community play in ecosystem services in managed crop and pasture lands***. We will apply the cosmic ray rover to delineate soil moisture dynamics and quantify soil and vegetation dynamics supported by established site phenocams and remotely sensed images. Our datasets will be curated and shared with the National Agriculture Library (NAL). We will also utilize our preliminary data to develop a USDA-NIFA grant that will be submitted in 2018, aimed at achieving our overall overarching objectives of increasing and maintaining ecosystem services in sustainable agroecosystems. We expect that by filling these gaps in knowledge regarding suitable sampling soil strategies, we can enhance the efficiency with which we conduct our long-term experiments. Findings will be published in peer-reviewed journals and presented at conferences in 2017-18. We expect to use our data to inform and implement best management practices for crop and beef production systems in Nebraska that take into account our soil spatial variabilities elucidated by their biological attributes. The project will also serve as an undergraduate interdisciplinary project for students.

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