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MANAGEMENT INTENSIFICATION AND TEMPORAL DYNAMICS SHAPE MICROBIAL COMMUNITIES IN A SOUTHERN PLAINS AGROECOSYSTEM

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MANAGEMENT INTENSIFICATION AND TEMPORAL DYNAMICS SHAPE

MICROBIAL COMMUNITIES IN A SOUTHERN PLAINS AGROECOSYSTEM

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF MICROBIOLOGY AND PLANT BIOLOGY

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Table of Contents

List of Tables

List of Figures

Abstract

Land use intensification has resulted in the conversion of complex natural systems into simpler, managed environments which has a considerable impact on physical and chemical soil properties as well as above and belowground diversity for crop production. The productivity of agricultural systems greatly depends on a number of ecosystems services provided by the soil biota which are directly and indirectly affected by land use. Important ecosystem services provided by soil microbial communities include biogeochemical cycling, plant nutrient availability, and organic matter decomposition, all of which contribute to the overall functioning of the soil environment. Soil microbial communities and soil properties are not only influenced by land use, but a combination of temporal dynamics including seasonal management, climatic variations, and their interactions. While detecting differences in the microbial DNA pools over shorter periods is challenging, microbial communities changes have been clearly documented over months, seasons, and years raising the question if sampling at a single time point is enough to describe microbial community differences especially concerning environmental changes and management practices. Therefore, understanding the consequences of such interactions on soil microbial composition, diversity, and function is essential for maintaining the productivity and stability of valuable soil ecosystems. Thus, this dissertation aimed to explore the interaction between land use and temporal variation on soil microbial communities under varying levels of management disturbance in a U.S. Southern Plains agroecosystem with the goal of better understanding the impact on microbial community diversity, function, and interactions in order to provide information to better inform future management decisions to sustain soil health.

At the beginning of this work, the study sought to seek out the microbial community responses to common agricultural land uses and how these responses differed temporally in a U.S. Southern Plains agroecosystem that included two grasslands and two croplands that ranged in management disturbance. Grasslands to cultivated soils represent a frequent land use conversion as well as two types of land uses that often distinctly affect soil microbial communities and soil properties. Together, the four land uses represent a gradient of increasing disturbance from native tallgrass prairie (TGP) < Old World bluestem (OWB) pasture < no-tillage (NT) canola < conventionally tilled (CT) wheat. By examining land use types within a single agroecosystem, the study was able to reduce the effects of soil type since all ecosystems had similar silt loam soils and decrease the exposure to different climate conditions with grasslands being approximately 2.7 km apart from cropland sites. First, this study showed that there were different responses of taxonomic and functional diversity of the bacterial communities with land use having a greater impact on taxonomic diversity, and sampling time and its interaction with land use being most important to functional diversity. Overall, differences in taxonomic diversity between land uses were driven by tillage management, sampling time, and the impact of air temperature. In comparison, temporal differences were driven by soil total nitrogen, rainfall, and soil nitrate all of which vary due to management input or seasonal climatic differences. Lastly, functional diversity had a stronger relationship with taxonomic diversity for CT wheat compared to phylogenetic diversity in the native TGP. This study emphasized the importance of the interaction between temporal dynamics and land use in influencing soil microbiomes as well as establishing the negative effect that tillage management has on bacterial community

diversity across a gradient of land management disturbance in a U.S. Southern Plains agroecosystem.

Next, the goal was to zoom in on the microbial community interactions in response to land use conversion for long-term cropland use by focusing on the native system (TGP) and the conventionally tilled wheat site. Complicated ecological relationships can be represented as networks that are useful for making relative comparisons across treatments to better understand ecosystem dynamics. While it is frequently investigated how microbial community diversity and composition are impacted by land use conversion and associated management practices, less is known about how these microbial networks change under the same scenarios which can have a critical effect on ecosystem functioning and stability. This study examined the temporal dynamics of soil microbial community molecular ecological network (MEN) complexity and stability in response to long-term cropland use. In summary, CT wheat land use increased network complexity as well as caused observable temporal changes in network complexity which did not occur under native land use. Network stability was also increased under CT wheat land use with network stability increasing with network complexity. Also, CT wheat network complexity was significantly influenced by soil water content, ammonium, and management practices while CT network stability was mainly impacted by different management inputs. The results of this study suggest that converting native land for long-term cropland use greatly increased network complexity and stability which also varied temporally likely resulting in network structures that could adapt quickly to the many management disturbances associated with cropland use. While the cropland soil microbial communities may have adapted to the effect of the

same repeated management disturbances, it remains to be determined if the microbial community and its ecosystem functions will become more vulnerable to other disturbances such as future environmental changes.

Lastly, the final study extended the exploration of soil communities in a U.S. Southern Plains agroecosystem to virus communities by monitoring virus and potential bacterial host abundance. Viruses are extremely abundant, present in most ecosystems, and evidence already exists that viruses impact soil microbes and soil biogeochemistry. Therefore, to truly understand soil ecosystem dynamics in relation to land use and time, we need to investigate how viruses could affect microbial community contributions to soil ecosystem processes. Three land uses were examined including conventionally tilled wheat, no-tillage canola, and the native tallgrass prairie. All land use systems had temporal differences in viral and bacterial abundance with abundance decreasing with increasing management disturbance. Virus and potential host abundance were also influenced by soil and environmental factors that were often reflective of management in each land use. Additionally, using metagenomic sequencing, this study showed that not only did virus abundance differ, but so did the viral community structure of the CT wheat cropland and the TGP. Together, the results suggest that land use and land management shape the physiochemical properties of agricultural soil which affect virus abundance, host abundance, and the structure of the soil viral communities.

Overall, the work in this dissertation provided valuable evidence on the microbial community response to land use and land management disturbance as well as the interaction with time in a U.S. Southern Plains agroecosystem. Intensive management disturbance greatly impacted the soil bacterial and viral communities reducing

abundance and diversity in addition to diminishing many soil properties generally associated with healthy soils. A more in-depth look at the microbial community interactions also revealed that long-term cropland use impacts network complexity, stability, and their interaction generating microbial communities that likely can quickly adapt to repeated management disturbance. Many of these findings support the idea that land use conversion and management disturbance has a negative impact on soil microbial communities and soil properties, while also providing new insight into other vital parts of soil communities (i.e. viruses) and looking beyond changes in microbial diversity to better understand how agriculture affects soil microbial communities.

Keywords: soil microbial community, microbial diversity, functional diversity, microbial ecology, virus abundance, viral metagenomics, agriculture, agroecosystem, land use, perennial grassland, cropland

Chapter 1 : Introduction

1.1 Agriculture

1.1.1 A brief history of agriculture

Agriculture is the basis of all civilizations. Without agriculture, early societies could not create large permanent settlements making it a prerequisite for people moving to more sedentary lifestyles (Tauger, 2016). Farming, or the practice of agriculture, consists of the cultivation of crops and raising of livestock to produce food, feed, fiber, and other desirable products. While agriculture is known to have been invented more than once in history (Solheim, 1972), the second agricultural revolution took place in Europe in the 1800s with the introduction of crop rotations, integration of livestock in crop production, and improvement of the plow system playing an important role for economic development (Andersen et al., 2016). During the last half of the nineteenth century, the ability to manufacture reliable and affordable machinery for agriculture was developed which allowed agricultural output to greatly increase (Hounshell, 1984). After mechanical advances came chemistry with the use of chemical fertilizer, insecticides, and fungicides, followed by a focus on soil makeup and analysis of agricultural products, both of which are still at the forefront of agricultural practices today. While these practices of agricultural intensification have successfully increased crop yields to meet the global food demand, it is now known that it is resulting in ecosystem degradation (Reid et al., 2005). This had led to agriculture across the world to be particularly vulnerable and functioning under significant strain. The developments over the twentieth and twenty-first centuries such as farming becoming dependent on technology and energy, environmental changes like global warming, and rapid population growth has

further complicated agriculture for future generations (Tauger, 2016). Together, such developments are leading agriculture to reach its global limits and generating a looming crisis, further stressing the importance of continued research on agriculturally impacted ecosystems.

1.1.2 Land use in a U.S. Southern Plains agroecosystem

An agroecosystem is the basic unit of study of agricultural ecology and involves a large number of alterations to a natural ecosystem in order to provide ecosystem services (Costanza et al., 1997). Agroecosystems generally consist of complex food chains and food webs interacting to create a single stable unit. Agriculture results in systems becoming more clearly defined in terms of biological and physicochemical properties and are simplified by losses of indigenous fauna and flora including the reduction in numerous natural ecosystem processes (Conway, 1985; Ding et al., 2013). Inversely, agroecosystems also become more complex through the input of human activity and management (Conway, 1985). Originally, agroecosystems could be characterized by decreasing diversity while increasing functionality by selecting for more productive crop varieties with the removal of unproductive species (Moonen and Bàrberi, 2008). They also tend to be associated with nutrient input in the form of organic or inorganic fertilizers. It is now known that such changes in land use have a long-lasting impact on soil nutrients, texture, and pH (Murty et al., 2002) that are often associated with the decreased plant diversity and management input (Lauber et al., 2008), changing the perspective on how biodiversity should be managed in agroecosystems. It was now proposed almost 25 years ago that agroecosystems can no longer focus on just above

ground productivity, but need to start disentangling the relationships between intensification, biodiversity, and function including soil biodiversity (Giller et al., 1997).

In the U.S. Southern Great Plains, agroecosystems often consist of varying land uses including rangelands, pastures, and croplands converted from natural ecosystems. Grasslands make up the greatest land cover in the subhumid to semiarid areas of the Great Plains (Ghimire et al., 2019) with native tallgrass prairies originally constituting the majority of the midwestern US (Fierer et al., 2013). Through fire suppression, the removal of native animal species, and plowing of native grasses, tallgrass prairies have been one of the most significantly destroyed major North American ecosystems (Fierer et al., 2013; Samson et al., 2004; Sims and Risser, 2000). Grasslands have large root systems generally exceeding that of above ground biomass (Yang et al., 2010) improving soil structure and increasing soil carbon (Kandeler and Murer, 1993; Woods, 1989) making them targets for agricultural cultivation. The term grassland is used to represent rangelands, native grasslands, and pastures all of which are used for agriculture to support livestock. Although, pastures typically involve more management than other grasslands such as the application of fertilizers and herbicides (Peterson et al., 2018). In the Southern Plains, Old World bluestem is commonly used in pasture sites because it is a productive perennial grass that does well under limited water conditions (Philipp et al., 2005). While pasture sites have resulted from the conversion of natural lands, changing more intensively management disturbed agricultural land uses into perennial pastures has positive impacts on the ecosystem such as reducing soil loss, restoring organic matter, and promoting soil structural stability (Haynes et al., 1991; Nath and Lal, 2017). Livestock grazing in grasslands can also contribute to greater soil nutrients through

animal waste (Vendramini et al., 2007). Grasslands including native systems are a key part of the agroecosystems in the U.S. Southern Great Plains, but grasslands only represent a portion of land uses in these systems.

In the United States, the majority of new croplands from 2008-2012 were originally grasslands with winter wheat being the dominant planted crop in the Southern Plains (Lark et al., 2015). More recently, crop rotations of winter wheat and winter-hardy cultivars of canola have drawn attention to control weeds, break disease in wheat, and increase grain yield for the following wheat crop (Bushong et al., 2012; Daugovish et al., 1999). Most winter wheat is grown under rainfed conditions and is managed in several ways including grain only (no grazing), graze-grain (dual purpose), and graze-out (no grain production) (Phillips et al., 1999). In the U.S. Southern Plains, the traditional management technique is conventional tillage (Hossain et al., 2004). Conventional tillage management includes plowing the soil and post-harvest removal of crop residues. Such practices result in loss of soil structure, soil erosion, and reduction of soil organic matter (Liu et al., 2010a). Due to these unfavorable impacts, conservation tillage practices such as reduced tillage or no-tillage with residue retention have gained attention as alternative management strategies. Results have shown conservation tillage practices to enhance soil quality by reducing erosion, enhancing soil moisture, increasing soil nutrients, and in some cases increasing crop yields (Hobbs et al., 2008; Ward et al., 2012). Therefore, in this region, it is common for a combination of conventional tillage and no-tillage practices to be used for winter wheat and canola crop production (Hossain et al., 2004). Yet, current crop production still relies heavily on management that involves chemical, mechanical, and biological inputs to support the population (Tilman et al., 2002), with the

long-term sustainability of crop production and the accompanying management practices under further investigation (Pretty, 2008; Tilman et al., 2002).

1.1.3 Defining a sustainable agricultural system

With an increasing population, the global food demand will continue to rise in the coming decades (Long et al., 2015) putting a greater emphasis on agricultural sustainability. Even though agriculture is working to improve management practices, it is still up for debate on how to quantify good management practices and what makes a system sustainable. Sustainable agriculture is generally defined as being able to meet the needs of the present generation without compromising the ability to meet the needs of future generations (World Commission on and Development, 1987). While sustainability must be addressed from several angles including economic and social (Goodland, 1995), agricultural research focuses on the environmental impact such as protection and effective management of natural resources. Ranges of indicators have been used to determine farm-level sustainability generally focusing on crop and soil properties under different management systems. For example, pest control, soil fertility, and soil erosion have been used as indicators on Malaysian farms (Taylor et al., 1993); while crop yield, frequency of crop failure, ground cover, organic matter, and soil depth were used in the Philippines (Gomez et al., 1997). Farmers in Utah relied on pest management, nutrient management, and field operations to create a farming index for sustainable agriculture (Drost et al., 1998). Similarly, a farm-level index in the UK was developed based on pest control, soil fertility, seed source, and crop management (Rigby et al., 2001). Although many different methods have been used to gauge sustainability, there are always several

common factors that appear to be important in most fields based on preserving natural resources from reducing management disturbances, but the majority of indicators previously used focus on aboveground health and abiotic soil characteristics ignoring soil microbial communities.

As knowledge has progressed on above and belowground interactions in agricultural soil systems, the idea of using soil microorganisms as soil health indicators has evolved as an area of interest for agricultural sustainability. It is believed there is great potential for using bacterial community composition or the relative abundance of specific taxa to understand the state of the soil environment (Hermans et al., 2017) because they are considered to be indicators of early changes in the quality of soil ecosystems (Kennedy and Stubbs, 2006). Previously, microbial populations have provided early signs of positive and negative transformations in soil ecosystems that preceded discernable changes in the physical and chemical properties of the soil (Nielsen et al., 2002; Pankhurst et al., 1997). Such strategy will likely be accomplished by examining changes at lower taxonomic levels that might not be visible at higher taxonomic ranks (Dohrmann et al., 2013), but it could also be largely dependent on the fact that terrestrial biomes are differently affected by agricultural management. In a Caatinga dry forest surrounded by agricultural lands therefore affected by water and nutrient use, almost all detected genera of Actinobacteria were enriched during drought periods potentially making them good indicators of "health" in arid soils in relation to irrigation practices (Lacerda-Júnior et al., 2019). On larger scales, meta-analyses of over 100 sites indicated bacterial communities and specific taxa were able to reflect changes in anthropogenically impacted soil environments (Hermans et al., 2017) and trends at the

community-scale held across tropical, temperate, continental, and arid biomes (Trivedi et al., 2016). As more studies are conducted looking at microbes as indicators of soil health, it will become clear if this is a reliable method for examining agricultural sustainability. If so, it could offer substantial benefits due to the speed and ease of data analysis in addition to less sampling-related disturbance compared to traditional chemical and biological measures (Lear et al., 2012). While using microbial communities to determine soil health is a promising direction in agricultural sustainability, more research needs to be done to determine if this is a viable method without forgetting about the importance of chemical and physical indicators in determining overall soil health.

1.2 Impact of agriculture on the soil microbial community

1.2.1 Introduction to microbes in soils

Soil microorganisms have been recognized for their importance for almost a century (Waksman, 1928), and despite their small size, make up one of the greatest forms of biomass on the planet. Soil frequently contains more than 1000 kg of microbial biomass carbon per hectare contending with forms of aboveground biomass including plants and animals (Fierer et al., 2009; Serna-Chavez et al., 2013). Not only are they highly abundant with cell estimates of up to 10^9 per gram of soil (Gans et al., 2005), but they are also extremely diverse with an estimated 10^4 species per gram of soil (Curtis et al., 2002). The original use of culture-based approaches greatly underestimated soil microbial diversity; however methodological advances have allowed for the discovery of an extensive diversity of microbes in soils providing researchers the ability to examine relationships between microorganisms and soil processes (Fierer, 2017). It is now known

that microorganisms play important parts in carbon sequestration, nutrient cycling, maintenance of soil fertility, and impact plant productivity (Fierer, 2017; Griffiths and Philippot, 2013). Yet, many of the important functions performed by soil microbial communities are threatened by a range of factors including climate change, soil degradation, and poor land management practices (Amundson et al., 2015). Therefore, despite the large body of literature on the impact of environmental factors and agricultural management on microbial communities, it is important to continue examining the dynamics of microbial diversity and associated functional traits as conditions continue to change and the demand for sustainable agricultural practices grows.

Taking into account the importance of microbes in global processes, it is believed that effective management of soil is one of the best lines of defense in reducing biodiversity loss and mitigating climate change (Cavicchioli et al., 2019). To be able to accomplish this task, ecologists need in-depth knowledge on the organisms present in soil and the environmental conditions such as topography, soil physicochemical characteristics, and climate that drive their activity; which has led to the continued detailed analysis of soil communities patterns around the world (Crowther et al., 2019). Soil communities are generally characterized by biomass measurements, taxonomic and phylogenetic diversity, taxonomic composition, functional group composition, and functional trait expression. The amount of microbial biomass in soil provides information not only on the abundance of organisms in a system but also offers insight into the ability of an area to cycle nutrients (Wang et al., 2003) acting as a baseline for determining the functional potential of the community (Crowther et al., 2019). Examining the relative

abundance of taxa is useful for identifying which groups of organisms dominate the system, and in combination with biomass measurements, can provide abundance estimates of specific taxa. Also, if the general functional traits of dominant taxa are known, it can provide initial evidence on the functional capacity of communities within a system as well as a direction for further functional research questions (Delgado-Baquerizo et al., 2018). However, at a fine-scale, taxonomic identity alone does not define function. It is the expression of the functional traits of the community that needs to be examined to determine what processes are taking place at any individual time or location. The differences in soil community composition and gene expression between systems are often determined by the combination of edaphic factors and climate (Noronha et al., 2017), but the introduction of anthropogenic disturbances (i.e. agriculture) could impact the influence of each factor. Even with all the studies on soil microbial communities, the ability of microbial diversity and function to fluctuate with time and space are often related to natural and anthropogenic disturbances making a comprehensive understanding of soil microbial community dynamics an enormous task (Nannipieri et al., 2003; Nannipieri et al., 2017; Nannipieri et al., 2020).

1.2.2 Spatial and temporal dynamics in the soil environment

Soil microbial communities are subjected to significant changes and disturbances that are of great importance on both temporal and spatial scales especially in the rhizosphere. Variations can be hard to determine since microbial communities can differ between areas meters to centimeters apart (O'Brien et al., 2016), and on larger scales studies have demonstrated that sampling space generally has the greatest impact on the microbial

community (Zhang et al., 2020). While soils are very heterogeneous, the implementation of agricultural management practices, specifically conventional tillage, results in the complete disturbance of soil in the rhizosphere (Peterson et al., 2019) making for a more homogenous soil structure than what is found in natural ecosystems. It has been shown that land conversion strongly negatively affects diversity creating microbial communities that are more similar across space (Rodrigues et al., 2013). Since soils are 3D dynamic environments that are constantly changing, they are not only affected by spatial differences but a fourth dimension of time which is generally less understood than spatial dynamics (Nannipieri et al., 2020). Soil microbial communities are subjected to short-term changes (i.e. hours to weeks), seasonal changes, and long-term trends all of which may be influenced by different factors such as freeze-thaw, changes in plant activity, and climatic change (Chernov and Zhelezova, 2020). In agricultural fields, temporal dynamics are also strongly linked to management practices (Spedding et al., 2004) as crops have specific growing seasons that are often accompanied by fertilizer, herbicide, and pesticide application. Yet, in several studies examining spatial and temporal dynamics, temporal variation is still usually less than spatial variation (Lauber et al., 2013; Uksa et al., 2014; Zhang et al., 2020). Absence of the detection of temporal variation may be related to the fact soil sampling is destructive so the exact location cannot be resampled (Fierer, 2017), the presence of relic DNA (Carini et al., 2016), and the lack of focus on living or active cells (Herzog et al., 2015; Zifcakova et al., 2016). Therefore, determining the contribution of spatiotemporal variations to microbial community compositional and functional differences is complex, and likely depends on the spatial and temporal scale in question as well as the land use being examined.

To understand how an ecosystem works, the effect of biotic and abiotic factors on ecosystem components needs to be determined. Many factors, directly and indirectly, influence microbial communities, and at a broad scale, several abiotic and biotic factors are regularly found to influence the bacterial community composition (Bahram et al., 2018; Fierer et al., 2009; Lauber et al., 2009). These key factors generally include soil pH (Lauber et al., 2009), temperature (Oliverio et al., 2017), nitrogen availability (Cederlund et al., 2014), and soil organic carbon content (Sul et al., 2013). Globally, bacterial biomass increases in areas of high soil organic matter content and lower pH; while bacterial richness appears to be the greatest in soils at midlatitudes where pH is relatively neutral and the soil carbon:nitrogen ratio is high (Bahram et al., 2018). Soil moisture availability has additionally been suggested as one of the best predictors of total soil microbial biomass at the global scale (Fierer, 2017). Functional gene diversity of bacteria peaks at midlatitudes with strong correlations to annual perception (Bahram et al., 2018), and is also driven by the interactive effects of edaphic factors and climate (Fierer et al., 2012b; Noronha et al., 2017). The majority of these large-scale studies focus on dynamics in natural systems and similar land use types, but intensive disturbances like agriculture can modify intrinsic soil properties resulting in significant impacts on soil moisture, pH, texture, nutrient status, and plant community composition (Lauber et al., 2008; Murty et al., 2002) potentially impacting the importance of specific factors on a local scale. When looking across a large transect including multiple land uses, microbial spatial distribution was impacted by soil properties as well as climate, topography, and land use, with areas of agricultural use having a local influence on microbial community variation (Xue et al., 2018). For example, in a study comparing monoculture cropping systems, the edaphic

properties shaped by land use were more important than the effect of specific crops on the bacterial communities (Bainard et al., 2016). The routine application of inorganic fertilizer in agricultural systems has been shown to reduce bacterial abundance and biodiversity while also changing the relative importance of environmental factors between the rhizosphere and bulk soil (Wang et al., 2017). Therefore, there is no single factor that determines the dynamics of a bacterial community especially at a local scale where different management practices are implemented. More thorough knowledge of the interactions between land use management, biotic and abiotic factors, and climate are needed to understand the impact on microbial communities to help determine the best management strategies for specific regions.

1.2.3 Further importance of land use and management

To further complicate predictions of soil microbial communities across landscapes, land use history may have long lasting effects on local community structure. Less is known about the persistence of land use legacies as there is a lack of long-term experiments addressing the topic. Although, some results have shown microbial community composition and/or function can persist over a growing season (Frindte et al., 2020), years (Hawkes et al., 2017), decades (Bond-Lamberty et al., 2016), or potentially longer for certain taxa (Andam et al., 2016). Such results emphasize that microbial community dynamics are also based on the intrinsic properties of the community assembly location and not just the current environmental conditions (Crowther et al., 2019). Soil communities have also demonstrated the ability to retain some functional characteristics after invasive species (Elgersma et al., 2011), natural disturbances (Fichtner et al., 2014)

and land use conversion (Kallenbach and Stuart Grandy, 2015). Similarly, areas with extensive cultivation histories impact microbial community structure long after the fields are no longer used for agriculture purposes (Buckley and Schmidt, 2001). Land use conversion and natural disturbances are additionally accompanied by changes in vegetation type and diversity. The impact on soil microbial communities by plants species is mixed with several studies linking microbial communities to changes in plant communities (Barberán et al., 2015; Peay et al., 2013), while others have observed little to no effect (Nunan et al., 2005; Tedersoo et al., 2016). It is likely that plants species in their native soils have a larger impact on the soil microbial community due to long-term co-evolution of plant–microorganism interactions compared to agricultural ecosystems (Philippot et al., 2013). In addition, a range of soil bacterial taxa may have the ability to associate with many plant species potentially extending the amount of time it would take to observe changes in microbial communities related to changes in aboveground vegetation (Crowther et al., 2014). Therefore, the degree to which land use history and the plant community impact the microbial community likely differs with bacterial group, soil type, and plant species.

While land use legacy may maintain some microbial taxa and ecosystem function, agricultural practices inevitably will impact the environment with many of the unfavorable effects being long-lasting and challenging to alleviate. Moving towards more sustainable agricultural management generally starts with implementing reduced or no-tillage systems with residue retention as well as crop rotations that have shown to maintain soil fertility, manage disease and pests, and promote crop yield (Karlen et al., 1994). Agricultural sustainability also depends on the ability to reduce the use of mineral

nutrients and pesticides with one of the overall goals being to improve the management of the soil microbiome. This is often approached through adapting plant genotypes to the environment to take advantage of the biotic and abiotic resources (Philippot et al., 2013) or amending soils and seeds through the addition of beneficial bacteria to promote crop production (Tilak et al., 2005; Timmusk et al., 2017). Previous research indicates that the soil microbiome can also be used to minimize soil erosion (Chiquoine et al., 2016) and suppress plant disease (Kinkel et al., 2011). Although, the interaction between the inoculated plant and soil microbes may be temporary and depends on the stage of growth of the plant (Sessitsch et al., 2003) meaning the microbes that are beneficial likely vary with plant types and growth stage. It should not be expected that amending the microbial community for a specific purpose will result in the improvement of soil structure, disease suppression, and crop growth. Therefore, there is no "ideal" community for sustainable agriculture as it can be very context-dependent, likely being contingent upon the specific crop, the biotic and abiotic challenges, and soil conditions (Fierer, 2017). This further emphasizes the importance of studying the soil microbiome under different management practices, crops, and soil conditions to determine regionally specific sustainable management practices.

1.3 Bacteriophages in soil

1.3.1 Viruses in natural systems

It was not until 1989 that viruses were recognized as being abundant in the environment with Bergh *et al.* showing that there were roughly 10 million virus-like particles (VLPs) per mL of seawater (Bergh et al., 1989). Since then, it has been estimated that viruses are

the most abundant biological entity on Earth (Suttle, 2005). The majority of knowledge on viral ecology has been generated from the study of natural viral assemblages in marine ecosystems. Advances in accurate enumeration techniques in marine microbial ecology (Hobbie et al., 1977) lead to direct counts of viruses (Bergh et al., 1989) which was fundamental to understanding marine environments. Through the study of aquatic ecosystems, it is now known that viruses are prevalent with a 2000-fold range in VLP abundance from the deep sea to freshwater marshes (Srinivasiah et al., 2008) with most viruses being phage-type viruses (viruses that infect bacteria) (Børsheim, 1993) generally ranging in size from 30-60 nm (Jacquet et al., 2010). It was also observed that viral abundance does not remain constant and fluctuates with shifts in host abundance (Sandaa and Larsen, 2006; Weinbauer and Suttle, 1996) mostly due to the productivity of the system during certain times of the year (Filippini et al., 2008; Weinbauer and Peduzzi, 1994). Due to the ubiquity and wide range of VLP abundance, viruses were then thought to be an active part of aquatic microbial communities with viruses now being recognized for their role in biogeochemical nutrient cycling (Fuhrma and Suttle, 1993), shaping microbial communities through viral lysis (Muhling et al., 2005), acting as one of the greatest genetic reservoirs (Paul and Sullivan, 2005) and mediating horizontal gene transfer contributing to microbial evolution (Millard et al., 2004). For example, marine and freshwater systems have linked host mortality due to viruses as an important control of microbial community composition and biogeochemical cycling (Middelboe et al., 2008), with it being shown that viruses in marine systems release 20-30% of the daily carbon production into the water by lysing host cells (Suttle, 2007; Wilhelm and Suttle, 1999). With all the important information about viruses in aquatic ecosystems, there is a

narrow understanding of viral distribution and the relationship between viruses and hosts in many natural environments (Wommack and Colwell, 2000). Specifically, areas that need further research include the mechanisms controlling viral proliferation and activity such as abiotic parameters (Jacquet et al., 2010) in differing ecosystems. Due to the large impact of viruses in aquatic ecosystems, it is thought that viruses will play equally important roles in terrestrial environments.

As the interest in viral ecology grows, studies are now expanding to terrestrial ecosystems, but little is still known about natural virus populations in soils compared to aquatic environments. Early soil studies focusing on VLP abundance have shown a range of 80-390 x 10^7 VLPs per grams dry soil in wetlands to 23 and 64 x 10^7 VLPs per grams dry soil in agricultural areas of Delaware (Srinivasiah et al., 2008; Williamson et al., 2005) with viral abundance often being reflective of host abundance. With the continued use of these methods, studies have demonstrated that VLP abundance often exceeds bacterial abundance, showing less variability in VLP abundance than their bacterial hosts, and being extremely abundant in a diverse range of locations and soil types (Srinivasiah et al., 2008). Although several studies examine viral and microbial abundance in soils at a single time point, few studies examine the change in abundance of these communities related to time and space as well as what factors may impact virus populations (Narr et al., 2017; Roy et al., 2020). Still, with the lack of VLP abundance dynamics, soil virus ecology is quickly shifting to trying to understand viral community composition and function through the use of sequencing technologies. Viral diversity is generally examined using metagenomic approaches since there is no single genetic element shared by viruses (Rohwer and Edwards, 2002). Viral communities in oceans often share a large

portion of viral genotypes across communities (Angly et al., 2006) compared to soils that appear to be locally unique with little to no overlap in viral genotypes across land types (Srinivasiah et al., 2008). Also, considering the importance of viruses in carbon cycling in marine systems (Fuhrman, 1999; Suttle, 2005; Suttle, 2007), similar roles are now being explored in soils (Trubl et al., 2018). Viruses not only impact their hosts through lysis but also metabolically influence their hosts through the expression of virus-carried auxiliary metabolic genes (AMGs) (Breitbart, 2012; Middelboe and Brussaard, 2017) which are genes used for host manipulation instead of viral replication. AMG classification is still in its early stages, but it is a promising method to examine the role of viruses in natural ecosystems. While techniques to explore autochthonous viruses in soils are constantly expanding and improving, little is still known about terrestrial virus abundance and community dynamics.

1.3.2 Survival of viruses in soils

Through the use of different lifecycle strategies, viruses not only influence host dynamics but impact their ability to survive in unique ecosystems. Bacteriophages have four different lifecycles including the lytic cycle, lysogenic cycle, pseudolysogeny (Miller, 2006), and chronic infection (Russel and Model, 2006), with the latter two being poorly understood in soils. Generally, lytic viruses quickly kill off host populations susceptible to infection by redirecting the hosts' metabolism towards the production of new viruses and lysing the host cell. After cell lysis, progeny must survive extracellularly as a free virus until coming in contact with a new host for this lifestyle to be successful (Weinbauer and Suttle, 1996). Lysogenic replication, on the other hand, involves the

suppression of lytic function once inside the host cell and instead usually exists in a symbiotic state (Ackermann and DuBow, 1987) either integrated into the bacterial chromosome as a prophage or as an extrachromosomal element. Soils are very heterogenous systems that can affect the encounter rate of viruses with host cells, potentially making a lytic lifestyle unfavorable in soils. It is thought that when host abundance is low or conditions are poor, lysogeny provides a way for viruses to survive (Smit et al., 1996; Williamson et al., 2002) which could be advantageous in soil systems where conditions frequently fluctuate. Although, a switch of lifestyle can be induced by an environmental signal such as DNA damage or an influx in nutrients resulting in lytic function and the production of viral progeny (Campbell, 2006). While lysogeny is believed to be favored in soil ecosystems, few studies have been done to determine the interaction of virus communities, their lifestyles, and changing ecosystem dynamics.

The survival of viruses in soil is further impacted by a range of soil and environmental factors that influence the activity of viruses and their ability to infect host organisms. Similar to their microbial hosts, viral activity and survival in soil are impacted by many factors including temperature, soil pH, clay type, ionic strength, organic matter content, and moisture content (Kimura et al., 2008); and the interaction with these factors may differ from that on their microbial hosts. Temperature is key to virus survival in soil with studies having demonstrated longer survival times, longer latency, and reduced burst size of viruses with lower temperatures (Leonardopoulos et al., 1996; Straub et al., 1992). Similar to temperate, soil pH affects host activity and virus survival. Soil pH directly affects phage-host interactions such as penetration and length of the latent period (Sykes et al., 1981). Indirectly, pH influences virus survival by affecting the electrostatic

properties of the soil surface altering viral absorption and increasing survival time (Kapuscinski and Mitchell, 1980). Viral survival is controlled by binding to soil particles that have positively and negatively charged sites as well as hydrophobic sites; and soil pH will influence the charge of both viruses and soils (Kimura et al., 2008). Clay particles also have the ability to shelter viruses from inactivation by shielding them from harmful environmental effects such as UV radiation (Vettori et al., 2000). The majority of viruses in soil bind to clay particles increase the survival time of free viruses in soils (Bitton et al., 1979; Moore et al., 1982). Clay particles and ionic strength work together to influence viral survival. The higher the ionic strength, the greater the ability of clay particles to absorb viruses preventing host infection while allowing virus persistence (Kimura et al., 2008). Soils that tend to be rich in organic matter do not absorb viruses as strongly since its presence weakens the electrostatic binding abilities normally found in highly clay-based soils (Zhuang and Jin, 2003). Using plaque formation studies, it was revealed that the successfulness of infection is dependent upon the substrate composition (Delisle and Levin, 1972). Virus hosts, specifically microbial communities, are heavily influenced by soil composition affecting the susceptibility of the host and/or the activity of the viruses. Lastly, while many studies have examined moisture content in relation to microbial communities, it was not until the early 2000s that a significant correlation between soil water content and virus-like particle abundance was discovered (Williamson et al., 2005). Also, as soils dry, viruses may irreversibly bind inactivating them in the environment (Yeager and O'Brien, 1979). Together, these soil and environmental factors govern the movement of viruses, the absorption of viruses, and the overall infectivity and survival of viruses in soils. Although, the majority of the known interactions were discovered from

the study of specific viruses with much to still be revealed about viral community interactions with the soil environment.

1.3.3 Methodological limitations of studying viruses in soils

With the growing interest in the role of viruses in terrestrial ecosystems, it has become clear that there is a lack of standardized protocol across the field. Varying methods have been used throughout the literature to estimate viral abundance (Ashelford et al., 2003; Narr et al., 2017; Swanson et al., 2009; Williamson et al., 2013; Williamson et al., 2005; Williamson et al., 2003), but due to the lack of a consistent method, the reasons for variations between studies cannot be fully determined. To date, viruses extracted from soils using sonication and vortexing have been the most widely used and efficient mechanical methods published (Narr et al., 2017; Williamson et al., 2013; Williamson et al., 2005), and has been the most reliable with a range of extraction buffers (Narr et al., 2017). Williamson and colleagues found that, overall, potassium citrate buffer was the most effective for removing viruses from soils, but, when examining the few studies comparing extraction buffers, soil type was often the determining factor (Williamson et al., 2005; Williamson et al., 2003). Many soil, environmental, and viral factors are known to affect the adsorption of viruses to soils such as type of clay minerals (Carlson et al., 1968; Moore et al., 1982; Moore et al., 1981), cation exchange capacity (Moore et al., 1982; Schiffenbauer and Stotzky, 1982), organic matter (Carlson et al., 1968; Moore et al., 1982; Moore et al., 1981; Zhuang and Jin, 2003), soil pH (Loveland et al., 1996; Taylor et al., 1981), and virus type (Dowd et al., 1998; Schiffenbauer and Stotzky, 1982; Zhuang and Jin, 2003). Furthermore, viruses and bacteria are often extracted from soils in

the same process then enumerated separately (Danovaro et al., 2002; Sawstrom et al., 2009; Williamson et al., 2013). However, the combined extraction of bacteria and viruses can be biased, resulting in the preferential extraction of one cell type (Williamson et al., 2013). Taken together, all factors contribute to the need for optimized protocols to reduce differences between studies for better comparisons of virus abundance across a rapidly growing field.

The use of different virus enumeration methods often results in different trends in abundance across soil samples. Plaque assays, transmission electron microscopy (TEM), and epifluorescent microscopy (EFM) have all been used to study autochthonous bacteriophages in soils. While plaque assays demonstrate viral activity, the method greatly underestimates viral abundance due to the need to isolate phage-host systems (Wommack and Colwell, 2000). Current TEM and EFM methods give more accurate VLP counts than culture-based methods, however, both still have disadvantages (Ferris et al., 2002; Williamson et al., 2013; Williamson et al., 2005; Williamson et al., 2003). EFM has become the standard enumeration technique, yet it cannot be absolutely determined that the dots being observed during direct counts are viruses (Breitbart and Rohwer, 2005), hence it is sensitive to false positives. TEM gives confidence that the particles being counted are in fact viruses, but the abundance is usually several times lower than with EFM (Williamson et al., 2003). Overall, studies of viral extraction and enumeration, especially in porous media environments, have been limited by methodology, reinforcing the need to develop uniform protocols.

Studies of virus communities are shifting greatly towards culture-independent methods since only a tiny fraction of viruses are culturable in laboratory settings. As

previously mentioned, the first challenge is removing viruses from the soil matrix which will likely be based on soil type (Pratama and van Elsas, 2018), followed by overcoming issues of low DNA yields and co-extracted inhibitors (Zielinska et al., 2017) during DNA extraction. Amplification methods are often needed due to low DNA yields, but many widely used amplification methods bias samples by overamplifying specific virus types (Binga et al., 2008; Karlsson et al., 2013; Kim and Bae, 2011). Although, more recent developments of amplification methods have shown promising results for maintaining the relative abundance of template viral DNA (Roux et al., 2016b; Trubl et al., 2018). This is important as amplification will to needed to understand viral variations and dynamics at smaller sampling scales where pooling larger viral samples will not be able to address critical ecological questions (Fierer, 2017). These methods generally apply to datasets that have been enriched for viruses such as single-virus genomes or viral metagenomes. However, viral sequences can also be mined from microbial genomes and metagenomes but might be biased toward viruses that infect the dominant host cell in the system (Roux et al., 2019a). While sequencing has been able to show diverse viral communities in a range of ecosystems, the majority of viruses cannot be taxonomically identified being referred to as uncultivated virus genomes (UViGs). UViGs make up the majority of available sequences in publicly available databases (Brister et al., 2015; Paez-Espino et al., 2017) further showing the fast growth of sequencing data, but at the expense of not being able to identify who is present in the community. Lastly, to be able to determine the role of viruses in soils, the hosts of the viruses need to be predicted. The most reliable host-range prediction method relies on sequence similarity but requires a closely related host genome to have been sequenced (Edwards et al., 2016). While other less specific

approaches exist, all techniques remain to be predictive therefore still need to be interpreted with caution. As virus studies continue to quickly multiply, many steps of the process need to be more consistent to provide a better framework for generating, analyzing, and reporting virus sequence data.

1.4 Focus and objectives

As previously mentioned, agriculture is the backbone of functioning societies providing goods needed for human survival. Yet, land use conversion for agricultural use has resulted in numerous environmental consequences including decreased soil organic matter, above- and belowground biodiversity loss, and alterations to ecosystem functions. With the continued population growth, the ability to sustain agriculture into the future without further environmental costs is becoming a critical issue resulting in a revived focus on understanding the impacts of agricultural activity on soil microbial communities. Therefore, this dissertation aimed at addressing how soil microbial communities were shaped by a range of agricultural land uses often found in a U.S. Southern Plains agroecosystem as well as the temporal dynamics of these microbial communities based on differences in soil properties, climate factors, and management input. Soil samples were collected from grassland and cropland ecosystems and examined using high-throughput sequencing technologies to characterize the diversity and composition of the soil microbial communities. Major results are summarized in the following chapters.

Chapter 2 focused on the temporal dynamics of soil bacterial communities over one year subjected to different disturbance intensities across a U.S. Southern Plains

agroecosystem. The four field sites included a tallgrass prairie (TGP), old world bluestem (OWB) pasture, no-tillage (NT) canola cropland, and conventional tillage (CT) wheat cropland. Soils were sampled every two weeks during the fall and spring months and once a month during summer and winter months, all of which were used to characterize the bacterial communities. Several of the sampling times were further analyzed using GeoChip functional gene array to examine the diversity in the functional genes of the soil bacterial communities. We aimed to address the following questions: i) Does land use with varying management disturbance and season shape soil properties? ii) How do land use and seasonal temporal dynamics interact to influence bacterial community diversity? iii) What roles do soil and environmental properties play in influencing bacterial community diversity between seasons and under increasing management disturbance? This study intended to determine the effect of land use and sampling time on the structural and functional diversity of soil bacterial communities as well as help provide insight on which management factors need to be improved in U.S. Southern Plains agroecosystems to support important ecosystem services.

Chapter 3 focused on examining the impact of land use conversion on the ecological relationships of microbial communities using molecular ecological network (MEN) analysis. This study investigated these relationships for the conventionally tilled wheat cropland and native tallgrass prairie system as this is one of the most common types of land use conversion that occurs in the U.S. Southern Plains as well as has the greatest difference in management disturbance of the four sites studied in the previous chapter. Soils were sampled monthly for a total of 19-months to understand whether or how land use affects the complexity and stability of soil microbial MENs over time. We aimed to

address the following questions: i) How does land use conversion from the native system to cropland use impact the complexity and stability of the molecular ecological networks (MENs) over time? ii) Does land use conversion from the native system to cropland use change the relationship between the complexity and stability of the MENs? iii) Are the relationships between complexity and stability of the MENs with environmental factors altered due to land use conversion and management practices? In general, this study set out to better understand the ecological consequences of converting native land into long-term croplands by focusing on the network features which may be affected by both time and space.

Chapter 4 focused on viruses in agricultural soils which are an important part of the overall diversity of soil ecosystems. Like their microbial hosts, soil viruses are also affected by the changes of physiochemical properties in soil ecosystems that occur due to land use conversion. This study investigated the temporal changes in virus and potential host abundances over one year in the native tallgrass prairie and two cropland sites, CT wheat and NT canola. We also aimed to address whether increasing amounts of land management disturbance (TGP < NT < CT) had a further effect on the influence of soil and environmental factors on viral abundance. Additionally, the impact of land use on viral community structure in the native tallgrass prairie and conventionally tilled cropland were inspected using metagenomic sequencing. Overall, this study examined whether land use, season, and soil and environmental variables had an observable impact on virus abundance and bacterial host abundance, and if these differences in abundance were also accompanied by differences in virus community structure.

In summary, this dissertation provided notable results on the effects of land use disturbance and the interaction with temporal dynamics of soil microbial and viral communities in a U.S. Southern Plains agroecosystem. Together, this information is critical to understanding the impacts of long-term agricultural land use on soil ecosystems which is needed when trying to develop sustainable agriculture practices.

Chapter 2 : Temporal dynamics of bacterial communities along a gradient of disturbance in a U.S. Southern Plains agroecosystem

2.1 Abstract

Land conversion for intensive agriculture produces unfavorable changes to soil ecosystems causing global concern. Soil bacterial communities mediate essential ecosystems processes making it imperative to understand their responses to agricultural perturbations. Here, we used high-throughput sequencing coupled with functional gene array to study temporal dynamics of soil bacterial communities over one year under different disturbance intensities across a U.S. Southern plains agroecosystem, including tallgrass prairie, old world bluestem pasture, no-tillage (NT) canola, and conventional tillage (CT) wheat. Land use had the greatest impact on bacterial taxonomic diversity, whereas sampling time and its interaction with land use were central to functional diversity differences. The main drivers of taxonomic diversity were tillage > sampling time > temperature, while all measured factors explained a similar amounts of variations in functional diversity. Temporal differences had the strongest correlation with total nitrogen > rainfall > nitrate. Within land uses, community variations for CT wheat were attributed to nitrogen levels, whereas soil organic matter and soil water content explained community variations for NT canola. In comparison, all measured factors contributed almost equally to variations in grassland bacterial communities. Lastly, functional diversity had a stronger relationship with taxonomic diversity for CT wheat compared to phylogenetic diversity in the prairie. These findings reinforce that tillage management has the greatest impact on bacterial community diversity with sampling time also critical.

Hence, our study highlights the importance of the interaction between temporal dynamics and land use in influencing soil microbiomes, providing support for reducing agricultural disturbance to conserve soil biodiversity.

2.2 Introduction

Rising human populations have resulted in the need for increased land conversion to heavily managed environments for greater food production. Yet, land use change represents one of the largest perturbations to soil ecosystems significantly impacting both aboveground and belowground communities (Ding et al., 2013; Lauber et al., 2008). Whole ecosystem diversity is generally diminished when natural land is converted to agricultural systems with lasting negative effects on soil health (Murty et al., 2002). In general, agricultural land use type regulates microbial diversity, plant diversity, and soil physicochemical properties (Calderón et al., 2001; Plassart et al., 2019; Zhang et al., 2020; Zhao et al., 2005). The effect of land use on microbial communities has become increasingly important since microbes represent the bulk of biodiversity in terrestrial ecosystems, perform essential ecosystem functions, and are fundamental to ecosystem stability (Bell et al., 2005; Choudhary et al., 2011; de Moraes et al., 1996).

While it has been established that changes in land use shift microbial community structure and diversity, there has been a renewed focus on observing these communities under an increasing gradient of disturbance intensities due to the quickly growing need for sustainable agricultural practices. Different intensities of soil disturbance creates unique environments that support microbes with those specific environmental requirements (Steenwerth et al., 2002). Although terrestrial microbial studies over large

spatial scales (Bahram et al., 2018; Plassart et al., 2019; Xue et al., 2018; Zhang et al., 2020) have demonstrated which soil and environmental factors are important for shaping microbial distribution patterns, they are unable to pinpoint the dynamics required to manage microbial communities at the local level. Agricultural management practices also vary locally with inputs such as tillage, pesticide and fertilizer use, crop rotation, and residue incorporation directly altering soil microbial biomass (Franzluebbers et al., 1995; Sparling et al., 1994) and community composition (Ding et al., 2013; Ishaq, 2017). This is critical as there is no ideal community type (Fierer et al., 2021), soil type, or soil characteristics (Bünemann et al., 2018; Lehmann et al., 2020) when trying to define a functional soil system. By directing attention to gradients of disturbance in a range of land uses commonly found in agroecosystems, local variation can be captured in soil and environmental properties, management type, and plant diversity, which may give insight into the complex dynamics shaping soil communities (Steenwerth et al., 2002).

Patterns of variability between land uses with increasing management disturbance have been studied extensively at single timepoints, but much less is known about the extent to which land use under a gradient of disturbance intensities interacts with temporal dynamics in altering soil bacterial communities. As seasons transition, variations occur in environmental factors such as solar radiation, temperature, and precipitation, all of which can affect microbial community structure and functions (Ishaq et al., 2020; Koranda et al., 2013; Lacerda-Júnior et al., 2019; Lauber et al., 2013). Several studies investigating soil microbial community changes in relation to temporal variability have observed community differences on a range of time scales many of which are associated with shifting environmental conditions (Lacerda-Júnior et al., 2019;

Lauber et al., 2013; Zhang et al., 2011). These variations in environmental conditions and community structure are often related to land management practices (Ghimire et al., 2019; Ishaq, 2017; Ishaq et al., 2020) and temporal changes in plant growth and development (Chaparro et al., 2014; Sayer et al., 2017). Specifically, plant growth alters rhizodeposition promoting microbial activity (Philippot et al., 2013) and modifying community composition by enriching specific microorganisms (Peiffer et al., 2013). Expanding on spatiotemporal studies that are specific to the local land use, plant community, and soil conditions are critically needed.

Functional diversity of the soil microbial community is equally important as compositional diversity when examining overall ecosystem diversity. Typically, a high structural and functional microbial diversity are thought to be fundamental to soil health, function, and sustainability by providing functional redundancy critical for ecosystem stability in the presence of stress and disturbance (Bell et al., 2005; Bérard et al., 2011; Foley et al., 2005; Kuan et al., 2006). The use of functional gene arrays (FGAs) or GeoChip has provided a way to examine relationships between microbial community structure and function by focusing on genes important to microbial processes like biogeochemical cycling and stress responses (He et al., 2010; He et al., 2007; Rhee et al., 2004; Shi et al., 2019; Tu et al., 2014; Van Nostrand et al., 2016; Wu et al., 2001). FGAs allow for a thorough analysis of essential ecological questions especially those concerned with microbial community responses to disturbances (Cong et al., 2015; Guo et al., 2020; Hazen et al., 2010; He et al., 2008; Shi et al., 2019; Zhou et al., 2014), including soil microbial community responses to land use, land management, and temporal changes (Berthrong et al., 2009; Reeve et al., 2010; Zhou et al., 2020). However, it remains

unclear how the functional capabilities of soil microbial communities change under a gradient of disturbance intensities and seasons.

To investigate the effect of land use with increasing management disturbance and season on the temporal dynamics of soil bacterial communities and its underlying mechanisms, we conducted a 12-month field study in agroecosystem land uses with a gradient of disturbance within the U.S. Southern Plains agroecosystem. The agricultural sites included two perennial grasslands and two annual croplands: a native tallgrass prairie (TGP), Old World Bluestem (OWB) pasture, no-tillage (NT) canola (*Brassica napus* L.) field, and conventional tillage (CT) winter wheat (*Triticum aestivum* L.) field. In this study, we focused on i) Does land use with varying management disturbance and season shape soil properties? ii) How do land use and seasonal temporal dynamics interact to influence bacterial community diversity? iii) What roles do soil and environmental properties play in influencing bacterial community diversity between seasons and under increasing management disturbance? We predicted that soil bacterial diversity would decrease with increasing management disturbance while seasonal differences would become more discernable with increasing management disturbance. Our results revealed that land use drove differences in taxonomic diversity while sampling time and its interaction with land use influenced functional gene diversity, and that the biotic and abiotic factors shaping bacterial community diversity also differed spatiotemporally with their importance varying with management intensity.

2.3 Materials and Methods

2.3.1 Site description & field sampling

The study was conducted at the United States Department of Agriculture, Agricultural Research Service, Grazinglands Research Laboratory at El Reno, OK, (35° 34.1' N, 98° 03.6' W; 414 m above sea level) from August 2016 to July 2017. Soil was collected from four sites: native tallgrass prairie (TGP), introduced (OWB) pasture, NT canola field, and CT winter wheat field. The grasslands and croplands were approximately 2.7 km apart. All four sites are included in the Southern Plains site of the Long-Term Agroecosystem Research (LATR) network (Kleinman et al., 2018; Spiegal et al., 2018). The soil type for the research area was Bethany silt loam (a fine, mixed, superactive, thermic Pachic Paleustolls). The study area has a temperate continental climate with summer months being characteristically hot and dry with a 30 year (1980–2010) average daily maximum and minimum air temperature of 22.5℃, and 8.8℃, respectively, and rainfall mostly occurring in May-June and September-October with an average annual rainfall of 860 mm (Bajgain et al., 2018; Fischer et al., 2012; Peterson et al., 2018; Peterson et al., 2021).

Native tallgrass prairie consists of mainly warm-season native mixed grasses. This mixture includes big bluestem (*Andropogon gerardii* Vitman.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), Indiangrass (*Sorghastrum nutans* (L) Nash), and switchgrass (*Panicum vergatum* L.). Old World bluestem (*Bothriochloa* spp.) is a monoculture pasture site that was established well over 20 years before this study was conducted. Both pasture sites had deep soils (> 1 m depth) and high water holding capacity (Bajgain et al., 2018; Zhou et al., 2017). During the sampling period, the TGP

was grazed by beef cattle for approximately five of the sampling months at a stocking density of 0.13 head/ha for 30 days and 0.83-1.06 head/ha for the remaining months. The OWB pasture was grazed for roughly eight of the sampling months at a stocking density ranging from 0.65-0.94 head/ha. Prescribed burns of the pasture sites are on a 4-year rotation with the most recent burning occurring in February 2014. The OWB pasture is managed using annual fertilizer and herbicide treatments (Peterson et al., 2018), while the native TGP is not fertilized. In these pastures, vegetation generally greens up in April and enters the senescence phase towards the end of October with peak growing season occurring during the May-June period (Wagle et al., 2017).

As a cool-season crop, winter wheat is the dominant cultivated ecosystem in the U.S. Southern Plains generally converted from native tallgrass prairies. Winter wheat has been planted in the study sites under CT management since the late 1990s. In Oklahoma, winter wheat fields are managed for multiple purposes such as grain-only, graze-grain, and graze-out. The CT wheat field was grain-only during the 2015-2016 growing season and graze-out (no grain production; cattle grazing from November through May) during the 2016-2017 growing season. Each year the seedbed was prepared for planting using a chisel plow treatment to a depth of 31 cm, which resulted in complete disturbance of soil and residue mixing (Peterson et al., 2019). The NT canola field was converted from a CT wheat field in 2015. It was grain-only wheat during the 2015-2016 growing season and on canola rotation during the 2016-2017 growing season as a part of the 4-year crop rotation (canola, grain-only wheat, graze-grain wheat, and graze-out wheat). It was the first year canola had been grown in the NT plot. Both croplands were left fallow from June to September being fertilized and planted between late August to mid-October.

Crops had fall and spring growing seasons and were dormant during the winter months. The CT wheat site was harvested in early June and the NT canola site was harvested in late June. Detailed management data have been previously published (Wagle et al., 2019).

Soil sampling began in August 2016 for all sites. Soil sampling was conducted every two weeks during the fall and spring months and once a month during summer and winter months, resulting in 20 sampling times per field between August 2016 and July 2017 for a total of 80 soil samples. During each soil sampling time point, eight cores roughly 20 meters apart were taken in a random walking pattern throughout each field at a depth of 0-15 cm using a 2.5 cm-diameter soil probe. Soil cores were pooled and homogenized at each sampling time for a representative sample of each plot. Soils were sieved to 2 mm to remove debris and stored at -80°C until analysis.

2.3.2 Soil properties & climate data

Weather data including average monthly rainfall, maximum air temperate, average air temperature, and minimum air temperature were gathered from an Oklahoma Mesonet weather station (http://www.mesonet.org/index.php/weather/local/elre) in El Reno (ELRE), Oklahoma. The Mesonet tower is located on the native TGP site used in this study (35° 32.9' N, 98° 02.2' W). Soil chemical analyses were performed at the Oklahoma State University Soil, Water and Forage Analytical Laboratory (https://agriculture.okstate.edu/departments-programs/plant-soil/soil-testing/). Tests included topsoil nitrate (TopN), soil organic matter (OM), soil total nitrogen (TN), and

ammonium (NH_4). Gravimetric soil water content (SWC) was determined by oven drying for ≥ 24 hours at 65ºC or until the weight no longer changed (Peterson et al., 2019).

2.3.3 Soil DNA extraction, PCR amplification, & sequencing

Microbial genomic DNA was extracted from 0.25 g of soil using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions and DNA was eluted with sterile water. For each soil sample, four technical replicates were extracted. DNA was quantified with the Qubit® dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) as described by the manufacturer's instructions. DNA dilutions of 2 ng/µL were prepared for use in PCR. PCR was performed using a two-step barcoding protocol (Herbold et al., 2015). For the first DNA amplification, primer pair M13 tagged 341F (5'-GTAAAACGACGGCCATACGGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3') were used (Klindworth et al., 2013). The second PCR step used a barcoded version of the forward primer and the 785R primer stated above. The PCR reaction for amplification was a 50 µL containing 0.1 µL of each primer (100 µM), 2 µl of template DNA, 25 µL of Phusion High-Fidelity PCR Master Mix with HF Buffer, and 23 µL of water. The PCR conditions were preliminary denaturation phase at 95 ºC for 5 min then 30 cycles at 95 ºC for 30 s, 55 ºC for 30 s, 72 ºC for 60 s, and final extension at 72 ºC for 10 min. PCR products were checked on a 1% agarose gel for amplification and purified using QIAquick PCR Purification Kit (Qiagen, USA) before the barcoding reaction. The PCR barcoding step was 30 µL containing 0.15 µL of reverse primer, 1 µL of the barcode forward primer, 5 µL of purified PCR product, 15 µL

Phusion High-Fidelity PCR Master Mix with HF Buffer, and 8.85 µL of water. The PCR conditions for the barcoding reaction were 95 ºC for 5 min followed by 6 cycles at 95 ºC for 30 s, 55 ºC for 60 s, and 72 ºC for 90 s, and a final extension at 72 ºC for 10 min. PCR product was then pooled and further purified before sequencing using an Illumina MiSeq platform (Illumina, USA) at the Oklahoma Medical Research Foundation (Oklahoma City, OK).

2.3.4 Sequence analyses

Raw FASTQ files were checked for quality with FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc) then demultiplexed and processed using QIIME (version 1.9.0) (Caporaso et al., 2010). Low quality sequences and chimeras were removed. Operational taxonomic units (OTUs) were clustered at 97% sequence similarity using the UCLUST function in QIIME. Technical replicates for each sample were combined to increase sequence number per sample and get one representative sample per time point. The OTU representative sequences were taxonomically identified using the SILVA 16S database. Sequences were rarefied to 12,000 sequences per sample based on the lowest sequence number sample to use for alpha-diversity calculated using the vegan package (Oksanen et al., 2019) in R version 4.0.3 (R Core Team, 2020). Beta-diversity was calculated using the vegan package using the unrarefied data.

2.3.5 Functional gene array

GeoChip 5.0S array containing ~60,000 probes per array (Shi et al., 2019) was used for functional gene analysis. Microarray analysis was conducted following previously described protocols (Shi et al., 2019; Van Nostrand et al., 2016). In short, three time points were chosen from the one-year sampling period to represent various sampling seasons from TGP and CT wheat. These two fields were chosen for further examination because CT wheat croplands are the most common type of land conversion of native prairie systems. The DNA extracted for sequencing was also used for this part of the study. Each DNA sample was purified using Agencourt AMPure XP (Beckman Coulter, California, USA) bead purification following the manufacturer's protocol. The quality of the DNA was determined using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) based on the A_{260}/A_{280} and A_{260}/A_{230} ratio, and DNA concentrations were quantified again using Qubit® dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA was labeled using random priming and cyanine dye, purified using Qiagen QIAquick purification kit per manufacturer's instructions and dried. Resuspended labeled DNA was mixed with hybridization solution, pipetted into the center of the well of the gasket slide (Agilent), the array assembled and sealed, and placed into a rotisserie hybridization oven to hybridize in the presence of 10% formamide at 67°C for 24 h. Once hybridization was complete, slides were washed and imaged using a NimbleGen MS200 microarray scanner (Roche NimbleGen, Madison, WI, USA).

GeoChip data were normalized and quality filtered with methods modified from previous ones (Shi et al., 2019; Van Nostrand et al., 2016). Probes flagged as outliers

(bad spots) were removed from all samples. Then, for each array, the sum of the signal intensity was calculated, and the maximum sum value was applied to normalize the signal intensity in each array producing a normalized value for each spot in each array. Normalized data was further denoised as follows. A probe signal is counted as low-rank in a sample if raw signal < 500, or SNR < 2, or SBR < 1.3, or CV > 0.8, or signal < 99% of detected probes, or signal < 50% of designed probes. Only the probes showing low-rank signals across all samples were removed as noise. Then, probe signals with SBR < 1.1 were filled with zeros, considered as undetected.

2.3.6 Statistical analyses

Differences in measured soil properties were compared across land use and season using Kruskal-Wallis rank sums test. The effects of land use type, seasonal sampling time, and their interactions with alpha-diversity and beta-diversity indices were analyzed using R. The main effects and interactions of land use and season on alpha diversity indices were tested using avop in the "lmPerm" package (Wheeler and Torchiano, 2016) in R. Tukey's post hoc test was used when significant values ($p < 0.05$) were returned for one of the main effects or interactions. ANOVA was used to compare the effect of soil properties to alpha-diversity within land use types. Principal coordinate analysis (PCoA) was performed using Bray-Curtis distance metrics. The statistical significance of season and land use on beta-diversity was assessed by permutational multivariate analysis of variance (PERMANOVA), using adonis in the vegan package (Oksanen et al., 2019). The same analysis was used for alpha- and beta-diversity of the functional community. Bacterial community composition differences were compared by field using adonis and

pairwise field comparisons. The same method was used for within field seasonal differences of bacterial community composition. To link beta-diversity to measured soil and environmental factors, Mantel test and multiple regression on distance matrix (MRM) were modified as previously described (Ning et al., 2020). In the modified Mantel and MRM, beta-diversity of spatial, temporal, or all pairwise comparisons was, respectively, subjected to a linear mixed model with random effect of intercepts in different seasons. The significance test was based on constrained permutation of samples considering the repeated measurement. The factors in the MRM models were forward selected based on adjusted R^2. Canonical correspondence analysis (CCA) was conducted to determine the effect of soil properties and environmental factors on the bacterial community within land uses and for the overall functional dataset. CCA models were considered significant when $p < 0.05$ and redundant variables had been removed (VIF > 15). Each variable was additionally checked for significance within each model. Variance partitioning analysis was conducted for bacterial community composition of each field based on CCA results. Variables were separated into three groups representing including nitrogen measurements (TopN, NH_4, TN), soil variables (OM, SWC), and climate factors (rainfall, air temperature). To examine differences in relative gene abundance based on GeoChip data, genes present in at least 50% of the samples across treatment were used. Response ratios were determined using an online available MicroArray functional gene microarray analysis system (http://ieg.ou.edu/microarray/) based on 90%, 95%, and 99% confidence intervals for land use and sampling time (He et al., 2012; Liang et al., 2016). Mantel test was also used to examine correlations between functional diversity, taxonomic diversity, and phylogenetic diversity. Correlation coefficients were compared

using a two-sided t-test. Spearman's correlation was used to look at relationships between functional groups and OTUs using cor.test in R.

2.3.7 Data availability

16S rRNA gene sequences were deposited in the Sequence Read Archive (SRA) database under BioProject accession number PRJNA816491.

2.4 Results

2.4.1 Changes in soil and environmental variables across land use and management gradient

Over the one-year sampling period, all the measured soil properties were significantly affected ($p < 0.001$) by land use (Figure 2.1a). Only properties influenced by climate (soil water content, SWC) and management practices (topsoil nitrate, TopN) significantly differed by season ($p < 0.05$). Notably, soil organic matter (OM) and soil total nitrogen (TN) decreased as management disturbance increased. The OM only differed by season for CT wheat. SWC significantly decreased ($p < 0.05$) in CT wheat with the lowest SWC of all sites being observed in CT wheat during January, whereas all other land uses had lows in SWC during summer months (Figure 2.1b). Minimum daily air temperatures occurred in December 2016 and January 2017, and maximum air temperatures occurred in August 2016 and July 2017. The greatest monthly rainfall was recorded in April 2017 (227 mm) and the lowest monthly rainfall in November 2016 (15 mm). Both croplands had higher averages of TopN that significantly differed from the less management disturbed grasslands ($p < 0.05$). Elevated levels of TopN were present during summer and

fall in both croplands (Table S2.1). Ammonium (NH_4^+) was significantly ($p < 0.05$) lower in NT canola than other land uses.

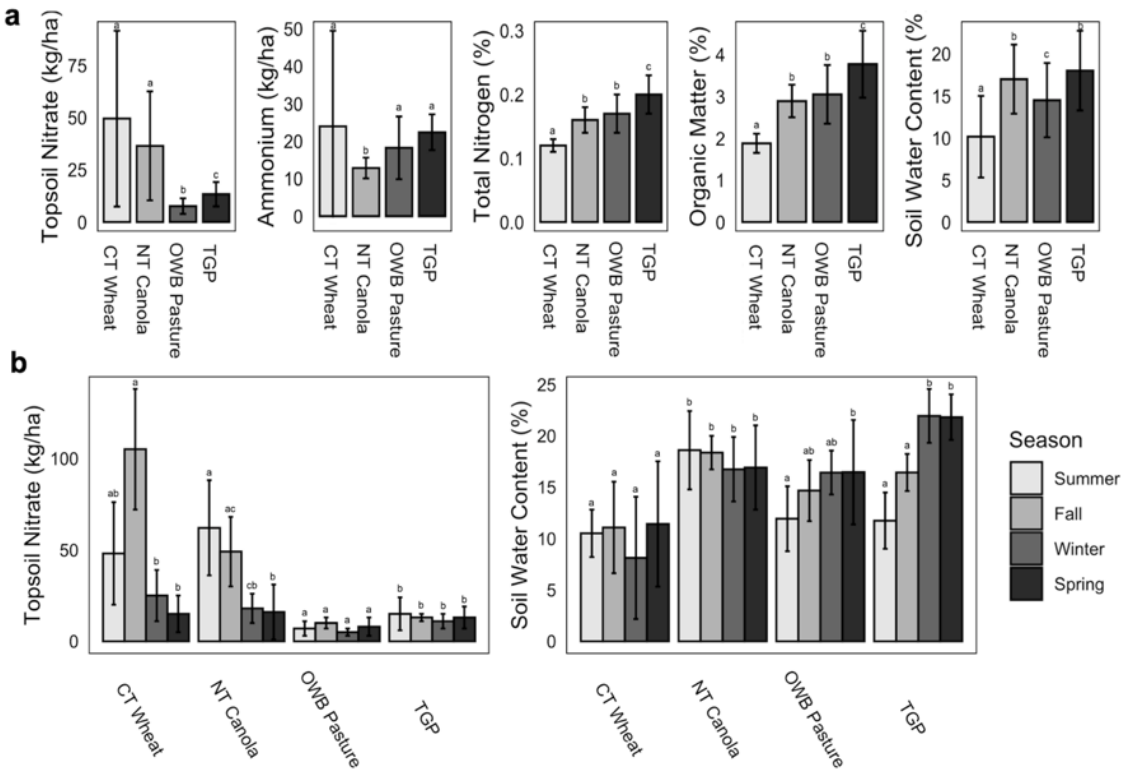

Figure 2.1 Soil chemistry within each land use type across one-year sampling period. a) Soil chemistry averages for each land use across one year. b) Factors that were significantly different by season across the whole land use gradient shown by season within land uses. Error bars represent the standard deviations. Letters represent significant differences of $p < 0.05$ between pairwise land use comparisons or seasons within land use. The same letter indicates no significant difference.

2.4.2 Impact of land use and seasonality on soil bacterial communities

To determine the effect of land use and season over the sampling period, α-diversity was calculated for the bacterial communities (Table 2.1). For all land uses, seasonal variability had a greater impact on α-diversity than land use with all indices significantly

different between seasons ($p < 0.001$). Overall, bacterial richness was lower in summer and fall than winter and spring, but no single land use had a more diverse or rich community throughout the sampling period. Shannon diversity was the lowest in the fall across all land use types, and fall was significantly different from other seasons ($p < 0.05$). The two fields that differed the most as far as management disturbance, TGP and CT wheat, were compared separately to see if land use differences were observed when focusing on the most different land use types and level of management disturbance (Table 2.1), interestingly significant differences were still driven by season.

Table 2.1 Bacterial community structural and functional differences in alpha-diversity based on land use and sampling time.

Alpha diversity	**Effects**	**16S**		**16S**		**GeoChip**	
		All Fields		TGP and CT		TGP and CT	
		F value	***p***	**F value**	***p***	**F value**	***p***
Chao 1	Field	0.517	0.672	0.896	0.350	-	-
	Season	12.05	**< 0.001**	5.196	**0.005**	-	-
Observed OTUs	Field	0.573	0.635	1.401	0.244	-	-
	Season	11.16	**< 0.001**	3.663	**0.022**	-	-
Pielou	Field	1.891	0.138	1.738	0.195	0.410	0.529
	Season	3.494	**0.020**	0.280	0.839	1.790	0.192
Shannon	Field	0.426	0.735	0.415	0.523	0.939	0.343
	Season	9.129	**< 0.001**	1.970	0.137	1.772	0.195

Bolded values indicated significant effects ($p < 0.05$) based on analysis of variance.
Season for GeoChip represents sampling time since only three time used across dataset.
Native tallgrass prairie (TGP) and conventionally tilled (CT) winter wheat

The effect of land use and season on the β-diversity of soil bacterial communities was examined using PCoA based on Bray-Curtis distance metric. The bacterial community structure of soils visibly separated by land use with CT wheat generally isolated from the other land uses (Figure 2.2). NT canola and OWB pasture were the

most similar in community structure. Each field had observable temporal differences in community structure, and the visible temporal differences increased with increasing management disturbance. PERMANOVA analysis supported the PCoA plot (Table 2.2), indicating that the structure of the bacterial community was significantly shaped by land use ($p = 0.001$, $R^2 = 0.2949$) and season ($p = 0.001$, $R^2 = 0.1067$), but the effect of season was not as strong as that of land use. When comparing just the TGP and CT wheat, the same significant differences were observed, but the effect of land use ($p < 0.05$, $R^2 = 0.3614$) on bacterial community structure was even greater.

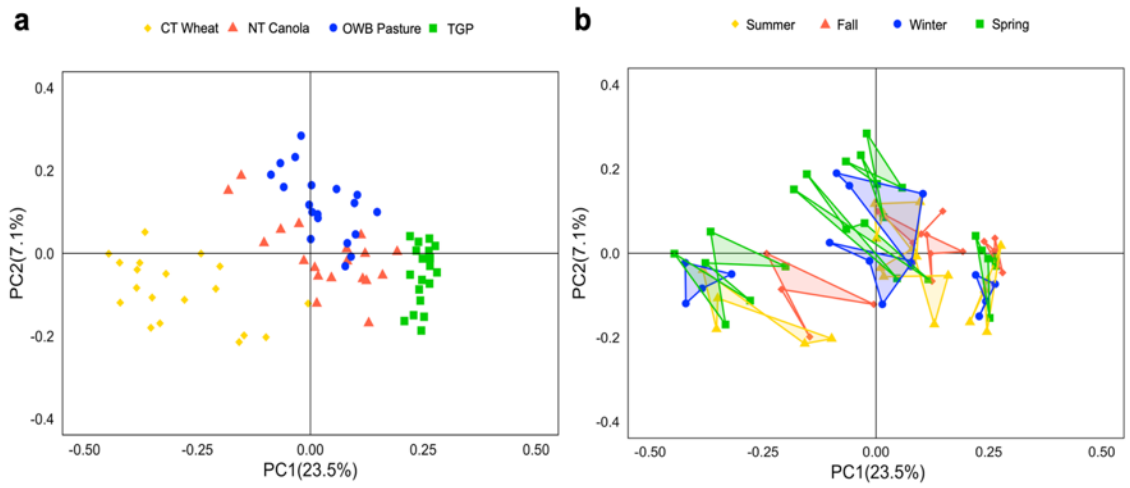

Figure 2.2 Principal coordinate analysis of Bray-Curtis dissimilarity for soil bacterial communities (16S rRNA gene) showing the differences in four fields along a management disturbance gradient types over a one-year sampling period. a) Differences in community structure between land uses. Land uses include conventionally tilled (CT) wheat, no-till (NT) canola, Old World bluestem (OWB) pasture, and native tallgrass prairie (TGP). b) Differences in community structure separated by season

Table 2.2 Effect of land use and season on bacterial community structure

Distance Metric	Effects	16S		16S		GeoChip	
		All Fields		TGP and CT		TGP and CT	
		R^2	*p*	R^2	*p*	R^2	*p*
Bray-Curtis	Field (F)	0.2949	0.001	0.3614	0.001	0.0842	0.005
	Season (S)	0.1067	0.001	0.1140	0.014	0.1363	0.034
	F × S	0.1102	0.104	0.0783	0.112	0.1049	0.011
Jaccard	Field (F)	0.2057	0.001	0.2394	0.001	0.0798	0.004
	Season (S)	0.0876	0.001	0.1125	0.018	0.1299	0.042
	F × S	0.1303	0.032	0.0928	0.083	0.1054	0.014

16S permutational multivariate analysis of variance (adonis) model was set up as dissimilarity ~ field * season. 16S analysis was done including 4 fields: TGP, OWB pasture, NT and CT. It was also performed using only prairie and CT since GeoChip only included those two fields. GeoChip permutational multivariate analysis of variance (adonis) model was set up as dissimilarity ~ field * season + block with permutation constrained by block to deal with effect of data on multiple arrays.

2.4.3 Responses of soil bacterial community composition across land use gradient

The soil bacterial community was dominated on average by several bacterial taxa across all land use types commonly found in soils including Actinobacteria (20.16% - 24.67%), Proteobacteria (21.20% - 24.36%), Firmicutes (9.68% - 18.03%), Acidobacteria (9.20% - 13.53%), and Chloroflexi (4.23 - 9.90%) accounting for over 75% of the relative abundance of each system (Figure S2.1, Table S2.2). The lower relative abundance phyla were comprised of Bacteroidetes, Gemmatimonadetes, Planctomycetes, and Verrucomicrobia, all of which made up at least 1% of the bacterial community in all land uses over the sampling period (Figure S2.2, Table S2.2).

Significant differences in the relative abundance of the main bacterial taxa between land use were evident at the phylum level ($p = 0.001$). The greatest number of significant differences of relatively abundant phyla were between CT wheat and TGP communities, while NT canola and OWB pasture had the least significant community

differences (detailed in Table S2.3). Within individual land uses, the phylum relative abundance of the bacterial communities differed significantly by season ($p < 0.01$) except for OWB pasture ($p = 0.066$). Markedly, significant changes in many phyla across seasons were unique to specific land use types (detailed in Table S2.4). At a lower taxonomic level, roughly 20% of the OTUs in each land use were not present in any of the other land uses with the smallest percentage of shared OTUs between CT wheat and the TGP (Figure S2.3a). Within all land uses, the greatest percentage of unique OTUs was observed during the spring (Figure S2.3b-e) corresponding to warming air temperatures, rainfall peaks, and resuming plant growth. CT wheat had the most shared OTUs during the fall and spring, which were the wheat growing seasons. TGP also had the most shared OTUs during the peak growing season for warm grasses during summer and fall. In general, as the amount of management disturbance increased between land uses, the bacterial communities became increasingly different at the phylum and OTU levels.

2.4.4 Effect of soil and environmental factors on soil bacterial diversity

While α-diversity indices were not significantly different across the land management disturbance gradient, they were affected by local soil properties that differed between land uses. The richness and diversity of the CT wheat bacterial community significantly ($p < 0.05$) decreased when there were high levels of TopN present. Similarly, the diversity and evenness significantly ($p < 0.05$) decreased in the presence of elevated TopN in NT canola. Only the richness in the TGP was significantly influenced by SWC.

There were no detectable relationships between soil variables and α-diversity for OWB pasture.

The influence of management, soil, and environmental factors on β-diversity was determined using Mantel test and multiple regression on a distance matrix (MRM) with correlations to individual taxa within land uses in Table S2.5. Overall, bacterial community differences were significantly driven by tillage > SWC > sampling time > minimum air temperature according to Mantel test (Figure 2.3a). Between fields (spatially), soil factors shaped by land use were significantly important including OM > TN > SWC. In comparison, sampling time had the greatest correlation to temporal community differences as well as OM and TN. Since potential significant correlations among factors could exist, MRM was further used to determine the contributions of different environmental factors on shaping bacterial community structure. In general, differences based on MRM were similar to that of Mantel test with tillage > sampling time > minimum air temperature being significant (Figure 2.3b). Notably, spatially related factors had a stronger impact ($R^2 = 0.62$) on the bacterial community than temporally ($R^2 = 0.46$). The same soil factors were key to spatial difference based on MRM and Mantel test (Figure 2.3c), with average air temperature also having moderate importance. Sampling time again had the strongest relationship with temporal differences (Figure 2.3d). Average rainfall > TN > TopN were also key to temporal community dynamics.

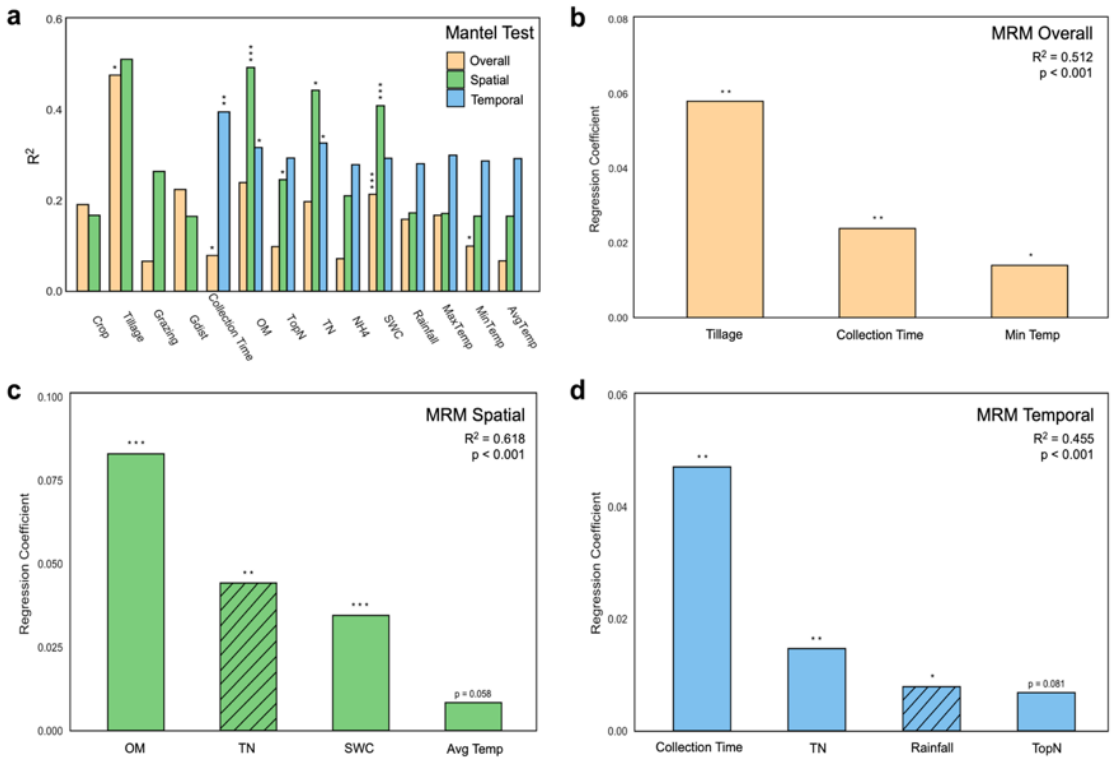

Figure 2.3 Effects of soil and environmental factors on soil bacterial community structure. a) Correlations on overall, spatial, and temporal differences based on Mantel test. b) Multiple regression on distance matrix (MRM) on overall community structure. c) MRM for spatial differences in community structure. d) MRM for temporal differences in community structure. Bars with diagonal lines represent negative regression coefficients. Gdist, geographical distance between sampling sites. Significance expressed as ***$p <$ 0.001, **$p < 0.01$, and *$p < 0.05$.

Each land use was then examined separately to determine if soil and environmental variables contributed equally to the variation in bacterial community structure. Based on CCA analysis (Figure S2.2), variance partitioning was used to determine if climate variables, nitrogen measurements, or other soil properties explained the most variations in community structure (Figure 2.4). Nitrogen measurements had the largest impact on the CT wheat bacterial community and interacted with the other soil properties and climate variables (Figure 2.4a). The majority of the variation of bacterial

communities was explained by the soil properties for the NT canola site (Figure 2.4b), with nitrogen measurements and climate variables having a minor interaction. For OWB pasture, the variation explained by all groups was similar, with soil properties and nitrogen measurements explaining almost the same amount of variation (Figure 2.4c). The distribution of the variation explained for TGP was similar to what was observed at the OWB site (Figure 2.4d), with all sets of variables having a relatively equal impact on the bacterial community structure. The greatest interaction of all variables was observed for the TGP. Therefore, management and sampling time had the greatest impact on diversity, and management greatly impacted the importance of different soil and climatic factors on shaping bacterial communities within fields.

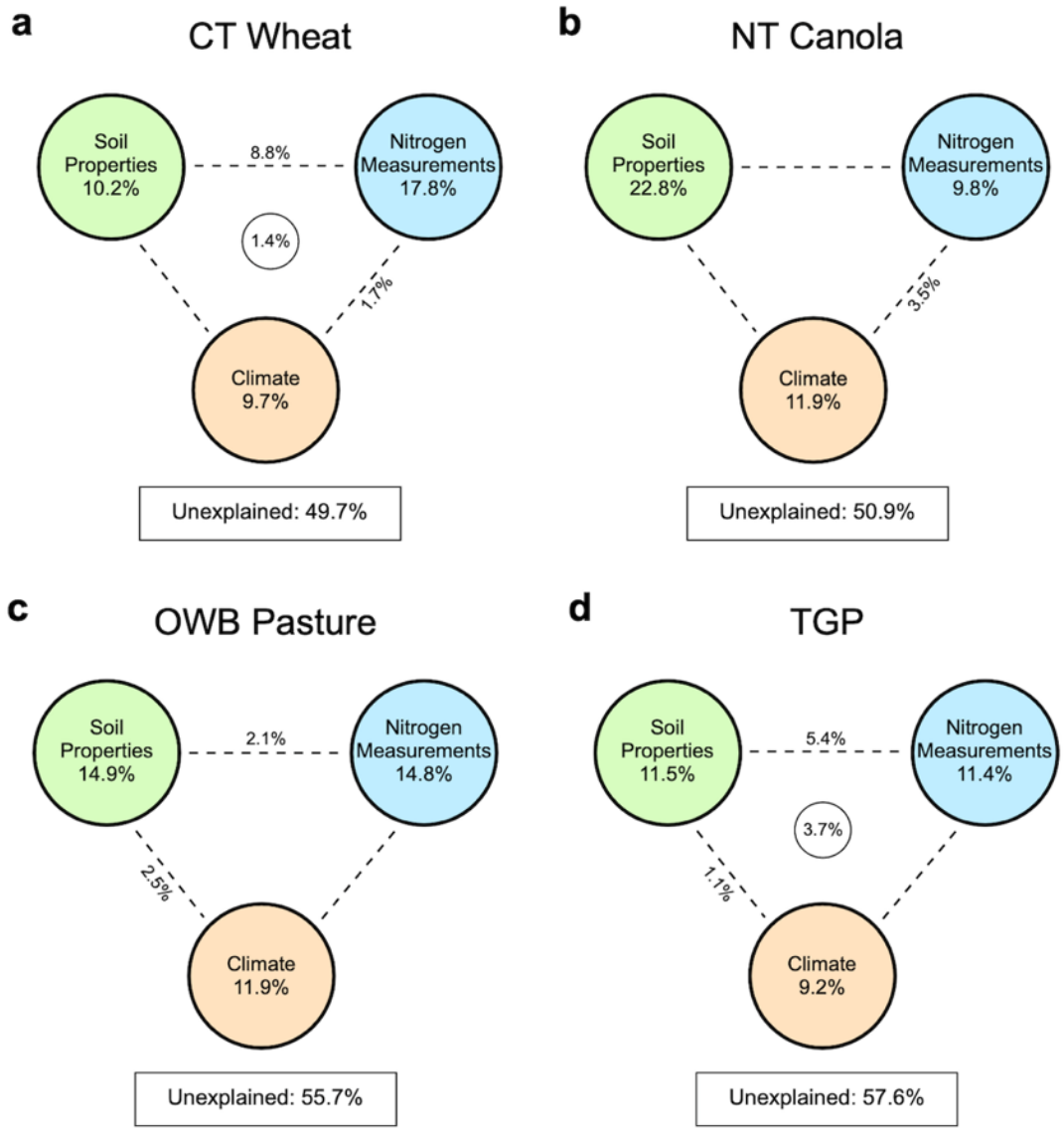

Figure 2.4 Variation partition analysis (VPA) of bacterial community structure explained by soil properties, nitrogen measurements and climate variables for each land use. Variable groupings include soil variables (SWC, OM), nitrogen measurements (TopN, NH_4^+, TN), and climate (rainfall, temp) variables. Total nitrogen (TN) was only included in the CT wheat nitrogen measurements based on the CCA results. Bacterial community data based on 16S rRNA gene sequencing data.

2.4.5 Functional community differences between tilled cropland and native prairie

Functional diversity at three distinct times across the one-year dataset was investigated using a functional gene array for the CT wheat and TGP. For α-diversity of the functional community, no significant differences were found based on land use or sampling time when looking at evenness and diversity (Table 2.1). Significant differences were observed for the functional β-diversity of the two fields. Land use, sampling time, and the interaction of the two factors were all significant in shaping functional diversity (Table 2.2). Interestingly, sampling time and the interaction of sampling time and land use had a stronger effect on the functional structure than land use alone.

To investigate the significant differences in functional community structure, response ratios were used to compare relative gene abundance between land use and sampling time. When comparing by land use, all genes that were significantly different were greater in the TGP. Comparisons of sampling time between land uses had significant differences in genes involved in carbon cycling, organic remediation, nitrogen, and metal homeostasis. Surprisingly, very few functional gene differences were observed when comparing the two fields during August (Figure S2.4). The greatest significant differences between the functional community structure of CT wheat and the TGP occurred during January (Figure 2.5a) when the relative abundance of almost all genes that were significantly different were greater in the TGP. Fewer significant differences in function were observed in May (Figure S2.4) than in January.

Soil and environmental factors also impacted the functional potential of CT wheat and TGP. The local diversity of the CT wheat field was impacted by OM, SWC, and temperature measurements. Evenness and diversity significantly decreased ($p < 0.05$) as

OM increased, while both indices significantly increased ($p < 0.05$) with increasing air temperature. An increase ($p < 0.05$) in local diversity was also associated with increasing SWC. No significant relationships were identified in the TGP land use. CCA was also used to explore the impact of soil and environmental factors on the functional community composition (Figure 2.5b). Nitrogen measurements (TopN, NH_4^+) appeared to have a greater impact on CT wheat and soil properties (SWC, OM) appeared to be more important for the functional structure in the TGP. Average air temperature (AvgTemp) had a more important relationship with the function of the TGP community than the CT functional community. Overall, OM grouped closer to the first axis where functional differences were observed based on land use, while AvgTemp and NH_4^+ were closer to the second axis where functional differences were separated by sampling time. SWC and TopN appeared to influence functional structure the most based on the interaction between land use and sampling time. Similarly, VPA showed a large amount of the variation in the functional structure to be unexplained (Figure 2.5c) and soil properties, nitrogen measurements, and climatic factors all explained a comparable amount of variation. Soil properties greatly interacted with climatic factors and nitrogen measurements. While all soil factors and climatic variables were important to variations in functional diversity, more links to these factors and local functional diversity were observable for the highly managed CT wheat field.

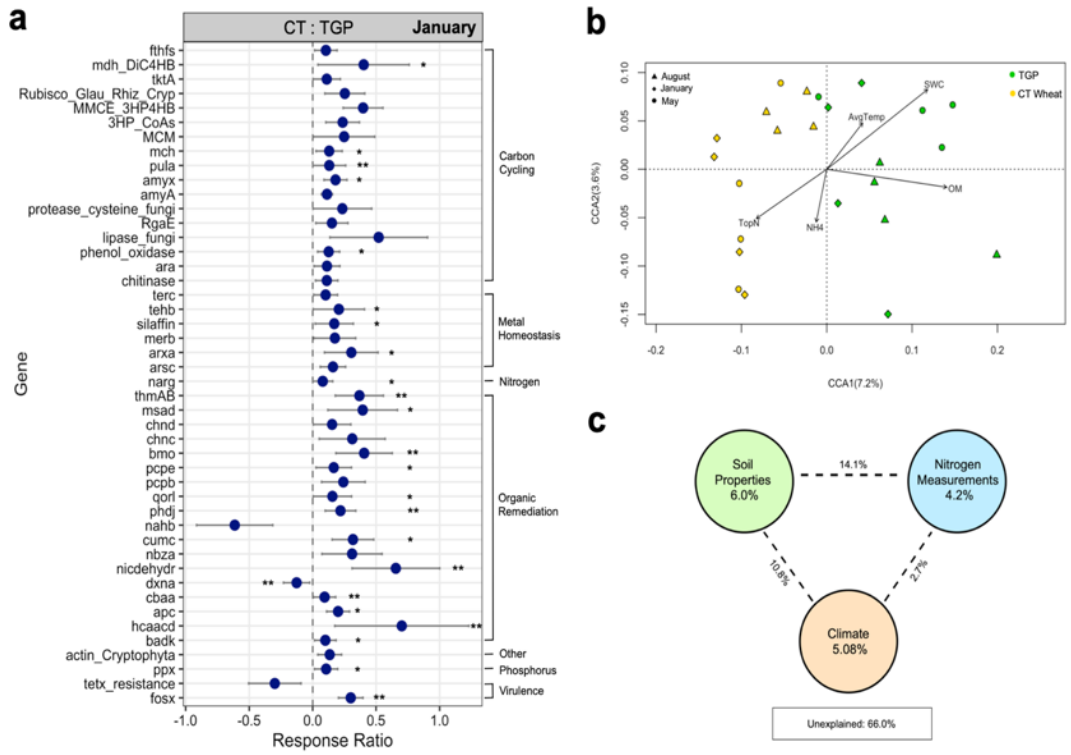

Figure 2.5 Functional differences between conventional tilled (CT) wheat cropland and tallgrass prairie (TGP). a) Differences in relative gene abundance during January between TGP and CT wheat functional community based on response ratio. All genes greater than 0.0 were greater in the TGP community. All genes present significantly different by 90% confidence interval, * 95% confidence interval, and ** 99% confidence interval. (b) Canonical correspondence analysis (CCA) examining the relationships between soil and environmental factors on function community structure using GeoChip data (c) VPA of functional community structure explained by soil properties (SWC, OM), nitrogen measurements (TopN, NH_4^+), and climate variables (rainfall, temp).

2.4.6 Links between structural and functional community in tilled cropland and native prairie

Mantel test was used to examine the relationship between taxonomic, phylogenetic, and functional bacterial community structure focusing on key functional groups (e.g. carbon cycling, nitrogen cycling, virulence). When considering overall community interactions for the TGP, almost all functional groups had a negative relationship with taxonomic

diversity (Figure S2.5a). In comparison, most of the relationships between functional groups and phylogenetic diversity were positive with a moderately significant association with methane cycling genes (r = 0.229, $p = 0.07$). The strength of the relationship between functional groups and phylogenetic diversity was significantly greater ($p < 0.001$) on average than that with taxonomic diversity for the TGP. For individual sampling times, a similar pattern was observed where the strength of the relationships between functional groups and phylogenetic diversity was greater than taxonomic diversity. For the CT wheat field, the majority of relationships between functional groups, taxonomic diversity, and phylogenetic diversity were negative (Figure S2.5b). The most significant relationships were found between functional groups and taxonomic diversity for CT wheat including carbon cycling (r = 0.26, $p = 0.05$), methane cycling (r = 0.31, $p = 0.02$), and organic remediation (r = 0.24, $p = 0.05$). The strength of the relationships was significantly greater ($p < 0.001$) between taxonomic diversity and functional groups on average. The correlation strength for each sampling time for CT wheat was greater between taxonomic diversity and functional diversity for individual sampling months as well. Primarily, functional structure was more strongly associated with phylogenetic structure in the TGP compared to CT wheat where functional structure was more closely related to taxonomic structure.

To look closer at relationships between structure and function, functional groups were compared to OTUs. Several OTUs that had a significant relationship ($p \leq 0.05$) with functional groups that were present in both fields (Table S2.6) with the direction of the relationship often varying between fields. For example, all the OTUs linked to carbon cycling, carbon degradation, and methane cycling in both fields had a negative

correlation for the TGP and a positive correlation for CT wheat likely related to their importance to the processes directly or indirectly within each field. OTUs significantly correlated to N fixation present in both fields were all *Bacillus* spp. and were present in a much greater abundance in CT wheat generally having highs in August and May compared to highs in just May for the prairie. One OTU (*Massilia* sp.) significantly correlated to organic remediation in both fields and had a higher abundance overall in the TGP with peaks in August whereas abundance was greatest in May for CT wheat. Significant OTUs were then compared within fields to determine if OTUs were significantly correlated to more than one function. When examining the functional groups of carbon cycling, nitrogen cycling, and organic remediation, the TGP had almost 60 OTUs that significantly correlated to all three processes compared to only three in CT wheat potentially reflective of how specialized the community is in the CT wheat field. The three OTUs in CT wheat all belonged to a different phylum, while the significant OTUs in the TGP included many species in the order Rhizobiales, Bacillales, and Solirubrobacterales.

2.5 Discussion

2.5.1 Impact of land use and seasonality on soil properties

Land use change, management intensification, and season have different effects on soil properties and thus impact microbial communities in different ways. In this study, we examined how the soil ecosystem was affected by an increasing amount of management disturbance across four land uses commonly found in the U.S. Southern Plains. Land use change and management intensification modify the soil environment and generally

reduces soil quality (Dunjó et al., 2003) as illustrated by the decrease in OM, TN, and SWC under tillage management compared to other land uses (Liu et al., 2010a). Reducing management disturbance resulted in several soil properties being indistinguishable between land uses further signifying that removing intensive management improves vital soil properties (Lauber et al., 2008). Meanwhile, it has been previously observed that land uses under comparable amounts of management resulted in similar edaphic properties when comparing cropland and non-cropland soil properties (Johnson et al., 2003; Murty et al., 2002), which may explain parallels between properties in NT canola and OWB pasture which received similar yearly management. Only soil properties related to climate and management significantly differed by season. Lower SWC was evident in times of low monthly rainfall or increased daily temperatures. For the croplands, soils exhibited highs of TopN during summer and fall due to fertilizer application, which is expected as management practices in agricultural fields that are largely seasonally dependent (Ishaq et al., 2020; Morrison-Whittle and Goddard, 2015). Overall, even though land use was the greatest determinant of soil properties, sampling time was also key for explaining differences in soil properties, especially as management disturbance increased.

2.5.2 Impacts of land use and seasonality on soil bacterial diversity

Determining how soil microbial community diversity is impacted across time and space is crucial for preserving soil health against continued environmental changes. The α- and β-diversity of bacterial communities were differently altered by land use and season. As has been observed in a similar study comparing land use types and temporal dynamics

(Lauber et al., 2013), season had the most significant impact on α-diversity with different land uses having greater diversity at varying times of the year. However, the interactive effect of season and land use on belowground diversity remains unclear as most studies emphasize spatial variability over temporal variability (Fierer, 2017). On large spatial scales, variation in α-diversity is not significantly explained by land use but rather soil properties (Plassart et al., 2019), with moisture and nutrient availability generally being the most notable factors (Koranda et al., 2013; Manzoni et al., 2012; Taketani et al., 2017). Increases in TopN in the croplands decreased α-diversity (Wang et al., 2017; Zhou et al., 2016), while SWC influenced α-diversity in the TGP. Both properties generally vary over shorter periods of time making them potentially better predictors of seasonal microbial community changes (Zhang et al., 2020). Even with the documented impact of season on α-diversity, it is thought that the importance of temporal dynamics is underestimated due to the presence of relic DNA (Carini et al., 2016), the response of different taxa to environmental changes (Fierer, 2017), and the lack of focus on living/active cells (Herzog et al., 2015; Zifcakova et al., 2016). Given that no land use had the greatest α-diversity throughout the whole one-year period and α-diversities were influenced by soil properties that vary seasonally, it is important to assess temporal dynamics when trying to determine differences in the microbial community.

The effects of land use were far more critical for regulating the β-diversity of the bacterial communities across the management gradient. The β-diversity of all land use types differed from that of the TGP (Figure 2.2), with tillage management having the most significant impact (Figure 2.3). Between land uses, several soil factors and air temperature were critical for differences in bacterial diversity (Figure 2.3), while the

distance between sites had no significant effect on the smaller scale of our study. Within fields, as management decreased, less variation in β-diversity was explained by the measured properties, of which the relative importance became more evenly distributed (Figure 2.4). No group of variables was clearly the most important to variations in TGP bacterial diversity. In comparison, slightly greater importance of nitrogen and other soil properties was found to be associated with variations in OWB pasture, possibly due to changes in microbial community composition and diversity from management disturbances in grasslands (Kennedy et al., 2005; Nacke et al., 2011). More variation in bacterial diversity was explained in the croplands. While both croplands were fertilized, soil N content was far more important to bacterial diversity in CT wheat presumably because fertilizer was applied with no residue cover and was directly incorporated through tillage. Soil properties that increased under NT management like SWC and OM explained more variations in the NT canola supporting that reduced management increases carbon storage and moisture availability (Derpsch et al., 2010; Díaz-Zorita et al., 2002). Sampling time was also a significant driver of diversity differences (Figure 2.3) with rainfall and soil nutrients again having considerable influence. This is consistent with previous studies where climate variables, soil moisture, and nutrient availability dictated temporal changes (Zhang et al., 2020). Although several factors were exclusive to shaping bacterial diversity based on time or space, SWC, OM, and TN continually appeared to be notable factors impacting the bacterial communities (Cederlund et al., 2014; Oliverio et al., 2017; Serna-Chavez et al., 2013; Sul et al., 2013), with land use type being critical to explain differences in diversity especially compared to the native system. It should be noted, that this was the first time canola was planted on the NT

cropland, which had previously been a long-term winter wheat system. While plant species can influence the microbial communities, many other factors in croplands likely outweigh the introduction of a new crop. In agricultural systems, crops are cultivated in various soils being impacted by the soil type, soil properties, and land management often reducing the importance of the rhizosphere microbial community for plant growth compared to native ecosystems (Philippot et al., 2013). Soil properties have also been shown to override the influences of crop type on soil bacterial communities (Bainard et al., 2016), with land use and management strongly shaping soil properties (Lauber et al., 2013; Lauber et al., 2008). Additionally, a mesocosm experiment using soil collected from long-term monoculture cropping systems determined that the cropping history of the soil was the main factor determining the microbial community composition when a new crop was introduced (Frindte et al., 2020). Together, these points help emphasize that the plant type during this single growing season was likely not responsible for the overall observed differences.

While much is still unknown about the relationship between taxonomic/phylogenetic and functional diversity, it is widely believed that increased diversity, including functional diversity, sustains soil functions and creates greater resilience to disturbance and stress (Tardy et al., 2014; Torsvik and Øvreås, 2002). Taxonomic/phylogenetic and functional diversity can also be differentially affected by soil and environmental properties. Based on results from the FGA analysis, land use and sampling time were both central in shaping the functional diversity of the CT wheat and TGP field, although land use alone had less of an effect than sampling time or the interaction of sampling time with land use. The reduced effect of land use on functional

diversity is likely due to shared taxa between communities leading to more similar functional traits (Fierer et al., 2013; Montecchia et al., 2015) and the redundancy of many biogeochemical gene families across microbial groups (Fitter et al., 2005). TGP functional diversity was associated with greater SWC, OM, and air temperature, and CT wheat functional diversity was associated with higher N content. Available N has been shown to significantly impact the active bacterial community and increase the number of taxonomic and phylogenetic groups that specialize in using N compounds (Herzog et al., 2015). We also attempted to uncover the correlations between taxonomic/phylogenetic and functional diversity, although deciphering such correlations is not straightforward. Functional diversity had stronger correlations to taxonomic diversity than to phylogenetic diversity in the CT wheat field, whereas in the TGP functional diversity had stronger relationships with phylogenetic diversity. It is possible that the CT wheat community remains more phylogenetically similar overtime, while the taxonomic community changes more rapidly. These types of patterns have been previously observed and suggested as warning signs of biodiversity loss due to environmental changes (Hewitt et al., 2010; Rodrigues et al., 2013) resulting from intensive management practices in agroecosystems.

2.5.3 Impacts of land use and seasonality on soil bacterial community composition

Throughout our study, the greatest management disturbance resulted in the greatest impact on the bacterial community as shown by the results of tillage treatment at both the phylum and OTU levels. The impact of land management especially tillage on bacterial community composition has been extensively documented (Lauber et al., 2008; Le

Guillou et al., 2019; Wang et al., 2020), and although less studied, season has considerable influence on composition as well (Degrune et al., 2017; Ishaq et al., 2020; Lauber et al., 2013). For all land uses, the most unique OTUs were present during the spring season. During spring, air temperatures begin to rise and rain increases. Temperature and moisture not only impact the physiological activity of bacterial communities, but also regulate plant activity, including rapid growth and increasing root exudates (Chernov and Zhelezova, 2020; Thomson et al., 2015). Such large seasonal changes are likely responsible for differences in community composition observed between land uses as well as the increase in bacterial richness during the spring season. Monitoring changes in microbial composition over time and in response to management is one of the best ways to determine sustainable agricultural practices as it can indicate early potential changes in soil functionality, although it is necessary to remember there is not one optimal microbial community composition.

To examine the functional gene community composition, relative gene abundances of the whole communities were compared between CT wheat and the TPG. Between the two land uses, the abundances of all genes that significantly differed were always greater in the TGP. Such differences are believed to be reflective of microbial functional gene abundance and diversity (Reeve et al., 2010), although gene presence does not necessarily mean the gene is being actively transcribed. More distinct differences in gene abundances between land uses were apparent when comparing specific sampling times (Figures 2.5, S2.4). In general, seasonal microbial community differences are usually more evident in agricultural soils compared to native soils (Lauber et al., 2013) due to seasonal management practices and plant activity. The greatest

differences occurred during January when plants in both fields were generally not active, air temperatures reached yearly lows, and CT wheat had the lowest SWC. The importance of soil water content in regulating microbial activities is well known with soil water content being a key abiotic factor linked to functional diversity (Liu et al., 2010b). Furthermore, the greater ground cover (*i.e.*, residues) during the winter in the TGP may help alleviate the stress of the colder temperature on the microbial community with greater plant litter amounts also increasing water infiltration and reducing soil evaporation (Weaver and Rowland, 1952). Therefore, the effects of reduced SWC and reduced ground cover could lead to decreased microbial diversity and activity under CT wheat. The least amount of differences in the functional gene community was observed in August. The tallgrass prairie mainly consists of warm-season grasses, therefore the plant community is in peak growth during this time likely releasing nutrients to support microbial activity. In comparison, the CT wheat field is tilled during the summer fallow season to incorporate residues for decomposition providing organic carbon and nitrogen again likely resulting in increased microbial activity (Frey et al., 1999; Peterson et al., 2019). Even though there were clear differences in the functional diversity of the microbial communities in relation to land use and sampling time, it is equally necessary to survey changes in functional gene abundance as shifts in diversity alone do not always result in differences in the biogeochemical functional ability of the soil microbial community (Cheneby et al., 2009; Hallin et al., 2009).

2.6 Conclusions

Environments in agroecosystems are continually modified due to land use and management practices that can, directly and indirectly, influence soil bacterial communities. Soil communities are exposed to variability in space and time, making no single biotic or abiotic factor the sole reason for shifts in bacterial community composition (Fierer, 2017), raising the need for continued research on a range of agricultural systems. In this study, we investigated the effects of land use and sampling time on the structural and functional diversity of bacterial communities as well as the interactions with soil and environmental factors in four land uses in the U.S. Southern Plains. First, our results indicated that land use, especially with intensive management, had the greatest impact on taxonomic diversity, while sampling time and time within a specific land use were more important for differences observed in functional diversity. Next, soil nutrients, particularly nitrogen, and soil water content were determined to be critical for variations in community taxonomic and functional diversity across land management and sampling time. Last, functional diversity was also reduced under intensive management with species likely being more specialized in function due to fertilizer usage and more strongly linked to taxonomic diversity than phylogenetic diversity. Although the impacts on functional and structural diversity may have different relationships with land use and sampling time, it is clear that both are important for structuring the interactions of edaphic properties, climatic factors, and bacterial communities. The results contribute to the idea that preserving microbial diversity should be one of the main focuses of sustainable agriculture. While these observations may be regionally specific, we recommend sampling around management practices (e.g., August)

as sampling in relation to a specific management practice or environmental change likely provides the most insight when trying to determine the impact on soil health. This is one reason why microbes show great promise as a soil health indicator as they can respond to disturbance before plant communities and soil properties (Delgado-Baquerizo et al., 2016). Additionally, we further recommend the use of no-tillage as it increased the total nitrogen, organic matter, and water content in the soil, in comparison to CT management which increased the reliance on nitrogen inputs generating a less diverse and likely more specialized bacterial community. Moving forward, continued monitoring of changes in bacterial communities within local land uses corresponding natural and anthropogenic disturbances will likely be most useful when trying to make informed decisions about managing soil health and ecosystem services.

Chapter 3 : Land use conversion enhances microbial network complexity and stability

3.1 Abstract

Soils harbor highly diverse microbial communities that are critical to soil health, but the spread of agriculture has caused extensive land use conversion resulting in negative effects on critical ecosystem processes. However, the responses and adaptations of microbial community interactions due to land use conversion have not yet been understood. Here, we examined the effects of land conversion for long-term cropland use on the complexity and stability of molecular ecological networks of soil microbial communities over 19 months. Despite reduced microbial biodiversity in comparison with native tallgrass prairie, conventionally tilled (CT) cropland use significantly increased network complexity such as connectivity, connectance, average clustering coefficient, relative modularity, and the number of species acting as network hubs and connectors as well as resulted in greater temporal variation of the complexity indices. Molecular ecological networks under CT cropland use became significantly more robust and less vulnerable, overall increasing network stability. The interaction between network complexity and stability was also substantially strengthened due to land use conversion. Lastly, CT cropland use decreased the number of relationships between network structure and environmental properties instead being strongly correlated to management disturbances. These results indicate agricultural disturbance generally increases the complexity and stability of species “interactions”, possibly as a trade-off for biodiversity loss to support ecosystem function when faced with frequent agricultural disturbance.

3.2 Introduction

Land use conversion largely due to agricultural expansion has considerably impacted ecosystem structure and function (Shaoqiang et al., 2004; Verchot, 2010). Grasslands often have deep, rich soils that support increased soil carbon making them targets for conversion for agricultural cultivation (Lark et al., 2019). Pointedly, temperate grasslands in the central U.S. have undergone one of the greatest anthropogenic transformations with habitat conversion greatly exceeding habitat protection (Hoekstra et al., 2005). From 2008 to 2012, roughly 77% of new croplands in the US were originally grasslands (Lark et al., 2015), and in the Southern Great Plains, these new croplands replaced approximately 11,000 km^2 of grasslands with winter wheat alone (Bajgain et al., 2018), the dominant crop in this area. This extensive ecosystem conversion has resulted in significant declines in soil health which also includes the effects on the soil biota and biotic processes (Doran and Zeiss, 2000).

Soil microorganisms are essential for providing many ecosystem services needed for agricultural production, but they are also very sensitive to land use changes and management disturbances (Trivedi et al., 2016). Numerous studies examining the response of microbial communities to agricultural land use and management show consistent results that increasing land use intensification significantly decreases microbial community diversity and shapes microbial community composition (Ding et al., 2013; Ishaq et al., 2020; Lauber et al., 2008). In addition, these studies also reveal that land use conversion substantially changes intrinsic soil properties such as soil moisture, pH, and nutrient status, all of which are known to further affect microbial community dynamics (Bainard et al., 2016; Fierer, 2017; Lacerda-Júnior et al., 2019). While many types of

agricultural management exist, tillage is one of the most common practices that causes the largest disturbance and has led to the greatest degradation of soil ecosystems (Liu et al., 2010a). Tillage physically disturbs the soil, breaks down soil structure, causes nutrient loss (Liu et al., 2010a; Six et al., 1999), and leaves the soil more vulnerable to climatic differences which results in more perturbation to soil microbial communities. While previous studies have been valuable for describing the impact of agriculture on community composition, diversity, and the role of biotic and abiotic factors in shaping these communities, few have investigated the potential interactions among soil microorganisms which may be more important to the functioning of complex ecosystems (Deng et al., 2012).

Individual populations of microbial species do not exist alone, but instead interact to form complex microbial communities (Barberán et al., 2012; Zhou et al., 2010), and these interactions represent a crucial dimension of microbial community ecology. The widely used method of ecological network analysis has proven to be a powerful tool to examine the interactions and organization of microbial communities (Barberán et al., 2012; Faust and Raes, 2012; Zhou et al., 2011a), provides a way to study community complexity and stability (Montoya et al., 2006; Yuan et al., 2021), as well as serves as a basis to quantify the contribution of microbial interactions to ecosystem functions and services. These association networks are commonly referred to as molecular ecological networks (MENs) as they are generally reconstructed based on molecular markers (Zhou et al., 2010). Network topological features have been shown to change with environmental conditions (Deng et al., 2016; Tian et al., 2018; Wu et al., 2016) and can be used to reflect the ability of the ecosystem to respond to such changes (Jia et al.,

2021). Recently, studies have investigated the interactions of complex microbial systems in response to anthropogenic activities including groundwater pollution (Deng et al., 2016), deforestation (Tian et al., 2018), nitrogen addition (Li et al., 2021), and climate warming (Yuan et al., 2021), but the effects on network interactions due to converting native land for long-term cropland use is still largely unknown. Yet, it is expected that introduced disturbances will significantly affect the assembly and overall composition of the soil microbial community (Yan et al., 2017), emphasizing the importance of preserving biotic interactions that are equally at risk as individual species to extinction due to anthropogenic disturbances (Pocock et al., 2012).

For these reasons, we set out to understand whether and how native land use conversion for long-term cropland use affects the complexity and stability of soil microbial community ecological networks by examining the temporal dynamics of soil microbial communities in a native tallgrass prairie (TGP) and conventionally tilled (CT) winter wheat site in the U.S. Southern Plains in El Reno, Oklahoma. While previous studies from this area have shown that land use and sampling time impact bacterial abundance (Cornell et al., 2021) and bacterial community diversity and composition (Cornell et al., unpublished) with increased management intensification having the greatest impact, it is not clear if the networks of the microbial communities will be similarly affected. In this study we aimed to address: (1) How does land use conversion from the native system to cropland use impact the complexity and stability of the molecular ecological networks (MENs) over time? (2) Does land use conversion from the native system to cropland use change the relationship between the complexity and stability of the MENs? (3) Are the relationships between complexity and stability of the

MENs with environmental factors altered due to land use conversion and management practices? We hypothesized that increasing habitat disturbance under cropland use would increase the complexity of species associations resulting in a more complex and stable network.

3.3 Materials and Methods

3.3.1 Study site and sampling strategy

This study included a native tallgrass prairie (64 ha) as the control site and a conventionally tilled winter wheat field (20.5 ha) as the treatment site located at the United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Grazing Research Laboratory (GRL) in El Reno, Oklahoma, USA (35° 34.1' N, 98° 03.6' W). Both sites are included in the Southern Plains site of the Long-Term Agroecosystem Research (LATR) network (Kleinman et al., 2018; Spiegal et al., 2018). El Reno, Oklahoma, has a temperate continental climate with summer months generally hot and dry and most rainfall occurring May-June and September-October. The average daily maximum and minimum air temperature of the study sites were 23 °C ± 8.7 °C and 8.9 °C ± 6.4 °C respectively, with an average total annual rainfall was 855 mm ± 44.7 mm over a 30-year period (1980-2010) (Bajgain et al., 2020).

The tallgrass prairie (TGP) plant community is predominantly warm-season mixed grasses native to Oklahoma including big bluestem (*Andropogon gerardii* Vitman.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), Indiangrass (*Sorghastrum nutans* (L) Nash), and switchgrass (*Panicum vergatum* L.). The soil is classified as Norge loamy prairie (Fine, mixed, thermic Udertic Paleustalf) with a high

water holding capacity. Grazing in the tallgrass prairie is under a year-round rotation by 50 cow-calf pairs being grazed for 30-day periods followed by 90-day rest periods. Prescribed spring burns are implemented on 4-year rotations as part of routine management. Burns generally occur in early spring before the initiation of growth of warm-season grasses. Recent prescribed burns occurred in February 2013 and February 2018.

Native tallgrass prairies are often converted into cultivated ecosystems dominated by the cool-season crop winter wheat in the U.S. Southern Plains. Winter wheat (*Triticum aestivum*) has been planted in the study site under conventional tillage (CT) management since the late 1990s. Concerning the studied period (June 2017-December 2018), the CT wheat field was grain-only during the 2015-2016 growing season and graze-out wheat (no grain production; cattle grazing from Nov/Dec through April/May) during the 2016-2017, 2017-2018, and 2018-2019 growing seasons. The soil in the wheat field was disced with a tandem disc harrow (4-5 in depth) in May 2017 and June 2018 post growing season. Before planting in late September, the field was fertilized, tilled, and seedbed prepared. Since wheat was never harvested for grain, a burndown herbicide was applied in May 2018 between the 2017-2018 and 2018-2019 growing season with the field being resowed in November 2018.

Soil samples were collected monthly from the native TGP and CT winter wheat site from June 2017 – December 2018. To collect soil samples representative of each field, eight soil samples were taken 20 meters apart along a diagonal transect in each field. Each replicate soil sample consisted of four soil cores pooled into a single representative replicate sample. Soil samples were taken using a 2.5 cm-diameter soil

probe at a depth of 0-15 cm. Soils were passed through a 2 mm sieve to remove debris and stored at -80°C until analysis.

3.3.2 Soil properties and climate data

Weather data was collected from an Oklahoma Mesonet station (http://www.mesonet.org/index.php/weather/local/elre) in El Reno (ELRE), Oklahoma. The Mesonet tower is located on the native tallgrass prairie used in this study (35° 32.9' N, 98° 02.2' W). Mesonet data included rainfall, maximum air temperate, average air temperature, and minimum air temperature. Soil chemical analyses were performed at the Oklahoma State University Soil, Water and Forage Analytical Laboratory (https://agriculture.okstate.edu/departments-programs/plant-soil/soil-testing/). Tests included topsoil nitrate (NO_3^-), soil organic matter (OM), soil total nitrogen (TN), ammonium (NH_4^+), and available phosphorus (P). Gravimetric soil water content (SWC) was determined by oven drying for ≥ 24 hours at 65ºC or until the weight no longer changed (Peterson et al., 2019). Soil pH was measured with a pH meter using soil:water (w/v) = 1:5 (Hendershot et al., 2007). Soil properties were measured for seven of the 19 sampling times being representative of different seasons over the study period. Soil properties were measured for all eight replicates.

3.3.3 Soil DNA extraction, amplicon sequencing, and analysis

DNA was extracted from 0.5 g of individual soil samples using an established protocol involving bead mill and SDS lysis (Zhou et al., 1996) combined with the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA). A total of 304

soil samples were processed in this study. The quality of DNA was assessed based on 260/280 nm and 260/230 nm absorbance ratios using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). DNA concentrations were quantified by PicoGreen using a FLUOstar Optima fluorescence plate reader (BMG Labtech, Jena, Germany). For microbial community profiling, the V4 hypervariable regions of 16S rRNA genes were amplified using the common primer pair 515F (5'- GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). A two-step PCR protocol was used and carried out in triplicate to minimize amplification bias as previously described (Ding et al., 2015). PCR products from triplicate reactions were then pooled and quantified using PicoGreen. An equal amount of DNA for each sample was further pooled and purified with Qiagen QIAqick gel extraction kit. Sequencing was carried out on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) using a 2 × 250 pair-end format.

Raw amplicon sequencing data were processed through a pipeline (http://zhoulab5.rccc.ou.edu:8080) by the Institute for Environmental Genomics at the University of Oklahoma (Wu et al., 2016) to check read quality, demultiplex reads, and remove primers. Reads were then processed using USEARCH-UNOISE3 (Edgar, 2010; Edgar, 2016) which has been shown to have a good balance between resolution and specificity for amplicon sequencing process (Prodan et al., 2020). Reads were merged as suggested by USEARCH documentation for 2 x 250 pairs with longer overlaps. Reads were quality filtered using 1 as the max expected error threshold and unique reads identified. UNOISE3 was then used for ASV-level denoising based on the default level minimum abundance. An ASV table was generated and resampled to the same

sequencing depth across all samples (27,000 sequences per sample). Taxonomy was assigned using the USEARCH suggested RDP training set.

3.3.4 Network construction and characterization

Correlation networks using a Random Matrix Theory (RMT)-based approach (Deng et al., 2012; Yuan et al., 2021; Zhou et al., 2010) were constructed for all sampling times resulting in a total of 19 networks for each site using the Molecular Ecological Network Analysis Pipeline (MENAP) available at the Institute for Environmental Genomics, University of Oklahoma (http://ieg4.rccc.ou.edu/MENA/). RMT distinguishes system-specific, nonrandom associations from random associations and thus yields association networks that are robust to random noise. Each of the networks was constructed independently, with nodes representing ASVs and edges representing tentative association relationships based on correlation between the abundance profiles of connected nodes. To increase the reliability of the predicted association relationships, only ASVs in at least 6 of the 8 replicates were used for the network construction. In short, ASV abundance data was central-log-ratio transformed to mitigate the effect of compositional bias (Aitchison, 1994; Carr et al., 2019), and Pearson correlations were used to calculate the correlation matrix followed by an RMT-based approach (Deng et al., 2012; Yuan et al., 2021; Zhou et al., 2010). In order to compare network topologies under the same condition, a uniform cutoff value (St) was used to generate microbial networks. The best cutoff value for all networks was determined by a scheme based on the generalized Brody distribution (Sabri et al., 2014). Then, an adjacency matrix was generated, containing only the correlations whose absolute values of coefficient

(correlation strengths) were larger or equal to St. Nodes in isolation after the cut (no correlation strength to other nodes ≥ St) were removed from the network. iDIRECT was applied to these networks to reduce the influence of indirect relationships (Xiao et al., 2022).

The potential contributions of environmental filtering or dispersal limitation in shaping network topology were tested. First, we determined the importance of soil factors, climate variables, and spatial distance between samples on the networked community structure using a CCA model followed by VPA. Next, we used a pipeline developed by Yuan et al. publicly available R and Python 3 script (Yuan et al., 2021) to detect taxon–taxon–environment co-variation links (Lima-Mendez et al., 2015) and links possibly caused by dispersal limitation. While such analyses provide insight on the relative importance of biotic interactions in shaping MENs, it is still not possible to prove to prove that the links are truly due to biotic interactions. For this reason, MENs are best for making relative comparisons between conditions or treatments (Zhou et al., 2011a; Zhou et al., 2015). Therefore, this study focused on comparing network differences between native land (TGP) converted for long-term cropland use (CT wheat).

A total of twenty-two network topological indices were calculated using the MENAP to characterize network topological structure (Deng et al., 2012). We focused on several indices including nodes, the total number of links, average connectivity (avgK), average clustering coefficient (avgCC), average path distance (geodesic distance, GD), connectance (Con), and modularity (M). All network properties were calculated individually for each random network. To test the significance of the constructed empirical MENs, 100 random networks corresponding to each network were generated.

The numbers of nodes and links in random networks were constant, but link positions were rewired randomly so that the rewired network was comparable to the empirical network (Maslov and Sneppen, 2002). The same suite of network topological properties was calculated with each randomization. The means and standard deviations of these properties from the 100 randomizations were calculated and compared with those from the corresponding empirical MENs. Networks were visualized using Cytoscape 3.8.2 (Shannon et al., 2003).

Network size and connectivity considerably varied among the MENs especially under CT wheat land use, therefore relative modularity (RM) was calculated. RM is considered to be more meaningful for comparing modular structures across different networks by measuring how modular a network is compared to the mean expected modularity (Thebault and Fontaine, 2010; Yuan et al., 2021). RM was calculated as the ratio of the difference between the modularity of an empirical network and the mean of modularity from the random networks over the mean of modularity from the random network (Thebault and Fontaine, 2010).

Each node was grouped into a topological role in the network based on its within-module connectivity (Zi) and among-module connectivity (Pi) (Guimera and Nunes Amaral, 2005). As used in previous studies (Olesen et al., 2007; Yuan et al., 2021; Zhou et al., 2011a), nodes were classified as network hubs (highly connected nodes within the entire network, $Zi > 2.5$ and $Pi > 0.62$), module hubs (highly connected nodes within the modules, $Zi > 2.5$ and $Pi \leq 0.62$), connectors (nodes that connect the modules, $Pi > 0.62$), and peripherals (nodes connected in the modules with few links, $Zi < 2.5$ and $Pi \leq 0.62$).

Module hubs, connectors, and network hubs are referred to as keystone nodes (Banerjee et al., 2019; Rottjers and Faust, 2019)

3.3.5 Statistical analyses for network complexity comparisons

To evaluate the differences of MENs over time in both land uses, the 22 topological indices calculated for each empirical MEN were used for principal component analysis using the 'prcomp' function in the stats package in R (R Core Team, 2020). Overall differences of network topological properties between land uses were compared using a Mann–Whitney U test in the stats package in R (R Core Team, 2020). To examine differences in module composition, Fischer's exact test was performed to identify preserved module pairs in networks (Yuan et al., 2021) (1) under CT wheat land use or native TGP land use over time and (2) between CT wheat and native TGP land use. P-values from the exact tests were adjusted using the Bonferroni procedure within each network. In short, if two modules in different networks consisted of large proportions of shared nodes (adjusted $p \leq 0.05$), they were considered preserved module pairs (Zhou et al., 2011a). The exact tests were performed in R with the 'fisher.test' function in the stats package, and p-value adjustment was done with the 'p.adjust' function in the stats package in R (R Core Team, 2020).

In order to assess the differences of the networked communities under CT wheat and native TGP land use, three non-parametric multivariate analyses of dissimilarity were performed including MRPP, ANOSIM, and Adonis based on Bray-Curtis distance using the R package 'vegan' (Oksanen et al., 2019) and visualized using principal coordinate analysis (PCoA). Mantel tests were also performed between networked community

structures and soil and climate variables using the R package ‘vegan’ (Oksanen et al., 2019). The taxonomic composition of the networked communities under CT wheat and native TGP land use were analyzed at the phylum and class levels. Mann–Whitney U tests were used to evaluate the changes in the average relative abundance of each taxa due to land conversion.

3.3.6 Network stability analyses

To determine whether and how land use conversion affects the stability of the constructed MENs, several indices were used to characterize network stability including robustness, vulnerability, node and link constancies, node persistence, and compositional stability. Detailed descriptions of the calculations can be found in Supplementary Table S3.8.

Network stability based on simulation includes robustness and vulnerability. The robustness of a MEN is defined as the proportion of the remaining species in the network after random or targeted node removal (Dunne et al., 2002; Montesinos-Navarro et al., 2017). For simulations of random removal, robustness was measured when 50% of random nodes were removed from each MEN. For simulations of targeted removal, robustness was compared when five module hubs were removed and when half of the modules hubs were removed since the number of module hubs differed greatly between networks. Vulnerability of each node measures the relative contribution of the node to the global efficiency. The vulnerability of a network is indicated by the maximal vulnerability of nodes in the network (Deng et al., 2012) and the global efficiency of a graph was calculated as the average of the efficiencies over all pairs of nodes. In ecological networks, efficiency explains the ability to spread information within a

network and is important to determine how quickly the effect of biological/ecological events spread to parts or the entire network (Yuan et al., 2021).

Network stability based on empirical data includes node constancy, link constancy, node overlap, node persistence, and compositional stability. Constancy measures the temporal stability of species. It is defined as μ/σ, where μ is the mean of abundance over time and σ is the standard deviation (Hautier et al., 2014). The constancy of node i was calculated as μ_i/σ_i. The abundance of species i at a certain time point was positive only if species i was in the MEN at that time point. Otherwise, the abundance of species i was considered zero for that time point and removed from subsequent analyses. The average of all the node constancy values were reported. Similar procedure was used to calculate link constancy. We let $l_{ij+} = 1$ if nodes i and j were positively linked in a network, $l_{ij-} = 1$ if nodes i and j were negatively linked in a network, and $l_{ij+} = l_{ij-} = 0$ if there was no link between i and j (Yuan et al., 2021). Again nonfinite values were removed from subsequent analyses. The average of all the link constancy values were reported. The number of overlapping nodes among multiple networks was calculated following previous methods by Hui et al. (Hui et al., 2014) where the higher numbers of overlapping nodes among networks indicated slower turnover of species composition in the networks with time points being referred to as "orders" (Yuan et al., 2021). The node persistence is defined as the proportion of coexisting species (over the total number of species) at an ecological regime (Landi et al., 2018). Node persistence was calculated as the percentage of nodes present in the network in consecutive monthly comparisons. The compositional stability evaluates the change in community structure over time (Zelikova et al., 2014). The compositional stability for the networked microbial communities was

calculated using the sample × ASV matrix. If community structure does not change, the stability index is equal to 1; while if community structure is completely different among time points if stability index is 0. Compositional stability was addressed as consecutive monthly comparisons as done with node persistence.

Overall differences in stability indices between CT wheat and native TGP land use were determined using Mann-Whitney U test. The relationship between node persistence and compositional stability for each land use was addressed using Spearman correlation. Spearman correlations were also used to associate soil properties, climate variables, and management input with network stability and complexity indices. The correlations with management data were calculated using Spearman's generalized equation due to repetitive values in the coded management data.

3.4 Results

3.4.1 Constructing molecular ecological networks

Molecular ecological networks (MENs) were constructed for each sampling month resulting in 19 networks per land use (Figure 3.1a, Figure S3.1) (Zhou et al., 2010). In general, the empirical MENs were significantly different than the random MENs (Table S3.2). The overall topological properties (Table S3.1) revealed that curves of network connectivity distribution were fitted well with the power-law model with R^2 values for CT wheat (0.75-0.95) and the TGP (0.72-0.85) indicative of scale-free networks, or that most nodes in the network have few neighbors while few nodes have many neighbors (Amaral et al., 2000; Ding et al., 2013). The networks also exhibited small-world characteristics with average path lengths (GD) ranging from 3.3-8.3 and 3.2-10.4 for CT

wheat and native TGP, respectively. Small-world networks have short distances between nodes meaning network nodes are always closely related to each other (Girvan and Newman, 2002). Modularity (M) values for CT wheat and native TGP were significantly greater ($p = 0.04$ and $p < 0.001$, respectively) than the corresponding modularity values for the randomized networks and the relative modularity (RM) was > 0 (M of empirical MENs greater than M of random MENs) which is evidence of modular networks.

The observed species co-occurrence patterns in the MENs could mainly be due to environmental filtering, dispersal limitation, and biotic interaction. We used CCA plus variation partitioning analysis (VPA) and the link test for environmental filtering and dispersal limitation (LTED) (Yuan et al., 2021) to determine the relative contributions of these ecological processes to species co-occurrence in the MENs. While CCA results indicated that soil and climate variables had a significant ($p \leq 0.05$) impact on the networked microbial communities (Table S3.3), VPA showed that the majority of variation (70.2%, Figure S3.2b) could not be explained by measured environmental variables (i.e., environmental filtering effect), and distance between samples within a land use only had a noticeable effect (8.9%) when considering interaction with soil properties. LTED suggested similar results of minor contributions from environmental filtering with less than 1% of links on average considered taxon-taxon-environmental covariates using the network correlation cutoff (Figure S3.3a). When the correlation threshold (|r|) was lowered, links due to taxon-taxon-environment covariates increased in the CT wheat on average, but were still relatively minimal (2.1% - 20.0%). In addition, dispersal limitations impacted less than 5% of links (Figure S3.3b) in the networks on average in both fields ($p \leq 0.05$, $r > 0$) based on LTED, and only 1.14% and 0.81% of

links on average where considered significant strong correlations ($p \leq 0.05$, $r \geq 0.5$) due to dispersal limitations. Collectively, these results all indicated MENs in this study were less likely due to environmental filtering or dispersal limitation, and therefore, it is most probable that biotic interactions could be the major driver shaping these networks.

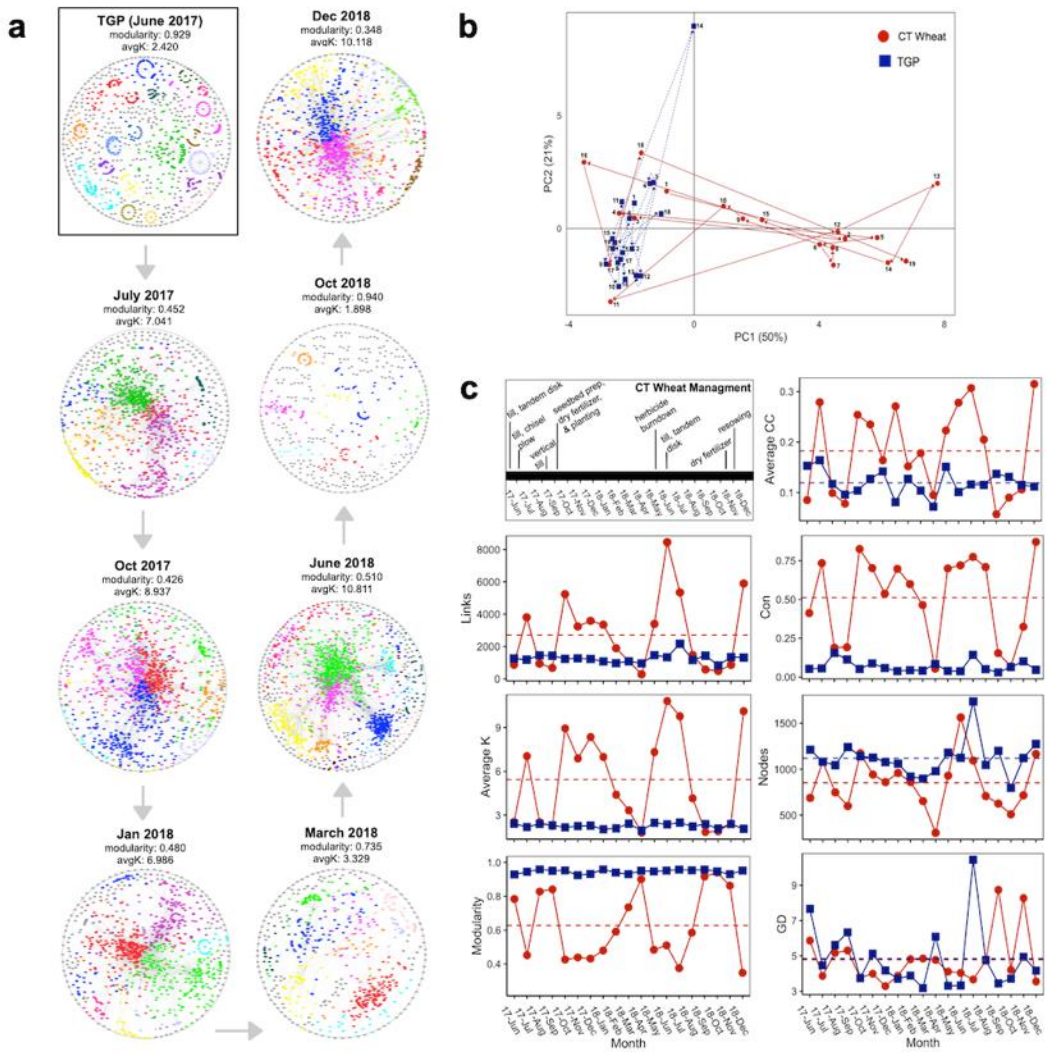

Figure 3.1 Temporal dynamics of soil microbial networks. a) Visualization of soil microbial networks over time. Temporal changes of MENs over 19-month sampling period represented by select sampling times. As networks on the native TGP systems remained relatively similar, a single visual representation of the control site is outlined in black. The other networks depict temporal differences of seven on MENs for the CT wheat land use. Large module with ≥10 node are shown in different colors, and smaller modules are shown in gray. b) Twenty-two network topological parameters were used for principal component analysis (PCA). Sampling times are labeled 1 through 19 representing consecutive sampling months. For example, 1 represents June 2017 while 19 represents December 2018. Arrows clarify the chronological order of the MENs. c) Temporal changes of the network topological parameters including nodes, links, average K, average CC, Con, and Modularity. Red circles represent networks under CT wheat land use and blue squares represent native TGP (control) land use. Overall differences between network topologies between land uses compared using Mann-Whitney U Test.

3.4.2 Networked community composition

The number of ASVs used for network construction (ASVs in 6 of 8 replicates) were on average 39% greater in the native TGP than CT wheat, and the resulting constructed networks were 24% larger. By contrast, 43% of ASVs made it into the constructed CT wheat networks compared to 34% of ASVs in the native TGP networks. Also, when considering ASVs in large modules (≥ 10 nodes), 72% of the nodes were in large modules in the CT wheat networks compared to 63% of nodes in the native TGP networks. Together, these results suggested that the CT wheat microbial taxa might associate more closely with each other than those of the native TGP.

The composition of the networked microbial communities significantly differed between the CT wheat cropland and native TGP as shown by principal coordinate analysis (Figure S3.2a). This was further supported by three non-parametric dissimilarity analyses (Table 3.1) that confirmed networked microbial communities significantly (p = 0.001) differed by land use as well as sampling time. Converting native land to long-term CT wheat use resulted in a significant ($p \leq 0.05$) increase in the relative abundance of Actinobacteria, Armatimonadetes, Bacteroidetes, Chloroflexi, Cyanobacteria, Gemmatimonadetes, Alphaproteobacteria, and Betaproteobacteria (Figure S3.4, Table S3.4) accompanied by significant decreases in the relative abundance of Acidobacteria, Thaumarcheota, Verrucomicrobia, and Deltaproteobacteria.

Table 3.1 Significance tests of the networked communities between CT wheat and native TGP land use.

Dataset	Factor	MRPP		ANOSIM		Adonis	
		δ	*p*	r	*p*	F	*p*
All	Land use	0.579	0.001	0.943	0.001	182.6	0.001
	Month	0.693	0.001	0.144	0.001	2.156	0.001
TGP	Month	0.457	0.001	0.726	0.001	4.043	0.001
CT Wheat	Month	0.533	0.001	0.443	0.001	3.778	0.001

Three different permutation tests were performed (MRPP, ANOSIM and Adonis) on the basis of Bray–Curtis distance.

3.4.3 Differences in complexity of MENs

Based on 22 different network topological properties, the microbial MENs under CT wheat and native TGP land use displayed noticeably different trajectories over the 19-month sampling period (Figure 3.1b; Figure S3.1). Compared with native TGP land use, CT wheat underwent many major management events. After the wheat spring growing season ended, the field was left fallow. It was tilled several times between June and September to mix the soil and prepare the seedbed (Figure 3.1c). Seedbed preparation was closely accompanied by fertilizer application and seed planting. For the 2018-2019 season, the field was left again fallow after the spring and prepared using herbicide and soil tillage. Finally, the field was fertilized during the fall and bare spots resowed in late November.

To determine how land conversion for long-term cropland use affected microbial network complexity, we closely examined several network topological properties. Network size (number of nodes; $p = 0.001$, W = 73) and modularity (M; $p < 0.001$, W =

5.5) significantly decreased under CT wheat, while average connectivity (average links per node, avg K; $p = 0.001$, W = 219), connectance (the proportion of realized links in all possible ones, con; $p < 0.001$, W = 345), and average clustering coefficient (the extent to which nodes are clustered, avg CC; $p = 0.077$, W = 242) strongly increased under CT wheat (Figure 3.1c). The majority of the native TGP network topological properties remained stable over the 19-month sampling period compared to the CT wheat cropland properties that had observable temporal variations. In addition, the relative modularity (how modular a network is as compared with the mean expected modularity, RM) of MENs was calculated as it is considered more meaningful for comparing modularity across networks. The RM was significantly greater ($p = 0.05$) under CT wheat compared to the native TGP. Notably, the RM of the CT wheat cropland significantly correlated ($p \leq 0.05$) with many of the network topological properties including nodes, links, average connectivity, average clustering coefficient, path distance, and connectance (Figure 3.2) while RM of the native TGP networks only significantly correlated ($p \leq 0.05$) with links and average connectivity. Together, these results indicated that MENs under CT wheat were more complex and experienced substantially more temporal variation than those of the native TGP over the sampling period, which coincided with and were likely due to the management that occurred in CT wheat field.

Variations in the structure of the microbial MENs could affect the network organization principles (i.e. modularity). Networks under CT wheat consisted of 189 large modules (modules with ≥ 10 nodes) accounting for 34.4%-91.2% of the node in each MENs while native TGP networks had 430 large modules totaling 52.4%-71.1% of the networked nodes (Table S3.1). Between CT wheat and the native TGP, there were no

preserved modular pairs (Table S3.5). In short, preserved module pairs are modules that contain a significantly large proportion of shared nodes when two modules in different networks are compared (Zhou et al., 2011a). The native TGP also did not have any preserved module pairs over the sampling period in comparison with 67 preserved module pairs for CT wheat, suggesting that CT wheat land use resulted in greater similarities in module identity.

Differences in network complexity could also impact the role of individual members within the network. The roles of each node were classified based on the within-module connectivity (Z_i) and among-module connectivity (P_i) (Zhou et al., 2010). A total of 433 and 637 module hubs (nodes highly connected to other members in a module) were identified for the CT wheat and native TGP networks (Table S3.6; Table S3.7), respectively. The CT wheat networks also consisted of 38 network hubs (nodes being both a module hub and a connector) and 456 connectors (nodes linking different modules). However, native TGP networks had no network hubs and only one connector for all networks. Together, network hubs, module hubs, and connectors are considered keystone nodes or nodes that play critical roles in shaping network structure (Banerjee et al., 2019). Of the 1,226 unique ASVs that acted as keystone nodes among all MENs, only 18 (1.5%) were found to be shared between both land uses. Additionally, of the keystone nodes within each land use, 17.7% acted as keystones in two or more of the CT wheat networks compared to only 3.8% in native TGP networks. Taken together, CT wheat land use altered the roles of members within the networks and resulted in a greater number of temporally preserved keystone nodes.

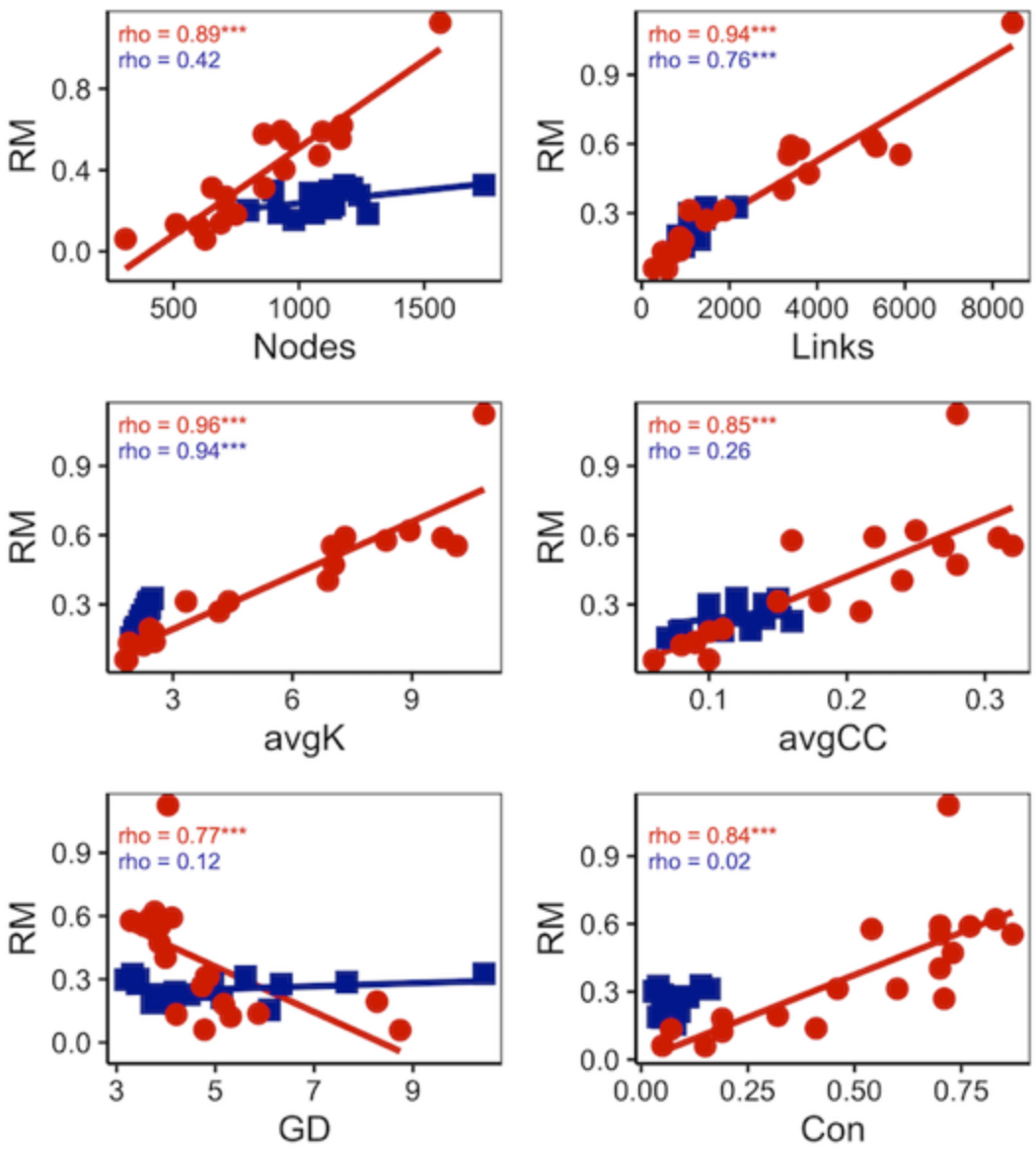

Figure 3.2 Relationship between network topological properties and relative modularity**.** Spearman correlations were used to compare network topologies including nodes, links, average K, average CC, GD, and Con to the relative modularity. Red circles represent networks under CT wheat land use and blue squares represent native TGP (control) land use. Correlation coefficients (rho) are shown in corresponding colors followed by "*" for $p \leq 0.05$, "**" for $p \leq 0.01$, and "***" for $p \leq 0.001$

3.4.4 Impact on stability of MENs

To determine whether and how land use conversion affected MENs stability, multiple stability indices were calculated based on simulations and empirical data. First, robustness or the resistance to node loss (Montesinos-Navarro et al., 2017) of the MENS was calculated based by simulating species extinction. Under random species loss (Figure 3.3a), the MENs had significantly higher robustness ($p < 0.001$, W=317) under CT wheat land use than the native TGP. When five module hubs were targeted for removal (Figure S3.5a), there was no significant difference ($p = 0.246$, W=221) in robustness. Yet, when 50% of module hubs were removed (Figure 3.3b), robustness of the MENs was significantly greater ($p < 0.001$, W=361) under CT wheat land use than the native TGP. Second, the vulnerability or the maximum decrease in efficiency when a single node was deleted from the network (Banerjee et al., 2018) was significantly lower ($p < 0.001$, W=47) under CT wheat land use averaging 0.11 ± 0.08 and averaging 0.25 ± 0.11 for the native TGP (Figure 3.3c). Third, while the temporal invariability of the community composition (Zelikova et al., 2014) was greater ($p < 0.001$, W=6825) under native TGP based on consecutive monthly comparisons (Figure S3.5b), more of the same nodes were present under CT wheat than the native TGP when any two pairs of networks were compared ($p = 0.02$, W= 53.5; Figure 3d). This held true for comparisons up to any six networks ($p < 0.005$). The compositional stability and node persistence strongly correlated under both CT cropland use ($p < 0.001$, rho =0.94) and the native TGP ($p < 0.001$, rho =0.89; Figure S3.5c), and the constancy (inverse of temporal variations) of nodes (Figure S3.5d) was greater ($p = 0.001$) under native TGP land use, while the constancy of links (Figure S3.5e) was greater ($p < 0.001$) under CT wheat land use.

Together, results from multiple stability indices suggest that the CT wheat cropland showed greater stability than the native TGP even though the networked community composition changed more between consecutive months.

Interestingly, there were significant correlations between network stability and network complexity that differed with land use. Overall, robustness, compositional stability, and node persistence significantly ($p \leq 0.05$) positively correlated with several network complexity indices under CT wheat (Figure 3.3e) while only robustness had significant positively correlations with network complexity for the native TGP (Figure 3.3f). Consistently, network stability indices under CT wheat significantly positively correlated with nodes, average connectivity, and relative modularity. Network vulnerability had a significant negative relationship with the majority of network complexity indices for CT wheat compared to no significant correlations for the native TGP. In general, CT wheat land use had a strong relationship between network stability and complexity that was not observed for the native TGP land use.

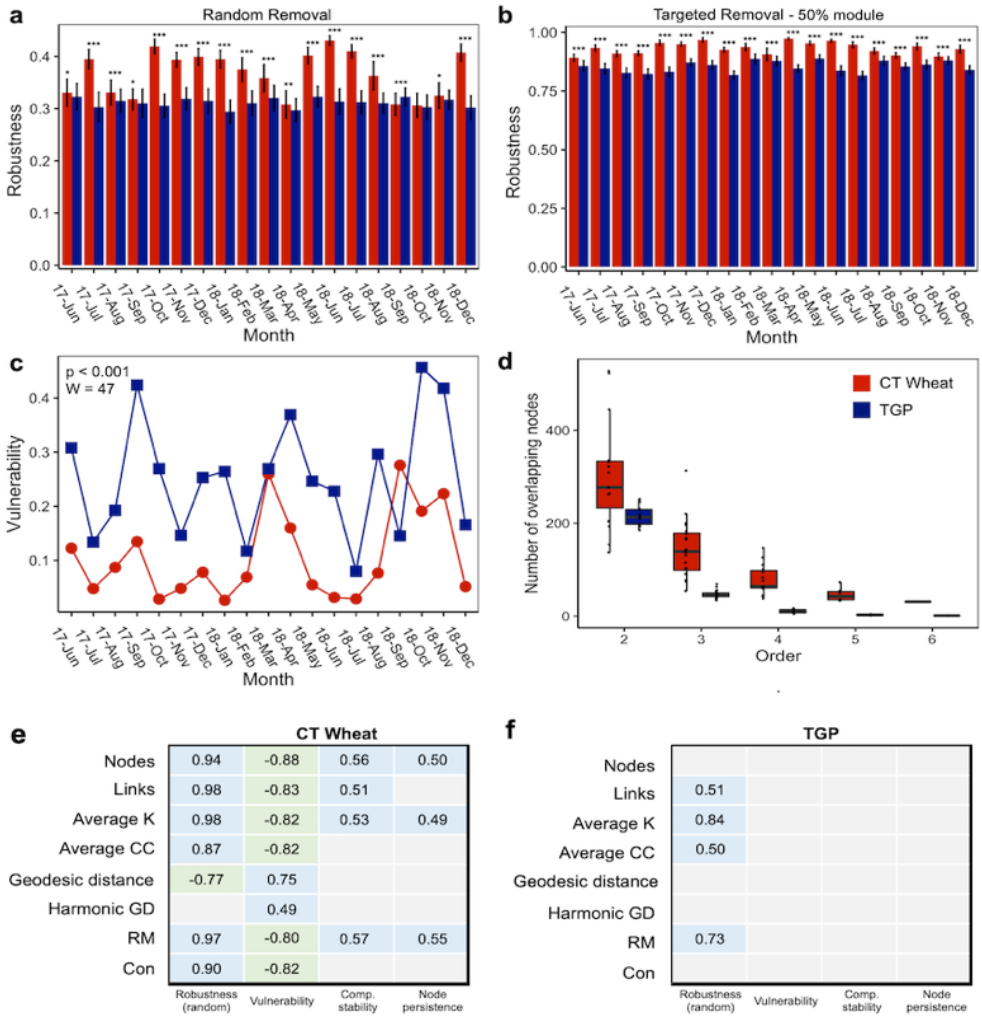

Figure 3.3 Temporal dynamics of network stability. a) Robustness measured by randomly removing 50% of taxa from each of the empirical MENs. b) Robustness measured by removing 50% of module hubs from each of the empirical MENs. For a and b, error bars represent the standard deviation of 100 repetitions of the simulation. Robustness for each timepoint was compared between CT wheat and native TGP (control) land use using a two-sided t-test. Significant differences are indicated by "*" for $p \leq 0.05$, "**" for $p \leq 0.01$, and "***" for $p \leq 0.001$. CT wheat shown in red and native TGP shown in blue. c) The network vulnerability of the empirical MENS measured by maximum node vulnerability in each network. d) The number of overlapping nodes under CT wheat and native TGP land use among different numbers of networks (that is, orders). For example, for order=2, the overlapping nodes were between any two pairs of networks; for order=3, they were among any three networks. Spearman correlations between network stability and network complexity indices under e) CT wheat and f) native TGP land use. Significant correlations ($p \leq 0.05$) are shown in blue for positive correlations and green for negative correlations. Inside the cells are the corresponding correlation coefficients. Non-significant correlations are shown in gray.

3.4.5 Interactions between complexity, stability, and the environment

An important question is whether the relationship between the complexity and stability of the networks with the environment are altered due to land use conversion for long-term CT wheat use. Land use conversion for CT wheat land use resulted in less importance of various environmental factors in shaping the networked community structure compared to the native TGP (Figure 3.4a,b). The TGP networked community structure was strongly correlated to all environmental factors except for nitrate (NO_3^-) and ammonium (NH_4^+). In comparison, the networked community under CT wheat was only strongly correlated with soil temperature, soil pH, and nitrate. CT wheat land use and likely its associated management also resulted in more negative correlations between pairwise comparisons of environmental factors than were observed for the native TGP.

Similarly, environmental factors played a less important role in influencing network complexity and stability of CT wheat land use than of the native TGP (Figure 3.4c). The TGP network complexity and stability were influenced by several environmental factors. Overall, OM, air temperature, and soil temperature had positive correlations with network complexity indices, while nitrate negatively correlated with complexity. Increases in SWC and NH_4^+ decreased TGP network stability by reducing node persistence, composition stability, and increasing vulnerability. For CT wheat, SWC had the greatest impact on the complexity of the MENs always having a negative correlation with complexity indices. Ammonium (NH_4^+) also significantly influenced CT wheat network complexity by increasing distance between nodes (GD) and decreasing node connectance (Con) when soil NH_4^+ was higher. However, CT network stability was

not as noticeably impacted by environmental factors with only rainfall having a positive effect on node persistence and SWC had a negative effect on network robustness.

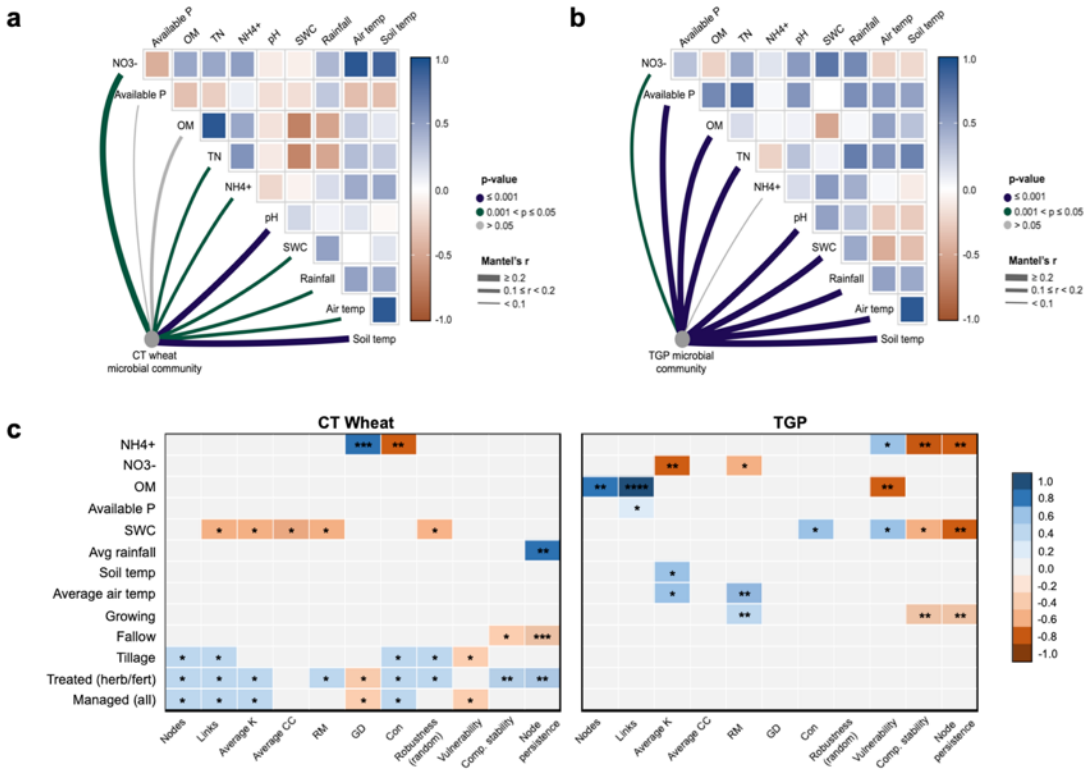

Figure 3.4 Associations between network indices, environmental properties, and management. Correlations of the networked community structures (Bray-Curtis distance) and soil and climate properties for the a) CT wheat and b) native TGP land use. Edge width corresponds to Mantel's r value and the edge color represents the statistical significance. Pairwise spearman correlation of the variables are shown with a color gradient representing the correlation coefficients. c) Spearman correlations between network stability and network complexity indices under CT wheat land use and native TGP (control) land use. Spearman's rho for significant correlations are depicted in a color gradient. The p-values of correlations are show in the color squares, "*" for $0.5 < p < 0.1$, "**" for $p \leq 0.05$, "***" for $p \leq 0.01$, "****" for $p \leq 0.001$. Non-significant correlations are shown in gray.

Notably, management practices had considerable impact on the soil microbial network complexity and stability of CT wheat land use. The TGP received minimal

management including cattle grazing and a prescribed burn, but no significant correlations were detected for either. Although, growing times of the perennial grasses negatively impacted native TGP node persistence and compositional stability (Figure 3.4c), management practices associated with CT wheat land use (including fallow, tillage, and herbicide/fertilizer application) significantly correlated with network complexity and stability, in particular, with compositional stability and node persistence. Overall, management input significantly increased network complexity and stability with soil tillage and herbicide/fertilizer application having considerable positive impacts.

3.5 Discussion

3.5.1 Microbial community network assembly

As an important part of many ecosystems, microbial communities and their "interacting" networks are inherently complex (Fuhrman, 2009). In this study, the architecture of the MENs was driven by several simple principles, sharing many common properties such as scale-free, small-world, and modular under CT wheat and native land use. Scale-free networks are highly non-uniform resulting in most nodes or species having few links or interactions while few nodes have many links (Barabási and Albert, 1999; Deng et al., 2012). In small-world networks, the short path length between nodes enables efficient, rapid communication between network members and allows disturbances to spread quickly through the network for swift reactions (Zhou et al., 2010) which is critical for responding to environmental changes. Lastly, a module in a network is a group of nodes that are highly connected within the group but have very few connections outside the group. Modularity in a microbial community has been suggested to arise for several

reasons including habitat heterogeneity, specificity of interactions, ecological niche overlap, resources partitioning, phylogenetic relatedness, and/or natural selection (Olesen et al., 2007); and it is important for minimizing the impacts of disturbance by containing the disturbance and damage at a local level (Kitano, 2004). Together, these properties have significant implications on microbial community dynamics and network topology (Barabasi and Oltvai, 2004; Kitano, 2004).

Although biotic interactions are a key part of regulating community assembly and disassembly (Galiana et al., 2018), environmental filtering and dispersal limitation, are also considered major drivers of microbial network patterns (Barberán et al., 2012). Yet, it remains challenging to disentangle these mechanisms and determine the importance of biological interactions in ecological community assembly (Zhou and Ning, 2017). Therefore, we used multiple statistical approaches to determine that biotic interactions could be the most important factor shaping soil microbial networks (Yuan et al., 2021). Nevertheless, soils are highly heterogenous environments making it exceptionally difficult to determine the involvement of unmeasured environmental variations, especially in agriculturally managed systems which are rapidly fluctuating environments (Trivedi et al., 2016). For this reason, biotic interactions should be at most considered putative biotic interactions (Yuan et al., 2021) as well as interpreted with caution since interactions are based on co-occurrence correlations (Blanchet et al., 2020).

3.5.2 Microbial community structure and keystone taxa

Networked community structure significantly differed between land uses with it being known that land use strongly impacts community structure (Lacerda-Júnior et al., 2019;

Lauber et al., 2013; Montecchia et al., 2015). Management intensification and the resulting environmental changes likely act as a deterministic filtering factor (Barnett et al., 2019; Guo et al., 2018; Zhou et al., 2021) generating dynamic changes to the microbial communities and their network structure. Similarly, the identity of the keystone nodes also differed between land uses. The native TGP keystone nodes overlapped with many of those previously detected in grasslands (Sphingobacteriales, Actinomycetales, Acidobacteria GP4) while the keystone nodes of the CT wheat land use corresponded to those for other agricultural lands (Rhizobiales, Solirubrobacterales) and plant-associated microbes (Acidobacteria GP1, Acidobacteria GP3) (Banerjee et al., 2018). Therefore, land use conversion for long-term cropland use not only shifted the microbial community structure but also changed the keystone nodes which drive community composition regardless of their abundance.

3.5.3 Network complexity and stability

While it has been recognized that changes in network structure affect the stability and functioning of an ecosystem (Dunne et al., 2002; Thebault and Fontaine, 2010), less is understood about this relationship in microbial ecology. In this study, we showed the soil microbial community response to long-term cropland use generated more complex networks and increased network topological temporal variability compared to native land use. Although the microbial community was more diverse and the networks were larger under the native TGP, the resulting networks were less complex suggesting that greater diversity does not necessarily mean greater complexity (Tu et al., 2020). This observation could arise for various reasons. For example, tallgrass prairies harbor greater

aboveground plant species diversity providing more diverse nutrient and energy sources for the belowground microbial communities compared to croplands where nitrogen fertilizers provide the majority of the nutrients (Philippot et al., 2013). Therefore, the more diverse environmental nutrient and energy may support the microbial community instead of supplies through complex species interactions (Tu et al., 2020). Another potential explanation could be greater functional redundancy due to higher microbial diversity in tallgrass prairies. Microbes often interact through function/metabolite preference (Tu et al., 2016), and higher diversity and functional redundancy of the microbial community reduces reliance on a few taxa and provides more opportunity for microbes to generate relationships within neighborhoods (i.e. modules). This could lead to greater modularity, reduced complexity, and the lack of persevered module pairs and keystone taxa as observed under native land use. In addition, the greater modularity in native land use is likely linked to stronger niche differentiation (Shi et al., 2016; Zhang et al., 2018) as the soils in native tallgrass prairies are generally a more heterogeneous and disconnected habitat compared to soils that are mixed by tilling creating a more homogeneous soil structure.

Network stability was enhanced under CT wheat land use with stability generally being important to dampen responses to environmental disturbance (Hudson and Henry, 2010; Tilman et al., 2006). The CT wheat networks were better able to withstand random and targeted module hub removal, indicating reduced network vulnerability. The networked CT wheat microbial community was also more consistent over time with significantly more shared nodes between networks, conserved modules, and conserved keystone nodes compared to native land use. Similar to macroorganisms (McKinney and

Lockwood, 1999; Smart et al., 2006), land use conversion can cause biotic homogenization of microbial communities (Rodrigues et al., 2013; Tian et al., 2018), which leads to greater similarity of communities over time and/or space (Olden and Poff, 2003). This could be a cause for concern if biotic homogenization is a result of the loss of endemic taxa as these taxa tend to have unique traits, and homogenization of these traits likely alters ecosystem function and reduces ecosystem resilience (McKinney and Lockwood, 1999; Olden et al., 2004). Furthermore, there are often important ecological relationships between the complexity and stability of a system (MacArthur, 1955; May, 2019) with native land use conversion for cropland use enhancing the relationship between network stability and complexity. Greater complexity could produce differential effects on stability creating a more resistant (Landi et al., 2018; Okuyama and Holland, 2008) but less resilient system (Pimm, 1984). Hence, while the CT cropland developed stable ecological networks after many years of cultivation, the networks also heavily rely on "interactions" to maintain stability, potentially leaving the networks vulnerable to cascade effects (Helbing, 2013) which could disrupt these interactions (i.e. complexity) and the network stability.

3.5.4 Impact of environment factors and management on MENs

Soil microbial communities are the most sensitive indicators of land use conversion and disturbance often being altered by soil properties (Fierer and Jackson, 2006), climate (Yao et al., 2017), land use intensity (Thomson et al., 2015), and plant communities (Guo et al., 2016). In the native TGP, we observed significant correlations between network complexity and stability with multiple environmental factors. Plant activity likely has a

substantial influence on the complexity and stability of the MENs as plants are thought to be highly important to microbial community dynamics in natural ecosystems due to the co-evolution of plant-microorganism interactions (Philippot et al., 2013). Plant productivity interacts with all the factors important to shaping native TGP networks including soil water content, nitrogen availability, organic matter, and temperature which in turn could, directly and indirectly, affect microbial interactions. For instance, grasslands are often nitrogen limited with the productivity of many plant communities relying on nitrogen availability (Vitousek and Howarth, 1991), and aboveground net primary production in grassland generally has a large response to increased water availability (Lauenroth et al., 2000). This was further supported by the decrease in stability during times of vegetation growth under native land use.

While nutrient content and water availability can act as robust environmental filters to strongly select for microbial communities (Chase, 2007; Huang et al., 2021), management disturbance could be equally if not more important to shaping microbial communities. Overall, the complexity and stability of the MENs in the CT cropland were more strongly correlated to management input than environmental factors. Summer-fallow decreased network stability, while the frequent disturbance of tillage, herbicide, and fertilizer input generally increased complexity and stability. Management disturbances also greatly contributed to the dynamic changes of the networks over time. Meanwhile, frequent fertilizer use in CT wheat cropland effects nitrogen (NH_4^+) content that was important for influencing CT wheat cropland network complexity (Lu et al., 2013; Zhang et al., 2018). Additionally, water availability is frequently limited in areas where wheat is grown and summer-fallow wheat ecosystems generally have reduced

water use efficiency (Lauenroth et al., 2000), thus, increased precipitation in the CT cropland community might disrupt existing microbial links. Although land use and the changes in soil properties may have changed the microbial community structure and diversity, repeated management disturbance in the CT cropland greatly influenced the ecological networks, generating more complex and stable MENs presumably because greater interactions are needed for the microbial community to quickly respond to management disturbances.

3.6 Conclusions

Determining the extent of microbial "interactions" and their changes due to land use conversion and management disturbance is a difficult issue to address and remains understudied. Therefore, we compared the soil microbial community temporal network dynamics in long-term cropland to the native tallgrass prairie land use. Similar to observations for microbial diversity, network features of the MENs differed due to land conversion and were temporally dynamic, especially under converted cropland use. Increased complexity of the MENs under CT wheat may have resulted from decreased microbial diversity, increased biotic homogenization, and/or greater niche sharing related to the more homogenous soil habitat of croplands compared to native land use. The stability of the MENs was also greater under CT wheat compared to the native TGP. On the one hand, frequent management disturbances stimulate dynamic responses that have led to greater complexity and stability of microbial "interaction" networks, making the ecosystem potentially less vulnerable to further disturbance. On the other hand, it remains unclear how resilient the community and the links between microorganisms would be to

non-management related disturbances, such as drought, wildfires, and long-term warming. In addition, the negative impacts of the biodiversity loss due to land conversion could far exceed the positive effects of greater complexity and stability of microbial "interaction" networks, resulting in even more vulnerable ecosystems to both management and non-management related disturbances. Considering the increasing intensity of anthropogenic disturbance and environmental change, preserving microbial diversity and "interactions" could be vital to maintaining critical ecosystem functions.

Chapter 4: Temporal changes of virus-like particle abundance and metagenomic comparison of viral communities in cropland and prairie soils

4.1 Abstract

During the last several decades, viruses have been increasingly recognized for their abundance, ubiquity, and important roles in different ecosystems. Despite known contributions to aquatic systems, few studies examine viral abundance and community structure over time in terrestrial ecosystems. The effects of land conversion and land management on soil microbes have been previously investigated, but their effects on virus population are not well studied. This study examined annual dynamics of viral abundance in soils from a native tallgrass prairie and two croplands, conventional till winter wheat and no-till canola, in Oklahoma. Virus-like particle (VLP) abundance varied across sites, and showed clear seasonal shifts. VLP abundance significantly correlated with environmental variables that were generally reflective of land use, including air temperature, soil nitrogen, and plant canopy coverage. Structural equation modeling supported the effects of land use on soil communities by emphasizing interactions between management, environmental factors, and viral and bacterial abundance. Between the viral metagenomes from the prairie and tilled wheat field, 1,231 unique viral operational taxonomic units (vOTUs) were identified, and only five were shared that were rare in the contrasting field. Only 13% of the vOTUs had similarity to previously identified viruses in the RefSeq database, with only 7% having known taxonomic classification. Together, our findings indicated land use and tillage practices

influence virus abundance and community structure. Analyses of viromes over time and space are vital to viral ecology in providing insight on viral communities and key information on interactions between viruses, their microbial hosts, and the environment.

4.2 Introduction

Viruses have been making their way to the forefront of ecological research for their significant roles in marine and terrestrial ecosystems, being found everywhere that life exists. Most knowledge on viral ecology has been generated from the study of natural virus populations in marine and freshwater ecosystems, where viruses have been shown to mediate horizontal gene transfer (Breitbart et al., 2004), help drive biogeochemical nutrient cycling (Fuhrman, 1999), and play a central role in controlling the total abundance, population dynamics, and evolution of their hosts (Liang et al., 2020b; Williamson et al., 2007). It has been estimated that viruses may be the most abundant biological entity on the Earth at 10^{31} viruses (Suttle, 2005), with soils providing one of the greatest reservoirs (Williamson et al., 2017). As a result, it is now predicted that viruses have equal ecologically valuable roles in terrestrial environments. Soils provide a more diverse habitat for viruses than aquatic environments due to their wide compositional range, spatial heterogenicity in terms of physicochemical properties, and management practices, allowing viruses to be exposed to many unique ecological pressures that are not present in aquatic systems (Jangid et al., 2008; Lauber et al., 2013; Schlesinger et al., 1990; Zablocki et al., 2016). Understanding the response of virus communities to such pressures is critical to the knowledge of soil ecology and important for ecosystem sustainability.

Natural land conversion is a prevalent practice that results in distinct effects on the soil characteristics and function of terrestrial ecosystems. Specifically, agricultural cultivation has significantly changed land use across North America, resulting in the depletion of native tallgrass prairies to 4% of their original land coverage (Claassen et al., 2011; Wright and Wimberly, 2013). The majority of new croplands in the United States were initially grasslands with roughly a fourth of the converted land planted with wheat (Lark et al., 2015), which is now the dominant annually cropped plant in the Southern Plains. Grasslands are important for preventing erosion, acting as carbon sinks and as a source of nitrogen fixation (Carlier et al., 2009). Converting previously natural land into arable soils results in above and below ground species loss, allowing species invasion, as well as introducing disturbances to soil and biological processes (Calderón et al., 2001; Ding et al., 2013; Peterson et al., 2019). Together, these anthropogenic activities act as environmental stressors greatly impacting soil ecosystems with little known about the effects on virus populations. Since viruses are highly abundant and influence microbial hosts, it is important to understand the impacts of land use and management practices on the soil viral community.

Estimates of viruses in terrestrial environments are the first step to identifying virus significance in soils since organisms that are present in large numbers generally play important roles in ecosystem function. Transmission electron microscopy (TEM) and epifluorescence microscopy (EFM) have been used in aquatic systems to show a range in viral abundance of 10^4 to 10^8 ml^{-1}, providing evidence that viruses are a prevalent component of marine and freshwater environments (Wilhelm and Matteson, 2008; Wommack and Colwell, 2000). Advance in epifluorescence microscopy resulted in an

approach to directly visualize virus particles in marine systems (Bergh et al., 1989; Hobbie et al., 1977). These previous discoveries have resulted in the development of methods to mechanically extract, microscopically enumerate, and quantify viruses from soils (Ashelford et al., 2003; Williamson et al., 2005; Williamson et al., 2003). Virus-like particle (VLP) abundance ranging from 10^7 to 10^9 VLPs g^{-1} soil has been observed in a diverse range of sites and soil types (Narr et al., 2017; Williamson et al., 2007; Williamson et al., 2005; Williamson et al., 2003). For example, more nutrient-rich soils found in forests and pastures generally have a higher viral abundance than soils from croplands and extreme locations such as Antarctica (Narr et al., 2017; Williamson et al., 2007; Williamson et al., 2005). The VLP abundance often exceeds bacterial abundance, with it being thought that viral abundance is dependent on the productivity of the hosts, as well as viral persistence (Sharma et al., 2002; Williamson et al., 2007; Williamson et al., 2005), but few studies examine these dynamics at seasonal or annual timescales in soils. While studies in marine environments have presented clear temporal dynamics in viral abundance and community structure (Brum et al., 2016; Jiang and Paul, 1994), limited research leaves much to be discovered about the spatiotemporal changes of viruses in soils of terrestrial ecosystems.

To compare differences of viral populations, it is fundamental to have an accurate assessment community composition. Investigations have come to rely on high throughput sequencing to evaluate diversity, population structure, and potential functional importance of whole viral assemblages. As studies of marine systems have reported a diverse population of DNA and RNA viruses (Angly et al., 2006; Breitbart et al., 2002; Hurwitz and Sullivan, 2013), most terrestrial studies focus on dsDNA viruses

or examine extreme landscapes such as polar (Zablocki et al., 2014) and desert regions (Adriaenssens et al., 2015; Zablocki et al., 2016). Comparisons of viral communities between soil and aquatic environments have implied that distinct habitat types consist of distinct viral communities (Angly et al., 2006; Srinivasiah et al., 2008; Zablocki et al., 2014). With advances toward optimized methods for studying terrestrial viruses, recent studies in a thawing permafrost gradient recovered roughly 2,000 viruses approximately doubling the number of known genera in the RefSeq database at the time (Emerson et al., 2018; Trubl et al., 2018) with the number of uncultivated virus genomes greatly surpassing the number of sequenced virus isolates in publicly available databases (Roux et al., 2019a). Such studies demonstrate that metagenomic analysis of a single environmental gradient has the ability to greatly expand the knowledge of terrestrial viruses. It also emphasizes the importance of including viral abundance and viral community structure in studies to fully understand the dynamics of soil ecosystems in response to environmental changes.

The objective of this study was to determine whether there were temporal changes in virus and their potential bacterial host abundance in three differently managed Oklahoma soils. Experimental sites included a native tallgrass prairie (never tilled or cultivated), conventional till (CT) winter wheat, and no-till (NT) canola. By using data from multiple sites, we also aimed to determine whether the abundance of the viral communities was affected by increasing amounts of land management by examining the influence of soil and environmental factors on VLP abundance over the 1-year sampling period. Furthermore, metagenomic analysis was used to examine the impact of land use on viral community structure in soils of the native prairie and CT cropland, which aimed

to examine whether viral abundance or community composition played a larger role in the observed changes in viral populations. Our results indicated that soil properties, plant canopy cover, and environmental factors such as air temperature, most of which are further controlled by land use and land management practices, are important in shaping virus-host interactions, along with virus and host abundance.

4.3 Materials and Methods

4.3.1 Sample sites

Soil samples were collected at the U.S. Department of Agriculture, Agricultural Research Service, Grazing Research Laboratory in El Reno, OK (35º34.19N, 98º 03.69W; 414 m above sea level), from August 2016 to October 2017. Samples were taken approximately every 4 weeks from a native tallgrass prairie (35º32.99N, 98º02.29W; 64 ha), conventional till (CT) winter wheat (35º34.19N, 98º03.39W; 27.5 ha), and no-till (NT) winter canola (35º34.07N, 98º03.5W; 20.5 ha). The croplands and prairie sites were ~2.7 km apart. The native tallgrass prairie was native, mixed species grassland managed by cattle grazing several months out of the year and spring burns on a 4-year rotation with the most recent burn occurring in 2014. The soil was classified as Norge loamy prairie (fine, mixed, thermic Udertic Paleustalf) with a high-water holding capacity and a depth of >1 m (Bajgain et al., 2018). Winter wheat fields represent a cool season crop that dominates in central Oklahoma in areas where tallgrass prairies have been converted to croplands. The soil type at the croplands was characterized as Bethany silt loam (fine, mixed, superactive, thermic Pachic Paleustolls) (Peterson et al., 2019). In Oklahoma, winter wheat fields are managed for multiple purposes (grain production and

cattle grazing). The CT wheat field was managed for grain production (grain-only) during the 2015-2016 growing season and graze-out wheat (no grain production; cattle grazing from November through May) during the 2016-2017 growing season. Each year the seedbed was prepared for planting using a chisel plow treatment to a depth of 31 cm, which resulted in complete disturbance of soil and residue mixing (Peterson et al., 2019). The NT cropland field was grain-only wheat during the 2015-2016 growing season and on canola rotation during the 2016-2017 growing season. No-tillage treatment was initiated in 2015 only (just a year prior to this experiment). Detailed management data have been previously published (Wagle et al., 2019). For each soil sampling time point, eight cores roughly 20 m apart were taken in a random walking pattern throughout each field at a depth of 0 to 15 cm using a 2.5-cm-diameter soil probe. Soil cores were pooled and homogenized to deal with soil heterogenicity and sieved to 2 mm to remove debris prior to analysis. Soils were kept on ice and directly transports to the lab where they were kept at 4ºC for virus extraction, while soils for bacterial and chemical analysis were stored at -80ºC. Samples for virus extraction were stored for a maximum of 48 h before processing. Not all soils were used in every experiment.

4.3.2 Environmental, soil, and plant data

Weather data were gathered from the Oklahoma Mesonet station (http://www.mesonet.org/index.php/weather/local/elre) in El Reno (ELRE), OK. The Mesonet tower is located on the native tallgrass prairie used in this study at 35º32.99N and 98º02.29W. Data used from Mesonet measurements included average rainfall, maximum air temperate, average air temperature, and minimum air temperature. Similar

weather data for croplands were collected from eddy covariance stations located in those fields. Soil chemical analysis was performed at the Oklahoma State University Soil, Water, and Forage Analytical Laboratory (http://soiltesting.okstate.edu/). Tests included topsoil nitrate, organic matter, total nitrogen, and ammonium. Gravimetric water content was determined by oven drying for 24 h at 65ºC or until the weight no longer changed (Peterson et al., 2019). Leaf area index (LAI) was measured nondestructively using an LAI-2200C plant canopy analyzer (LI-COR Inc., Lincoln, NE), and the percent canopy cover (Canopy%) was determined using the Canopeo app. The aboveground biomass was collected destructively from five randomly located 0.5 x 0.5m^2 quadrats within each field at 2-week intervals during the active growing season. Dry biomass weights were recorded after drying samples in forced-air oven at 70ºC for a minimum of 48 h (Wagle et al., 2019).

4.3.3 Bacterial extraction and qPCR

Bacterial genomic DNA was extracted with a Quick-DNA fecal/soil microbe miniprep kit (Zymo Research, Irvine, CA) according to the manufacturer's protocol with the exception of eluting DNA with sterile water. For each pooled soil sample, four subsamples were used for extractions. DNA was quantified with a Quibit dsDNA BR assay kit (Thermo Fisher Scientific, Waltham, MA) as described by the manufacturer's instructions. DNA dilutions of 2 ng/µl were prepared to use for downstream analysis. qPCR was performed to estimate bacterial abundance based on the copy number of 16S rRNA genes using an Applied Biosystems 7300 real-time PCR system (Thermo Fischer Scientific). All four replicates were run for each sampling time point and collection site. PCR was performed

in a total volume of 30 µl that contained 15 µl of Power SYBR Green Master (Thermo Fisher Scientific), 2 µl of DNA template, and 100 nM concentrations of primers 27F and 519R (Lane et al., 1985; Weisburg et al., 1991). The qPCR thermocycling steps included 95ºC for 10 min, followed by 40 cycles of denaturation at 95ºC for 45 s, annealing at 55ºC for 45 s, and extension at 72ºC for 1 min. The C_T (threshold cycle) and 10-log-fold standard curves were used to estimate bacterial abundance in soils by converting C_T values into estimates of bacterial cells present in 1.0 g of soil in each technical replicate. The amount of template DNA used for qPCR and the amount of soil used for each DNA extraction were accounted for in abundance estimates. Estimates were then converted to cells per gram of dry weight. Negative controls had no detectable amplification.

4.3.4 Virus-like particle extraction

Viruses were extracted from soil samples using an adaptation of the method by Williamson et al. (Williamson et al., 2005; Williamson et al., 2003). In short, 5.0 g of fresh soil was weighed into acid-cleaned 50-ml glass test tubes containing 15 ml of sterilized potassium citrate buffer (10 g of potassium citrate, 1.44 g of Na_2PO_4, and 0.25 g of $KHPO_4$ [pH 7.0] per liter) stored at 4ºC. Viruses were mechanically separated from soil samples through sonication. Each tube was sonicated using a Branson 5510 ultrasonic for a total of 10 min with vortexing at high intensity for 20 s every 2 min. Samples were centrifuged at 8,000 *x g* for 25 min at 4ºC to sediment large soil particles. Supernatants were filtered through a 0.2-µm syringe filter (GE Healthcare Life Sciences, Marlborough, MA) to remove bacteria and other large particles, and the filtrate was

collected into sterile 15-ml polypropylene tubes and stored at 4ºC. Three subsamples were used for VLP extraction from each composite field sample.

4.3.5 Epifluorescence microscopy quantification of VLPs

For VLP enumeration, 1 ml of viral extract that had been diluted at a 1:4 ratio with sterile deionized water was vacuum filtered through a 0.02-µm Anodisc filter (25mm diameter, Whatman International, Ltd., Maidstone, England). A 0.45-µm filter (Pall Life Sciences, Port Washington, NY) was used for support. Anodisc filters were stained with 500 µl of 2.5 *x* SYBR gold (Invitrogen/Thermo Fisher Scientific, Waltham, MA) in the dark for 15 min. Excess SYBR gold was vacuumed through, and filters were washed with 1 ml of sterilized TE buffer. Filters were then mounted on glass slides using 30 µl of antifade solution on the coverslip (Williamson et al., 2003) and analyzed by epifluorescence microscopy using an Olympus BX61 motorized system microscope with an attached DP71 digital camera (Olympus Corp., Center Valley, PA). Three slides in total were made for each field and time point, one from each replicate extraction. The number of VLPs were counted manually in 10 fields per slide at *x* 1,000 magnification. The average VLP counts were calculated from the grand mean of the replicate filters per gram of dry soil (Williamson et al., 2005; Williamson et al., 2003).

4.3.6 Virus dsDNA extraction and sequencing

Large soil samples (~500 g) were collected from the native tallgrass prairie and conventional tillage winter wheat site in October 2017 for viral DNA extraction. Using 200 g of fresh soil per field, soil samples were treated as described above to extract VLPs

for the purpose of DNA extraction. VLPs were then pelleted using an Optima LE-80K Ultracentrifuge (Beckman Coulter, Brea, CA) and a SW 28 Ti swinging bucket rotor at 50,000 rcf for 2 h at 4ºC in thin-wall, Ultra-Clear, 38.5-ml centrifuge tubes (Beckman Coulter). For each soil sample, six tubes containing 0.2-µm-filtered supernatant were centrifuged. Pellets were resuspended and combined in 200 µl of potassium citrate buffer. Samples were treated with DNase (100 U/ml) to remove any free contaminant DNA before lysing the virus particles (Lopez-Bueno et al., 2009). DNase reactions were stopped by incubating samples at 65ºC for 10 min in the presence of 0.5 M EDTA. Viruses were lysed using 1 volume formamide, 0.1 volume 2 M Tris-Cl, and 0.05 volume 0.5 M EDTA at 37ºC for 30 min (Sambrook and Russel, 2001b). DNA was then collected by PEG precipitation as described by Sambrook and Russell (Sambrook and Russel, 2001a). Pelleted DNA was resuspended in 200 µl of sterile water. dsDNA was extracted by using a Quick-DNA fecal/soil microbe miniprep kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions with the exception of removing the bead-beating lysis step. DNA was quantified using a Quibit dsDNA BR assay kit (life Technologies/Thermo Fisher Scientific) as described by the manufacturer's protocol. DNA was sequenced using Illumina HiSeq PE150 technology at the Oklahoma Medical Research Foundation.

4.3.7 Bioinformatic analyses

Raw reads for each metagenome were evaluated for quality using FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and duplicates were removed by using CD-HIT (Li and Godzik, 2006). Reads were then quality trimmed and

filtered using the NGCS QC Toolkit (Patel and Jain, 2012). IDBA_UD (Peng et al., 2012) was used for metagenome assembly using default parameter and keeping contigs 500 bp or larger. Using CyVerse, assemblies were processed with VirSorter to determine viral sequences using the Virome database (Roux et al., 2015). Sequences from VirSorter categories 1, 2, 4, and 5 were kept (Emerson et al., 2018; Roux et al., 2015). Contigs of >10 kb were selected and clustered into vOTUs using the CyVerse app ClusterGenomes (v1.1.3) with the parameters 95% average nucleotide identity and 80% alignment fraction of the smallest contig (Roux et al., 2016a). vOTU relative abundance was estimated by mapping reads using Bowtie2 (Langmead and Salzberg, 2012) with multimapping and zero mismatches (Daly et al., 2019). vOTUs were only considered present in a sample if at least 75% of a contig was covered. To normalize each data set for comparison, the total number of base pairs mapped were divided by the vOTU sequence length and divided by the total number of base pairs in the metagenome (Trubl et al., 2018). Bubble plots of the relative abundance of vOTUs was constructed using ggplot2 in R version 3.6.1 (R Core Team, 2020). Taxonomic classifications were determined by vContact2 by producing viral clusters (VCs) based on viral predicted proteins with pairs of sequences with a similarity score of >1 being clustered into viral clusters (Bolduc et al., 2017; Daly et al., 2019; Roux et al., 2016a). Reference sequences that coclustered with soil viral sequences from the present study were used to predict the taxonomy using the last common ancestor approach and if the taxonomy of reference genomes within a VC differed, majority rule was used (Daly et al., 2019). The network was then visualized and imaged using Cytoscape v3.8.0 (Shannon et al., 2003). MetaProdigal was used to predict open reading frames (ORFs) for the shared vOTUs. The predicted proteins were then compared the

viral RefSeq database using a minimum bitscore of 50 using blastp. Protein searches were also done using NCBI virus (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/), which includes virus sequences not available in the RefSeq database. Up to the top 500 query results for each ORF was compiled into a custom database, and blastp was used again to compare the proteins to the custom database. Results from both searches were compared to determine the best match for each gene prediction.

4.3.8 Statistical analysis

Principal-component analysis was performed using soil chemistry data for the three collection sites in R version 3.6.1 (R Core Team, 2020). To test for significant differences of soil chemistry between sites, data were checked for normality than analyzed using the aovp function in the lmPerm R package. Differences were considered significant base on a P value of < 0.05. T-tests were used to compare plant biometrics data for the fall and spring growing season. Spearman correlations were calculated using the cor.test function to determine the relationship between viral abundance, microbial abundance, soil properties, and other environmental factors. Correlations were done separately for each field due the difference in soil chemistry for each sampling site. The rho value for moderate to very strong correlations range from 0.4 to 1.0, while significant correlations were determined by a P value of ≤ 0.05. Relationships for the abundance, soil chemistry, and air temperature were further examined with structural equation modeling (SEM) using the lavaan package in R. Tallgrass prairie data were treated as the control with CT and NT management were used as treatments.

4.3.9 Data availability

Raw metagenomic data for each viral metagenome was deposited in the Sequence Read Archive (SRA) database under BioProject accession number PRJNA669149.

4.4 Results

4.4.1 Soil, plant, and environmental properties

Land use and land management had considerable impact on soil properties (Figure 4.1). All measured soil properties were significantly different between at least one set of sites ($p < 0.05$). Significant differences between field comparisons varied with specific soil properties. Organic matter (OM) and total nitrogen (TN) were significantly different ($p < 0.001$) in pairwise comparisons between all fields. OM and TN levels in soil decreased with increasing levels of management input. Croplands had significantly higher ($p < 0.05$) topsoil nitrate (TopN) compared to the native tallgrass prairie. The CT wheat field had TopN of 49 kg ha^{-1} on an average and was as high as 160 kg ha^{-1}. The NT canola field had higher level of TopN (37 kg ha^{-1}) compared to the native tallgrass prairie (13.5 kg ha^{-1}) on average. Ammonium (NH_4^+) levels were only marginally significantly lower ($p = 0.059$) in the NT cropland (13.9 kg ha^{-1}), while NH_4^+ levels were only slightly greater in the CT cropland at 24.0 kg ha^{-1} than in prairie soil at 22.4 kg ha^{-1}, on average. Nitrogen fertilizer was applied in both croplands during planting, while native prairie was not fertilized.

From August 2016 to September 2017, monthly rainfall ranged from lows of 14.99 mm during November 2016 and highs of 227.08 and 252.22 mm during April 2017 and August 2017, respectively. Over the growing season of wheat and canola

from October 2016 to May 2017, no severe drought was observed, with the sites receiving ~508 mm of rain. Overall, CT wheat had the lowest soil water content ($p <$ 0.001) of all the fields. The tallgrass prairie had soil water content (SWC) of 18% and the NT cropland SWC was 17% on average, whereas the CT cropland site had an average of 10% with SWC as low as 3%. However, lower SWC was recorded during winter (dormant period for the crops).

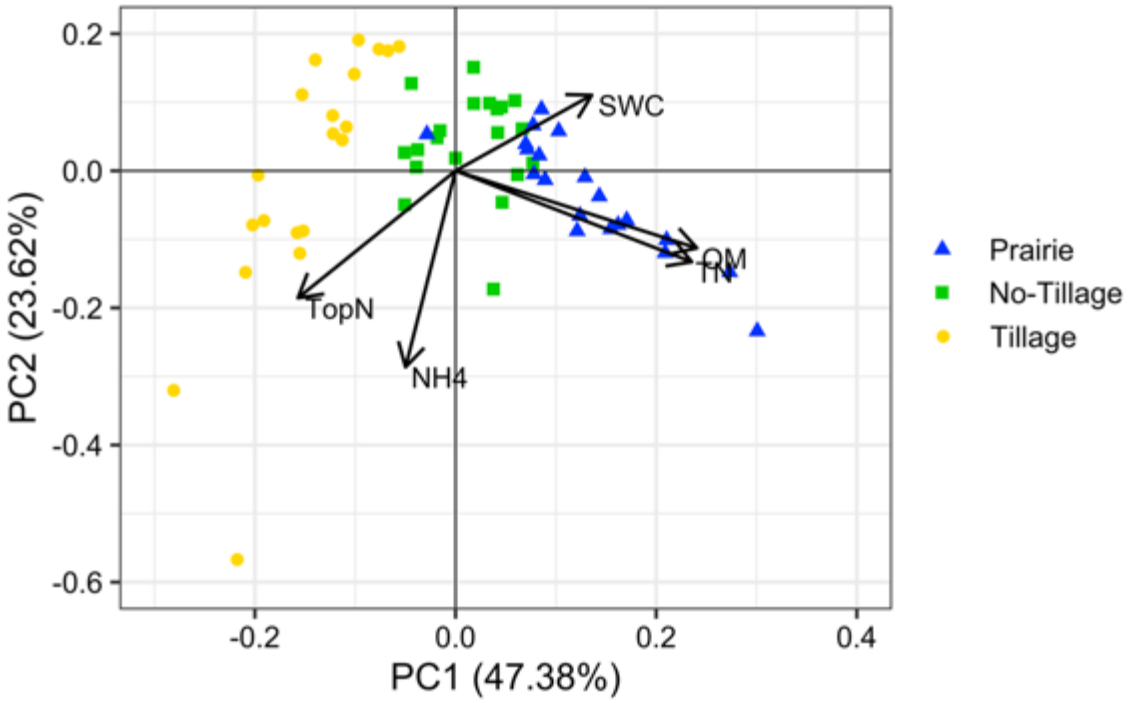

Figure 4.1 Comparison of soil properties that significantly varied between land use and land management based on principal component analysis. Study sites include native tallgrass prairie, no-till canola, and conventional till wheat. Soil properties data were collected from August 2016 to July 2017.

Air temperature reached a maximum during the summer months in August 2016

and July 2017 with minimum air temperatures during winter in December and January. Annual dynamics of soil temperature varied with sites and growing seasons, often differing based on land use type due to contrasting seasonality of crops and native prairie. Wheat was planted on September 12 and grazed from 15 November 2016 to 9 May 2017. Canola was planted on 3 October 2016 and harvested in June 2017. Plant biometrics measurements were taken during the fall 2016 and spring 2017 at both croplands. Higher values of leaf area index (LAI), biomass, and canopy cover percentage were observed before and after winter since both crops were dormant during winter. By mid-November, LAI reached ~5 m^2m^{-2} for canola and ~3 m^2m^{-2} for wheat, while canopy cover percentage was > 95 for canola and > 80 for wheat. Vegetation growth in croplands increased again with increasing air temperature in spring, with canopy cover percentage > 70 in both fields and LAI of ~3 and 3.5 m^2m^{-2} for canola and wheat, respectively, in April. Native prairie vegetation greened up in April and entered into senescence phase at the end of October. Croplands had higher soil temperatures during the summer compared to the tallgrass prairie because croplands were left fallow from June to September, while summer was peak growing season for the prairie.

4.4.2 Temporal dynamics of VLP abundance and its influencing factors

The VLP abundance was substantially altered due to land use and land management practices ($p < 0.0001$). Over the sampling period, VLP abundance ranged from 2.63 x 10^8 to 2.51 x 10^9 VLP g^{-1} dry weight among the three sites (Figure 4.2a). The greatest difference in abundance was observed between native prairie and CT wheat ($p < 0.001$).

There was also a significant difference for VLP abundance between native prairie and NT canola ($p = 0.001$) and both croplands ($p < 0.05$). The average abundance was 1.66×10^9 VLP g^{-1} in prairie soil, 1.01×10^9 VLP g^{-1} in NT canola, and 5.75×10^8 VLP g^{-1} in CT wheat. The tallgrass prairie had the greatest VLP abundance during all sampling months. The CT wheat had the lowest abundance except for July (fallow period) where VLP abundance was greater than that of the NT canola. Seasonal variations were observed with significant changes in abundance related to sampling month ($p < 0.01$) at all sampling sites. The shifts detected in the croplands overall followed the same seasonal dynamics with lower abundance observed during winter (December through February), and peak VLP abundance in March (i.e., the period of rapid vegetation growth with rise in temperature). This was supported by the most pairwise significant differences ($p < 0.05$) being observed for January and February in the winter and March and April in the spring. Prairie soil also had lower VLP abundance in February and elevated VLP abundance during the spring months, March through May (i.e., greening up and rapid growth of prairie vegetation) that was further supported significant pairwise difference ($p < 0.05$) in abundance between sampling months. The highest standard deviation was observed in the tallgrass prairie site at 4.80×10^8 VLP g^{-1}. In comparison, croplands had lower standard deviations of 4.31×10^8 VLP g^{-1} and 2.88×10^8 VLP g^{-1} at NT canola and CT wheat, respectively.

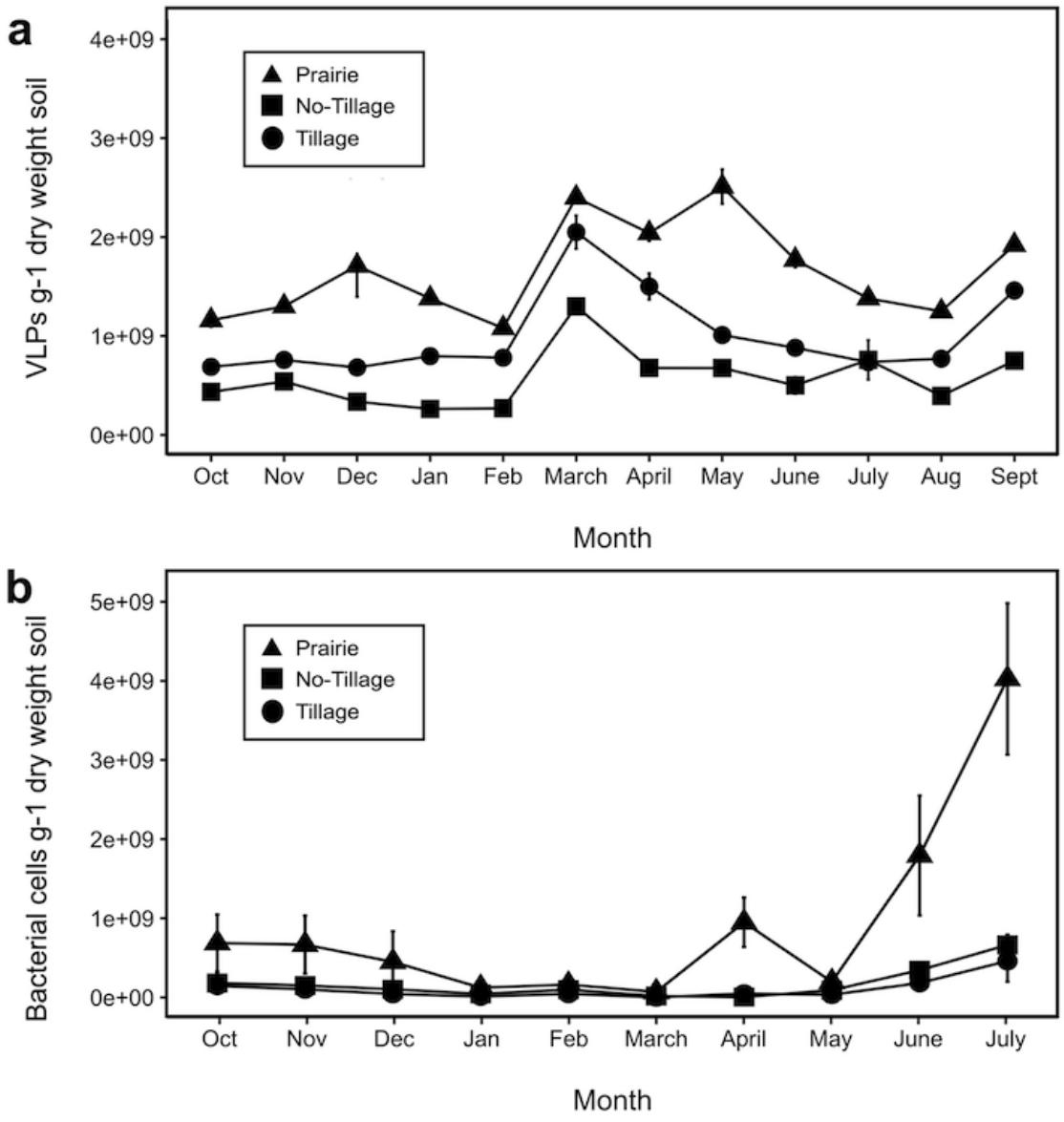

Figure 4.2 VLP and bacterial abundance between different land usage and land management. (a) VLP abundance over a 1-year sampling period from October 2016 to September 2017. VLP abundance was calculated based on the dry weight of soil. (b) Bacterial cell abundance at corresponding sampling dates for VLP samples. Only time points of bacterial abundance that overlap with VLP abundance are shown in the figure.

Spearman correlations were calculated to determine which soil, plant, and environmental factors potentially influenced VLP abundance for individual sites (Table 4.1). No highly significant correlations were observed between tallgrass prairie

parameters and VLP abundance. SWC and VLP abundance at the prairie site had the strongest correlation, but it was not significant (rho 0.40, $p = 0.0993$). The NT canola had a significant negative correlation between VLP abundance and TopN (rho - 0.65, $p = 0.0204$), and highly significant positive relationship between VLP abundance and leaf area index (rho 1.00, $p < 0.001$). The VLP abundance had correlations with several different factors at the CT wheat field. The VLP abundance showed a moderately strong correlation with air temperature (rho 0.49, $p = 0.0531$), and significant positive correlations with plant biometrics such as plant biomass, leaf area index, and canopy height. The only significantly negative correlation (rho - 0.56) was overserved between ammonium and VLP abundance at the CT wheat field.

Table 4.1 Influence of soil, plant, and environmental factors on VLP abundance within fields based on Spearman correlations

	Native Tallgrass Prairie		**No-Till**		**Conventional Till**	
	Rho	***p***	**Rho**	***p***	**Rho**	***p***
Topsoil Nitrate	0.01	0.5101	**-0.65**	**0.0204**	-0.37	0.1492
Organic Matter	0.14	0.6504	-0.03	0.4669	0.15	0.3438
Total N	0.05	0.5539	-0.04	0.4527	-0.04	0.4561
NH_4^+	0.07	0.4206	-0.12	0.6243	**-0.56**	**0.0449**
SWC	*0.40*	*0.0992*	0.04	0.4485	0.28	0.2215
Avg Rain	0.22	0.2596	-0.09	0.3952	-0.09	0.3952
Min Temp	0.09	0.3892	0.06	0.4314	*0.43*	*0.0834*
Avg Temp	0.10	0.3767	0.03	0.4656	*0.49*	*0.0531*
Max Temp	0.02	0.4785	-0.01	0.5086	*0.48*	*0.0591*
Avg Soil Temp	0.12	0.3685	-0.18	0.3508	0.47	0.1027
Plant Biomass	-	-	0.40	0.3000	**1.00**	**< 0.001**
LAI	-	-	**1.00**	**< 0.001**	**1.00**	**< 0.001**
Canopy Cover	-	-	-0.50	0.3333	0.50	0.3333
Canopy Height	-	-	-	-	**1.00**	**< 0.001**

Correlation coefficients with $p < 0.05$ are indicated in bold, coefficients with $0.1 > p > 0.05$ are indicated in italics. Dashes (-) represent missing data.

Topsoil nitrate (kg/ha), organic matter (%), total nitrogen (%), NH_4^+ (kg/ha), gravimetric soil water content (%), daily average soil temperature at 6 cm depth, leaf area index (LAI), canopy height (cm), canopy cover (%), dry plant biomass (kg/m^2).

Structural equation modeling (SEM) was used to further estimate the direct and indirect relationships between the soil variables and VLP abundance. SEM results were similar to those observed in the Spearman correlations (Figure 4.3). The VLP abundance was indirectly influenced by land management practices that directly influenced TopN, and SWC. Bacterial abundance also had significant positive influence on the overall VLP abundance ($p = 0.034$). TopN was positively influenced by NH_4^+, air temperature, and land use, while SWC had a negative effect on nitrate levels. Lastly, SWC had significant negative relationships with land management and average air temperature with tillage land use having the strongest effect.

4.4.3 Temporal dynamics of bacterial abundance and its influencing factors

Bacterial abundance was significantly different ($p \leq 0.001$) among the sites ranging from 10^5 to 10^9 bacterial cells g^{-1} dry weight (Figure 4.2b). Bacterial abundance was significantly less in CT wheat ($p \leq 0.001$) and NT canola ($p \leq 0.05$) than that of the tallgrass prairie. On average, the tallgrass prairie had an abundance of 6.87×10^8 cells g^{-1} dry weight, followed by NT canola (1.37×10^8 cells g^{-1} dry weight) and CT wheat (7.50×10^7 cells g^{-1} dry weight). Both croplands had lower standard deviations (i.e., 1.88×10^8 cells g^{-1} dry weight for NT canola and 1.41×10^8 cells g^{-1} dry weight for CT wheat) than that of the tallgrass prairie (1.02×10^9 cells g^{-1} dry weight). Significant seasonal shifts ($p < 0.05$) were detected at all sampling sites. In the tallgrass prairie, bacterial abundance in spring was significantly different from that of summer and winter, while June and July were the most significantly differently from other sampling months. Spring was also significantly different than summer and winter in the NT canola site along with

significant differences between bacterial abundance during summer and fall. Significant seasonal differences only occurred in bacterial abundance during the summer for CT wheat.

Table 4.2 Influence of soil, plant, and environmental factors on bacterial abundance within fields based on Spearman correlations

	Native Tallgrass Prairie		**No-Till**		**Conventional Till**	
	Rho	***p***	**Rho**	***p***	**Rho**	***p***
Topsoil Nitrate	**0.40**	**0.0392**	0.06	0.3930	0.13	0.2975
Organic Matter	**0.45**	**0.0235**	**0.53**	**0.0079**	**0.71**	**0.0002**
Total N	**0.49**	**0.0143**	**0.50**	**0.0125**	**0.69**	**0.0003**
NH_4^+	0.16	0.2448	0.04	0.4399	-0.05	0.4177
SWC	-0.07	0.3908	*-0.32*	*0.0821*	**-0.53**	**0.0082**
Avg Rain	-0.01	0.5172	-0.31	0.1775	-0.32	0.1701
Min Temp	0.24	0.1550	-0.03	0.5501	-0.04	0.5600
Avg Temp	0.17	0.2310	-0.11	0.6775	-0.13	0.7044
Max Temp	0.19	0.2118	-0.13	0.7131	-0.17	0.7651
Avg Soil Temp	0.21	0.1900	**0.68**	**0.0469**	0.21	0.2322
Plant Biomass	-	-	-0.21	0.3233	0.07	0.5605
LAI	-	-	**0.71**	**0.0454**	0.29	0.7327
Canopy Cover	-	-	**0.66**	**0.0481**	0.37	0.2342
Canopy Height	-	-	0.10	0.5636	-0.49	0.1644

Correlation coefficients with $p < 0.05$ are indicated in bold, coefficients with $0.1 > p > 0.05$ are indicated in italics. Dashes (-) represent missing data.
Topsoil nitrate (kg/ha), organic matter (%), total nitrogen (%), NH_4^+ (kg/ha), gravimetric soil water content (%), daily average soil temperature at 6 cm depth, leaf area index (LAI), canopy height (cm), canopy cover (%), dry plant biomass (kg/m^2).

Correlation analysis was performed to examine the relationship between the soil and environmental factors in relation to bacterial abundance for each sampling site Table 4.2). All fields had significant correlations to at least one factor, and the correlations differed from those observed in comparison to VLP abundance. For the native prairie, bacterial abundance had significant positive correlations of moderate strength to TopN, OM, and TN (rho 0.40, rho 0.45, and rho 0.49, respectively). Both croplands had a

significant positive correlation between bacterial abundance, soil organic matter, and total nitrogen. At the NT canola, bacterial abundance also had a positive significant relationship with soil temperature, leaf area index, and canopy cover. SWC showed a negative relationship with bacterial abundance in NT canola soil (rho - 0.32, $p = 0.0821$) and a significant negative effect in CT wheat soil (rho - 0.53, $p = 0.0082$).

The SEM revealed similar results as observed from Spearman correlations (Figure 4.3). Several factors appeared to have an influence on bacterial and VLP abundance. Land use had direct significant impact ($p < 0.001$) on bacterial abundance that was not observed for VLP abundance. Land use also had indirect impacts on bacterial abundance by significantly directly impacting NH_4^+, TopN, and OM, which further influenced bacterial abundance. In addition, average air temperature had a significant positive interaction with bacterial abundance. While bacterial abundance had a positive impact on VLP abundance, VLP abundance had a significant direct negative impact on bacterial abundance ($p = 0.001$).

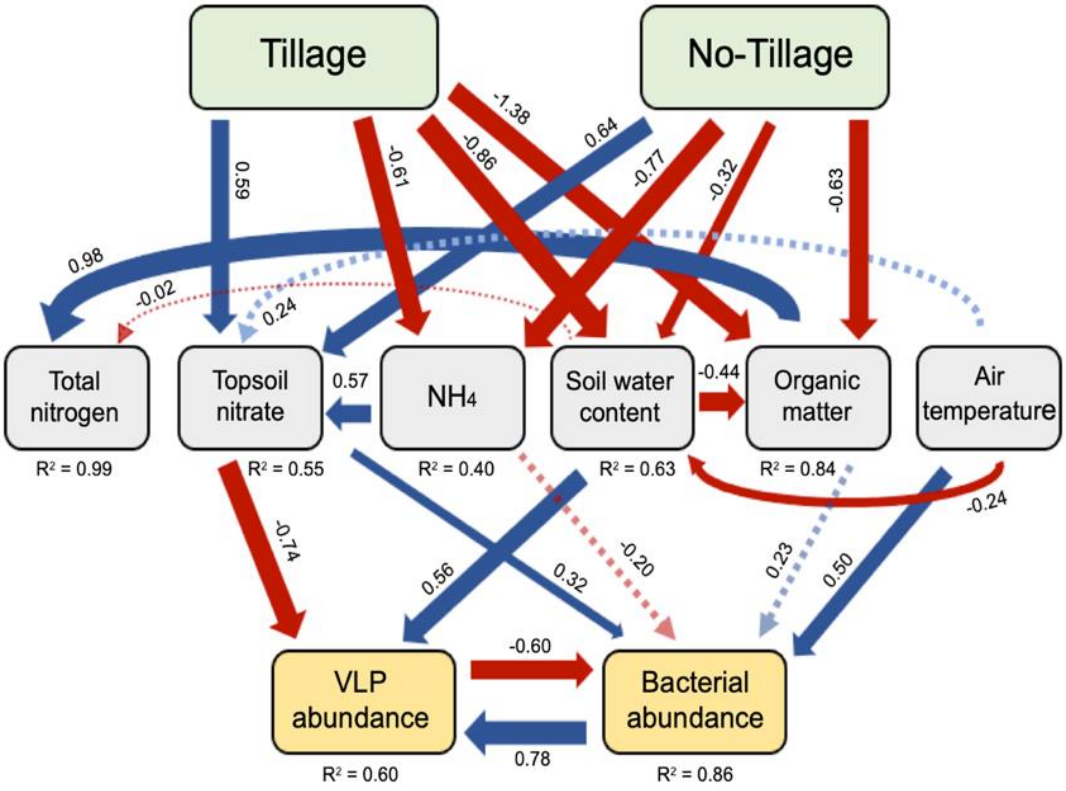

Figure 4.3 Relationship between VLP abundance, bacterial abundance, land management, and soil and environmental factors based on structural equation modeling. Solid arrows indicate factors that had p-values of < 0.05. Dashed arrows indicate factors with marginal nonsignificant relationships ($p < 0.1$). Red arrows represent negative relationships, while blue arrows represent positive relationships. Native tallgrass prairie was used as the control, with the two management practices acting as treatments.

4.4.4 Differences of DNA viral communities between tallgrass prairie and tilled wheat field

The tallgrass prairie and CT wheat field soils were chosen for metagenomic sequence analysis as they had the greatest differences in VLP abundance, bacterial abundance, and differed the most as far as management input. DNA viral genomes were extracted from purified filtrate enriched with virus particles and sequenced using Illumina technology. A large amount of soil per sample was used for virus extractions

and DNA concentrated to avoid amplification methods that might bias sequencing results (Kim and Bae, 2011). Metagenome assemblies of viral reads showed observable differences between two sites. The prairie soil virome consisted of 657,863 contigs and the CT wheat field soil virome included 274,051 contigs (Table 4.3). VirSorter predicted 375 contigs from the CT wheat assembly and 6,856 contigs from the prairie assembly to be possible viruses (≥ 1 kb). In total, 1,272 viral contigs were over 10 kb, which were used to determine viral operational taxonomic units (vOTUs). For the two data sets, only a little over 3% of the sequences formed clusters with more than one sequence, resulting in 1,231 vOTUs based on 95% average nucleotide identity (ANI) and 80% alignment fraction relative to the shorter sequence. Although the prairie assembly was three times larger than the CT wheat assembly, it had roughly 10-fold more vOTUs identified in the soil virome (Figure 4.4a). The majority of the vOTUs were unique to land use type with only five vOTUs shared across assemblies. The relative abundance of the shared vOTUs also differed between the land use types (Figure 4.4b). When one of the shared vOTUs was abundant in the CT wheat virome the abundance was reduced in the prairie. The opposite was true as well with vOTUs abundant in the prairie virome being rare in the CT wheat virome. While the richness and Shannon's diversity index were greater in the prairie, the evenness of the community based on Pielou's evenness index was relatively similar in the prairie and CT wheat field: 0.925 and 0.908, respectively.

Table 4.3 Summary of soil viral metagenomes

	Total no. contigs	Total bp	Max contig length (bp)	N50	VirSorter ≥ 10kb	Total no. vOTUs
Tallgrass prairie	657,863	831,434,430	227,057	1,450	1,145	1,231
Tillage wheat (CT)	274,051	260,104,506	350,802	905	127	

Virome assembly data provided only includes contigs at least 500 bp in size. VirSorter results represent contigs ≥ 10kb that were identified as possible viruses from categories 1, 2, 4, and 5. Size selected sequences were then used to cluster viral OUTs using 95% average nucleotide identity and 80% alignment fraction.

vOTUs were grouped into viral clusters (VCs) that were used to predict taxonomy of the viral sequences collected from soils in El Reno, OK. Together, the data will be referred to as the El Reno viruses or vOTUs based on the soil collection location. VCs roughly represent genus-level taxonomy of sequences grouped with a similarity score of at least 1 as previously described (Daly et al., 2019). Relationships of VCs, including the El Reno vOTUs in comparison to sequences in the RefSeq viral database, are presented in a gene-sharing network (Figure 4.5). Of the VCs formed, 34% contained vOTUs from the tillage and prairie soil. The majority of the clusters containing El Reno viruses did not cluster with known viruses in the database and instead formed VCs with sequences in their own data set. Five of the VCs consisted of known viruses from the RefSeq database and El Reno vOTUs. VC_15 consisted of three subclusters, one of which contained all El Reno viruses, and all subclusters grouped closely in the network meaning taxonomically the vOTUs most likely are the same at the family level but not genus-level. The 66 vOTUs in VC_15 were identified as belonging to the family Siphoviridae. One vOTU each belonged to VC_20 and VC_50 belonging to the genera Xp10virus and Ydn12virus,

both in the Siphoviridae family, respectively. VC_135 belonged to Ssp2virus containing two vOTUs and VC_140 belonged to Pepy6virus containing 15 vOTUs. Only one vOTU within VC_118 was identified in the Myoviridae family belonging to the genus Msw3virus. Another 14 VCs containing 76 vOTUs consisted of El Reno viruses that clustered with unclassified viruses in the RefSeq database. The rest of the vOTUs either clustered with other samples in the El Reno data set or were identified as singletons (no significant shared similarity to other protein sequences) or outliers.

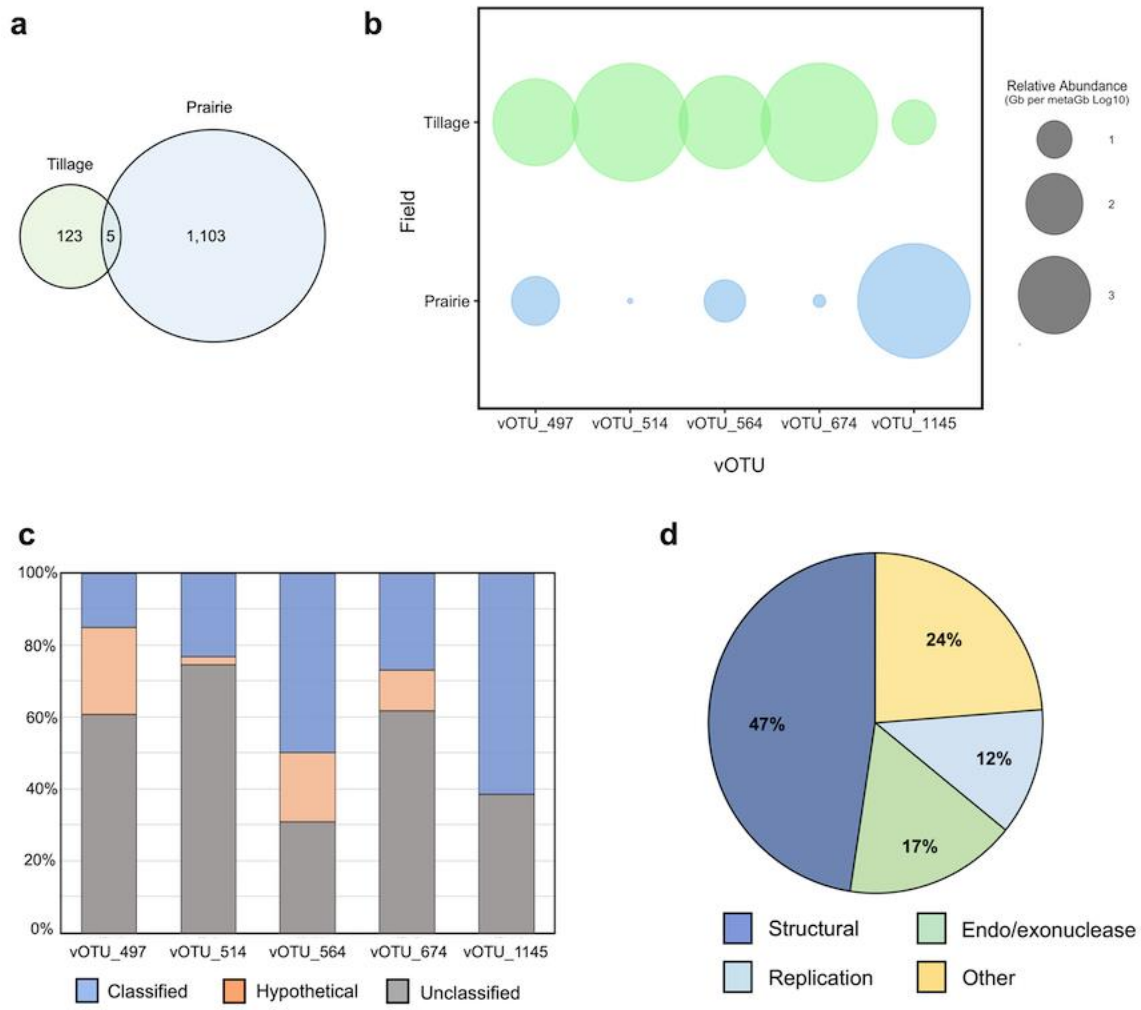

Figure 4.4 Overlap of viral community structure in soils from different intensities of land management. vOTUs were only considered to be present in an assembly if the vOTU had at least 75% sequence coverage. (a) Depiction of vOTUs in each virome and the number of shared vOTUs between the native and tillage viromes. (b) Bubble plot of the relative abundance of the shared vOTUs in the two viromes. vOTU abundance was normalized by the vOTU length and size of the individual assemblies then standardized by the minimal size of metagenomes (bp) across all samples. (c) Bar graph of portion of predicted genes identified in shared vOTUs. Unclassified genes had no high-quality matches in currently database. (d) Main groups of genes represented from shared vOTUs based on currently available virus protein sequences.

Viral OTUs shared between the tallgrass prairie and CT wheat field were compared to known viral sequences to try to determine gene function. Protein coding genes predicted per contig ranged from 13 to 43, with 28 genes predicted on an average. The majority of the predicted genes for the shared vOTUs were not able to be classified or associated with proteins that had no functional identification (Figure 4.4c). Of the 141 predicted proteins, 42 were identified and 17 were classified as hypothetical proteins. When looking at the classified viruses, the majority were identified as structural proteins including capsid, tail, and baseplate proteins (Figure 4.4d). Proteins related to replication mainly consisted of DNA polymerase and helicase, and endo- and exonucleases made up 17% of the predicted genes. Proteins classified as other did not have enough genes predicted to form clear groups, but several notable proteins include DNA methyltransferase, phage integrase, and hydrolase.

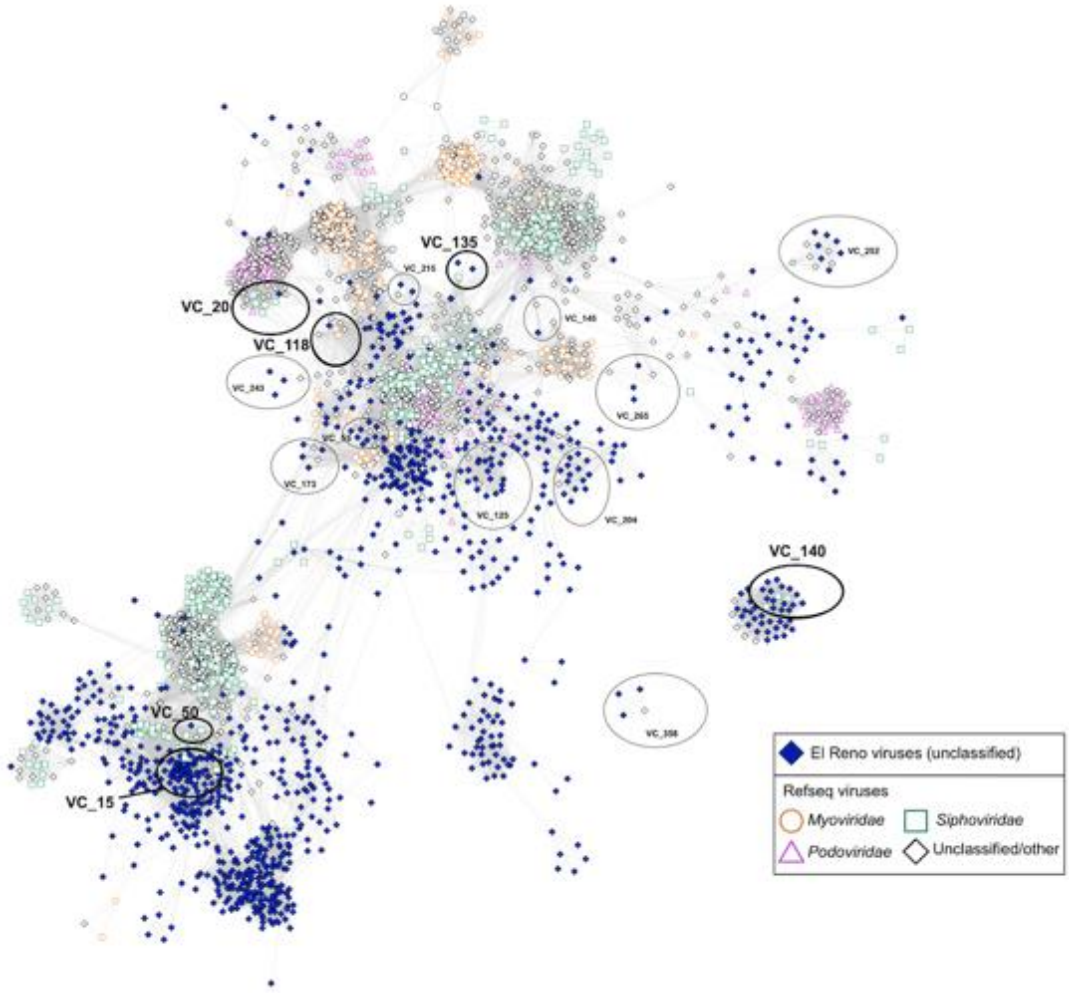

Figure 4.5 Gene sharing network of El Reno vOTUs clustered with RefSeq viral sequences. Solid colors indicate sequences from El Reno virus data set. Nodes are depicted as shapes of various colors that correspond to virus families within the RefSeq database. El Reno viruses in viral clusters that clearly grouped closely in the network on circled and labeled. Clusters in bolded circles represent those with El Reno viruses that could be taxonomically identified with the family-level taxonomy depicted. Viral clusters that contained only El Reno vOTUs and that did not interact with the main network are not pictured.

4.5 Discussion

4.5.1 Virus abundance.

In this study, we investigated VLP abundance over a 1-year period in soils from different land use and management intensities. VLP abundance ranged from 10^8 to 10^9 (g^{-1} dry soil) during the sampling period being consistent with previous research that showed abundance ranging from 10^7 to 10^9 (g^{-1} dry soil) from soils of differing land uses and times of the year (Ashelford et al., 2003; Narr et al., 2017; Williamson et al., 2013; Williamson et al., 2005; Williamson et al., 2003; Yu et al., 2018). Specifically in agricultural soils, VLP abundance has been observed between 1.0 x 10^8 to 1.1 x 10^9 g^{-1} dry soil (Narr et al., 2017; Roy et al., 2020; Williamson et al., 2005; Williamson et al., 2003), which is comparable to the average VLP abundance observed in the croplands in this study. Based on the average VLP abundance for sampling sites, abundance decreased with increasing amounts of land management. The tallgrass prairie had the highest VLP abundance and CT winter wheat soil had the lowest VLP abundance except during one collection month when abundance spiked above that of the NT canola soil (Figure 4.2a). According to management data, this is potentially reflective of soil tillage a few weeks prior to the collection that took place during the fallow period. Tillage is a physical disturbance to soil systems that increases soil erosion, loss of soil organic carbon, and loss of aggregate stability (Lal et al., 2007). However, tillage has also been shown to accelerate microbial activity (Young and Ritz, 2000), and increased virus abundance is often thought to occur when there are increased bacterial host activity and abundance in the system (Kimura et al., 2008; Maranger and Bird, 1995). This idea was supported by the SEM results of this study where bacterial abundance had a direct

positive influence on VLP abundance, and bacterial abundance was directly affected by land use. While increased bacterial host activity and abundance could be responsible for increased VLP abundance in soil, it is not possible to distinguish whether the increased VLP abundance that was observed during certain times of the year was a result of active virus production or virus survival related to physical and/or biological factors (Williamson et al., 2005) in specific soil systems. However, in general, viruses are often impacted by similar factors as their hosts, which affects virus-host interactions (Hurst et al., 1980).

The structural model based on VLP abundance from all study sites showed a clear relationship with nitrate and soil water content both of which were directly influenced by land use. Soil water content has been previously detected to influence virus abundance in other soil systems (Williamson et al., 2013; Williamson et al., 2003). The soil water content was higher in the prairie and NT canola than in CT wheat, both of which had greater VLP abundance. No-till management can reduce water evaporation from soil and increase water infiltration and soil water content due to plant residues on the soil surface. Survival and inactivation of viruses in soils is often strongly related to wetting and drying of soils (Kimura et al., 2008). Work examining virus survival has shown wetter soils result in virus persistence (Hurst et al., 1980; Straub et al., 1993). The CT wheat field had the lowest soil moisture throughout the year due to more water loss through evaporation from soil pores that are exposed directly to radiation. These dry conditions could further contribute to reduced counts in CT soils (Kimura et al., 2008). It is speculated that viruses are likely to passively distribute with water, and due to their size are expected to be present in micro- and nanoscale soil pores (Kuzyakov and Mason-Jones, 2018). The

increased presence of organic matter can improve soil water holding capacity and overall soil structure (Oades, 1984). The organic matter content of the soil is also considered to be linked to increased VLP abundance. While no direct interaction was found between OM and VLP abundance in this study, organic matter was directly influenced by land use and impacted bacterial host abundance (Figure 4.3). The greater levels of organic matter in prairie soil may further explain the increased virus survival, partially due to the input of fertilization from grazing cattle throughout the year. The CT wheat field also had grazing cattle for a portion of the year, but there was no observable effect on virus abundance. Other more intense management practices likely had a greater overall impact on the virus community. Previous studies have reported agricultural soils having lower VLP abundance than nutrient-rich forest and pasture soils, both of which were associated with organic matter and water content (Narr et al., 2017; Roy et al., 2020; Williamson et al., 2005). Land use and management practices also greatly affect soil temperature. Although little is known about the persistence of autochthonous viruses in soils, laboratory incubation experiments introducing nonnative viruses to soil demonstrated temperature was a key factor controlling virus survival in soil with survival often being favored at cooler temperatures (Hurst et al., 1980; Straub et al., 1993; Yeager and O'Brien, 1979). Tallgrass prairies accumulate an enormous amount of biomass leading to thick groundcover in contrast to croplands where above ground biomass is removed yearly, exposing the soil. More groundcover is present in an NT system where residues are left on the soil surface compared to CT management where residues are incorporated into the soil. Overall, tillage management leaves the soil more vulnerable to the elements for a larger portion of the sampling year. Based on correlation analysis, larger plant

related measurements reflective of land cover have a positive relationship with virus abundance. The presence of crops and crop residue may have played a role in virus survival by relieving stress especially in the form of high soil temperatures throughout the year. Although this is just one possibility, increased virus presence could also be based on greater bacterial host abundance (Srinivasiah et al., 2008; Srinivasiah et al., 2015) or virus lifestyle choice based on host nutrient availability (Pradeep Ram and Sime-Ngando, 2010; Wilson and Mann, 1997). While already known to influence microbial communities (Lauber et al., 2008), nitrogen levels in the soil also impacted the virus populations. Nitrate was an important factor linked to overall virus abundance (Figure 4.3). The response to different nitrogen sources based on Spearman's correlations in relation to viral abundance in the two croplands might be reflective of the differences in the viral and host community composition and their function in the soil system. This is supported by our model that confirms the important influence on nitrate in the soil by NH_4^+, air temperature, and management practices. Together, this study and prior studies of viral diversity demonstrate that local environmental conditions have a strong effect on the viral community (Lachnit et al., 2019; Narr et al., 2017; Roy et al., 2020; Srinivasiah et al., 2013). Therefore, it is highly likely that viral abundance and community structure are in part shaped by the biotic and abiotic factors influenced by land use and land management practices.

The VLP abundance data show temporal variation over the 1-year collection period that was often observed during specific months instead of across seasons. A 12-month study by Narr et al. also detected seasonal differences and changes in abundance over time in the majority of their sampling sites (Narr et al., 2017). When looking at

growing seasons alone, a similar study found viral abundance to be roughly constant from May to July and September to November in a range of agricultural treatments (Roy et al., 2020). Overall, this resembles what is observed during the growing seasons of both croplands in this study (Figure 4.2a), but the largest temporal difference we observed in VLP abundance occurred in March when the weather begins to warm up and crops resume actively growing. Long-term studies examining VLP abundance in soil are very limited; therefore, more studies are needed to support these initial findings. However, variation in seasonal VLP abundance was recorded early on in viral studies in seawater (Hobbie et al., 1977). Many marine viral studies have observed changes in abundance by an order of magnitude between the winter and the summer months (Cochran and Paul, 1998; Jiang and Paul, 1994). While temporal differences were observed in our study, the scale of the change in soils appears to be much smaller than that of marine systems (Narr et al., 2017; Roy et al., 2020). The amount of variation in VLP abundance throughout the year differed for each field. The temporal responses were larger in prairie soil, but all fields had lower abundance in winter months and higher abundance during March. Although VLP abundance had similar temporal dynamics, the differences observed in the magnitude of the variation are likely due to increased management activity that continually disturbs the system, including the microbial hosts needed for virus production.

4.5.2 Bacterial host abundance

Bacteria are speculated to be the most common hosts for viruses in environmental samples, explaining why bacterial abundance is often examined in combination with viral

abundance. In this study, estimates of bacterial abundance ranged from 10^6 to 10^9 bacterial cells g^{-1} soil. Although approaches differed, this is consistent with other investigations that determined bacterial abundance to be ≥ 10^6 g^{-1} soil (Roy et al., 2020; Williamson et al., 2013; Williamson et al., 2005). As seen with VLP abundance, there were significant differences between bacterial abundance in the cropland soils in comparison to the tallgrass prairie soils. Changes in abundance followed the same structure observed in the viral communities such that bacterial abundance decreased with increasing land management. Considering the idea that most viruses present in soils are bacteriophages, it is not unexpected that the observed population abundance for viruses and bacteria responded in a similar manner to land use and land management practices. Numerous studies in marine systems have looked at bacterial abundance and its relationship to viral abundance. In these systems, VLP abundance is highest in coastal environments and lowest in deep-sea waters in general (Wommack and Colwell, 2000). These variations in abundance are often correlated with microbial production and the productivity of the system (Kimura et al., 2008; Williamson et al., 2003). Soil studies have demonstrated similar relationships where organic-rich soils with higher moisture have greater prokaryotic cells present than that of dry low organic content soils; the latter of which generally results in a much greater presence of virus than prokaryotic hosts (Williamson et al., 2007; Williamson et al., 2005; Williamson et al., 2003; Wommack et al., 2015). As seen here using SEM, the positive direct influence of bacterial abundance on VLP abundance suggests increased productivity of bacteria is advantageous for viruses, while the negative direct effect of VLP abundance on bacterial abundance implies increase in virus abundance is unfavorable for the host population. Most current

soil studies do not look specifically at bacterial cell counts, but a large number have shown that soil microbial communities are considerably affected by changes in land use and land management (Bossio et al., 1998; Johnson et al., 2003; Lauber et al., 2008; Steenwerth et al., 2002). Such changes in land use and management also have a significant effect on soil and environmental factors (Ashelford et al., 2000; Kimura et al., 2008; Murty et al., 2002), all of which could contribute to the differences in bacterial abundance observed between the different sites.

Microbial activity and biomass have been shown to respond to multiple influences, including organic matter, soil management, and other abiotic factors (Calderón et al., 2000; Calderón et al., 2001; Narr et al., 2017; Williamson et al., 2005). Our data also indicate that many factors, including ground cover, soil nutrients, soil water content, and temperature, all of which are influenced by land use and land management, have significant interactions with soil bacterial abundance. The CT cropland had the most significant correlation between soil water content and bacterial abundance. The water content fluctuated more in the croplands than the prairie soil over the sampling period. Notably, bacterial abundance was negatively related to SWC, while VLP abundance had a positive relation with SWC, which may reflect virus production in the soils. Most often higher moisture in soil supports an increase in bacterial activity and abundance (Prado and Airoldi, 1999; Skopp et al., 1990), but an increase in the activity of a typically starved host infected with a virus can lead to induced virus production and host lysis (Kimura et al., 2008; Miller and Day, 2008). It is also possible that increased water in the soil dilutes or mobilizes the microbial hosts especially in the loose soil of the tillage site,

although this would be expected to be accompanied by an even greater runoff of viruses (Williamson et al., 2014).

Soil microbes play an important role in nutrient cycling, decomposing organic matter, carbon mineralization, and plant nutrient availability (Haney et al., 2008; Paul and Clark, 1989). These differences in functional activities of microbes are impacted by land cover which differs substantially between land uses. In addition, land cover has been found to regulate microbial structure by affecting soil conditions such as organic matter (Bissett et al., 2011; Moon et al., 2016). Bacterial abundance was strongly influenced by organic matter and total nitrogen at all sites based on Spearman's correlations, both of which strongly decreased with land cover and increased with management intensity. Land cover is also known to be a controlling factor of soil temperatures that is overall influenced by air temperature. Bacteria and virus survival in soil is often temperature dependent, and optimal temperatures can differ between hosts and their associated phages (Kimura et al., 2008). In the cropland sites, the directions of the relationship with temperature overall differed for bacterial and viruses. The changes in soil bacterial abundance may result from prophage induction triggered by increased temperature resulting in host cell lysis. Virus production can be induced by an environmental signal such as host DNA damage, resulting in the lytic function of lysogenic viruses and the production of progeny (Campbell, 2006). For example, DNA damage can induce an SOS repair mechanism initiating the lytic pathway of virus replication in lysogens (Weinbauer and Suttle, 1999). Alternatively, it could be caused by selective mortality of different microbial groups which have been recently shown to be triggered by bacterial quorum sensing signals inducing a lysogenic to lytic switch in samples collected from agricultural

soils (Liang et al., 2020a); any of these could result in the different response in abundance to changes in soil and air temperature by the bacterial and virus populations. Nitrate and ammonium both were key in determining bacterial abundance based on SEM and Spearman's correlations. These two forms of nitrogen, especially at elevated levels from fertilizer input, impact soil processes and shape microbial community structure (Fierer et al., 2012a). While the changes observed depend on the specific land use and management practices, sampling site overall appears to have the largest impact on soil factors which affects the below ground community dynamics.

There were observable seasonal shifts in bacterial abundance at all sampling locations over the 1-year study period. The lowest bacterial abundance was recorded in August and the following winter months for all fields similar to the temporal lows in VLP abundance. Increases in bacterial abundance were observed in fall and spring extending into early summer. While all the fields had similar shifts in abundance throughout the year, the magnitude of the changes varied greatly. Comparable results were detected in agricultural soils in Michigan where bacterial abundance stayed relatively stable and quickly returned to these stable levels when fluctuations in abundance occurred, but this study did not account for differences in bacterial abundance during winter and summer months (Roy et al., 2020). The same marine studies that observed seasonal changes in VLP abundance also observed similar changes in bacterial abundance, but of smaller magnitude than that seen for viruses (Cochran and Paul, 1998; Jiang and Paul, 1994). Although the results are from contrasting systems, the same general variations appear to be present in the recent studies of soil systems. Further

studies of combined viral and bacterial abundance are needed to determine the seasonal effects on virus and host interaction in
terrestrial systems.

4.5.3 Viral community

The scarcity of studies examining viral communities especially in terrestrial environments is usually attributed to the absence of a genetic marker sequence, such as those used in identifying bacterial communities (Breitbart and Rohwer, 2005). Certain viral taxonomic groups share conserved genes which allow them to be used as targets to study specific viral groups (Srinivasiah et al., 2013), but a less targeted approach needs to be used to look at the whole community. Fingerprinting methods have allowed for fast analysis and higher sample throughput for screening viral communities but lack information on viral abundance and identity (Narr et al., 2017). For these reasons, most examinations of viral community structure rely on metagenomic approaches. Studies have recently started to focus on optimizing protocols for viral metagenomic analysis from terrestrial environments in order to create a standardized method for viral communities to be compared across environments (Daly et al., 2019; Roux et al., 2019b; Trubl et al., 2019). However, it should still be taken into consideration that soil, environmental, and viral factors are known to affect the adsorption of viruses to soils (Kimura et al., 2008), and virus extractability from soil can be further impacted by the extraction method (Narr et al., 2017; Williamson et al., 2013; Williamson et al., 2005). Viral metagenomics provides more than just sequence data by offering insight into

biogeographical distributions, community structure, and ecological dynamics (Breitbart and Rohwer, 2005).

In order to determine the possible impacts of viruses on soil microbial communities, it is critical to study autochthonous viruses using cultivation-independent approaches to assess community composition. Current bioinformatic tools were used to characterize viruses in soils under different levels of management intensity. These recent tools have provided a way to use assembled virus fragments that have not been previously cultivated or identified in phylogenetic and diversity studies (Roux et al., 2019a). In El Reno soils, the majority of identified viral sequences did not cluster together, suggesting the majority of the sequences represented unique virus species or vOTUs. The large assembly and greater number of viral species in the native tallgrass prairie is also consistent with the observations of greater VLP abundance in the prairie soil. The term viral operational taxonomic units (vOTUs) has been proposed at the formal way of classifying species rank virus groups in order to streamline the area of viral ecology and prevent confusion between various terms used across studies (Roux et al., 2019a). There was also very few shared vOTUs between the two land use types as has been previously observed in other habitat gradients (Trubl et al., 2018), supporting the idea that viral communities are influenced by the environment in which they are found.

Clustering methods of comparing new viral data sets to known viruses in available databases provides a way to examine relationships between identified and unknown viruses while assigning taxonomic classification to uncultivated virus genomes (UViGs) (Roux et al., 2019a). UViGs represent the majority of virus sequences in available databases due to the use of metagenomic and metatranscriptomic studies

(Brister et al., 2015; Paez-Espino et al., 2017; Roux et al., 2019a; Roux et al., 2016a). By clustering the vOTUs from El Reno with publicly available viruses, we were able to identify 86 of vOTUs from assembled viromes with another 76 vOTUs grouping with unclassified viruses in the RefSeq database. The majority of vOTUs from CT wheat and native prairie soils had no genetic similarity to viruses in the current databases. Similar results have been obtained in other studies where only 8.5 to 24.3% of viral sequences were identified in Chinese agricultural soils (Han et al., 2017), 9.8% in polar freshwater (Aguirre de Cárcer et al., 2015), and 15% thawing permafrost harbors (Trubl et al., 2018). In combination, these studies reveal the limitations of examining viral communities showing most viromes consist of predominately unidentifiable sequences. This was further exemplified when examining proteins in a subset of the El Reno vOTUs, where less than half of the predicted genes were identified based on currently available sequences. Each field's taxonomic profile differed by the presence of specific bacteriophage families and the relative abundance of taxonomically identified viruses. Siphoviridae was the dominantly identified group in both viral communities with Podoviridae not being identified in either virome, but due to the lack of identified viruses in the El Reno viromes it is hard to determine which specific viruses are abundant in the community. Although, it does appear that each virome is distinct to the collection site with there being little overlap in the viral community structure, which could be partially due to the technical issues associated under sampling and reproducibility (Zhou et al., 2015; Zhou et al., 2013; Zhou et al., 2011b). When examining the shared vOTUs, unique function was not observed most likely due to the lack of predicted gene identification. One shared vOTU highly present in the tilled soil contained methyltransferase genes

which are known to be a powerful gene regulator in bacteria and have been proposed as a life cycle regulator in phages (Bochow et al., 2012). Switching life cycles in soils subject to frequent disturbances could be an important and distinctive function in frequently disturbed soils such as intensely managed croplands. Comparably, earlier soil viral metagenomic data have revealed that viral assemblages are locally unique, and medium type is most likely the driving force behind observed differences when comparing viral communities (Han et al., 2017; Srinivasiah et al., 2013). More specifically, the texture and physiochemical factors may influence the community more than distance between sites (Han et al., 2017), supporting the idea that viral abundance and community structure are influenced by various soil and environmental factors which are known to be affected by land use and land management practices. Although there were clear observable differences in VLP abundance, vOTU abundance, and community structure in the two fields, further work is required to determine whether similar environmental factors and seasonal differences are influencing the community structure over time as was observed for virus abundance.

4.6 Conclusions

In each land use system, there were clear temporal differences in viral and bacterial abundance over the 1-year sampling period. The abundance of viruses and potential hosts both decreased with increasing amounts of management input with the prairie site continually having greater abundance than the croplands. There were also observable seasonal differences in abundance with similar trends for virus and bacterial populations. Various soil and environmental factors influenced viral and

host abundance which was often reflective of management activities in each system. When examining DNA viral communities in the prairie and tilled wheat field, there were clear differences in community structure and vOTU relative abundance with the native tallgrass prairie containing more unique viral species. There was also minimal intersection of the viral community structure between land use types. This study suggests that the different levels of land management impacted the soil properties and environmental effects on the belowground communities especially abundance. Overall, our results implicate land use and land management as driving factors of shaping the physicochemical properties in agricultural soils which influence not only the abundance of virus and host communities but the structure of the soil viral communities. Global or large-scale studies are needed to identify whether such interactions between management, environmental factors, and viruses are a general rule across all agricultural systems.

Chapter 5 : Summary

This dissertation presented evidence based on field studies supporting that soil microbial communities are influenced by land use and associated management practices, specifically in a U.S. Southern Plains agroecosystem. Changes were observed in microbial community composition, diversity, abundance, functional potential, and network complexity and stability. The responses of the soil microbial communities were closely linked to land use type, soil physicochemical properties, seasonal climate differences, and the level of management disturbance. Different land uses resulted in distinct soil microbial communities with tillage management having the greatest overall impact on community dynamics. Conventional tillage causes the largest disturbance of most management practices to microbial communities since it mechanically turns over the soil with many areas trying to implement less intensive practices. As the interest in agricultural sustainability grows, detailed research on local microbial community dynamics is needed to inform management decisions to retain and restore soil health. Therefore, this research focused on examining soil microbial communities under common land uses in the U.S. Southern Plains to determine the effects of current land use and management practices over time.

The impact of intensive agriculture on soil microbial communities is of significant worldwide concern since microbes play important roles in nutrient cycling, carbon storage, and plant productivity. The application of conservation agriculture practices such as reduced or no-tillage has produce mixed results on soil health often being dependent on other factors such as climate and soil type. Thus, determining the impact of land use and management practices at a local scale will give greater insight into microbial

community dynamics needed to develop sustainable practices. Four common land uses in a Southern Plains agroecosystem including two croplands and two grasslands were examined over one year to examine microbial communities dynamics across a disturbance gradient. We observed that land use had the greatest impact on taxonomic diversity, and sampling time and time within a specific land use were more important for differences observed in functional diversity. The most disturbed fields became more dependent on nitrogen likely related to a dependence on fertilizer input compared to less disturbed fields where soil properties, nitrogen measurements, and climate were roughly equally important for explaining bacterial community differences. Intensive management disturbance and fertilizer usage were also possibly responsible for the reduction in functional potential observed in the tilled cropland compared to the native tallgrass prairie. This study provided critical information on the compositional and functional diversity of soil microbial communities across a gradient of management disturbance in an agroecosystem and elucidated the importance of minimizing management disturbance to improve soil health including the diversity of the bacterial communities.

After it was established that microbial communities significantly differed with time and land use, the next aim was to determine if the potential microbial interactions were similarly affected using molecular ecological network (MEN) analysis. This study focused on the conventionally tilled wheat field and native tallgrass prairie to investigate whether and how land conversion from native land use to long-term cropland use impacted the complexity and stability of the networks. Overall, we observed an increase in network complexity, network stability, and temporal variation under CT cropland use compared to native land use. Although microbial community diversity was greater under

native land use, the networks were less complex including fewer “interactions” and greater modularity. Soil properties, climate, and plant growth had the greatest impact on the complexity and stability of the native TGP networks, while different types of management disturbance were highly correlated to CT cropland network differences. Together, this study showed land conversion also influences the soil microbial community interactions with the microbial community under long-term cropland use adapting to withstand management disturbances and becoming heavily reliant on “interactions” to support ecosystem function.

With insight on the impact of land use disturbance on microbial community diversity, functional potential, and network interactions, we then focused on the viral community under different land uses. Viruses are abundant members of microbial communities with important roles in influencing microbial community structure and function. Here, we examined three land uses over one-year, focusing on the native tallgrass prairie and the two croplands with different amounts of management disturbance. As the amount of management disturbance increased, the abundance of viruses and potential bacterial hosts both decreased. Temporal differences were also observed in virus and bacterial abundance mostly differing by season. Not only did the abundance of viruses differ by land use, but so did the viral community composition with the native tallgrass prairie having more unique viral species. Overall, this study implicated land use and land management as driving factors of shaping the physicochemical properties in agricultural soils which influenced the abundance of viruses, the abundance of bacterial hosts, and the structure of the soil viral communities.

From the beginning, civilization has been dependent on food, fiber, and other goods produced by agriculture. Yet, current agricultural practices are threatening our soils and microbial communities needed for the health of ecosystems and human populations. While certain soil properties are generally always relevant when trying to assess microbial community dynamics such as soil water content and nutrient availability, the significance can often be context dependent as vegetation types may have different growth requirements and climate varies by region. Therefore, this dissertation aimed to explore the impact of land use and time on microbial communities in the U.S. Southern Plains in hopes to improve our understanding of the large effect microorganisms can have on the function of agricultural ecosystems.

Listed below are the individual manuscripts, published or in preparation, in relation to this dissertation. Chapter 2 and 4 in this dissertation presented contents in the published journal articles 1 and 3 below that has been reformatted for this dissertation. The Publisher of the journal granted the author to reuse these published materials in this dissertation by copyright policy.

As research is highly collaborative, it is important to mention my contributions to the published worked presented in this dissertation. For all studies, I collected and processed the soil samples, performed the laboratory work, carried out the statistical analyses, and wrote the original drafts of the manuscripts. Plant biometrics and land history data was provided by collaborators at the USDA, and soil chemistry was determined by the Soil, Water and Forage Analytical laboratory at OSU as mentioned in the methods of each research chapter. Co-authors, especially Dr. Ya Zhang, helped with editing manuscript drafts and made suggestions on additionally analyses to further improve the work presented here.

Peer reviewed journal papers published:

1. **Cornell, C.R.,** Zhang Y., Van Nostrand, J.D., Wagle, P., Xiao, X., and Zhou, J. 2021. Temporal changes of virus-like particle abundance and metagenomic comparison of viral communities in cropland and prairie soils. mSphere, 6(3): e0116020.
2. Deng, J., Frolking, S., Bajgain, R., **Cornell, C.R.**, Wagle, P., Xiao, X., Zhou, J., Basara, J., Steiner, J., and Changsheng, L. 2021. Improving a biogeochemical model to simulate microbial-mediated carbon dynamics in agricultural ecosystems. J Adv Model Earth Syst, 13(11): e2021MS002752.

3. **Cornell, C.R.**, Zhang, Y., Ning, D., Wu, L., Wagle, P., Steiner, J.L., Xiao, X., and Zhou, J. Temporal dynamics of bacterial communities along a gradient of disturbance in a U.S. Southern Plains agroecosystem. mBio, In Press.

Manuscripts in preparation:

4. **Cornell, C.R.**, Zhang Y., et al. Land conversion enhances microbial network complexity and stability. In preparation.

Appendix A : Supplementary Tables

Table S2.1 Soil properties as affected by season and land use type. Values represent averages over seasons and standard deviation within each land use.

Land Use	Season	Topsoil Nitrate (kg/ha)	Organic Matter (%)	Total Nitrogen (%)	NH_4^+ (kg/ha)	Soil Water Content (%)
CT Wheat	Summer	48 ± 28	1.66 ± 0.11	0.11 ± 0.01	46.9 ± 52.5	10.50 ± 2.31
	Fall	105 ± 33	1.80 ± 0.21	0.12 ± 0.01	27.9 ± 7.4	11.07 ± 4.45
	Winter	25 ± 14	2.06 ± 0.12	0.13 ± 0.01	17.2 ± 12.1	8.11 ± 5.94
	Spring	15 ± 10	1.89 ± 0.19	0.12 ± 0.01	11.2 ± 3.2	11.41 ± 6.09
NT Canola	Summer	62 ± 26	2.56 ± 0.21	0.14 ± 0.01	13.1 ± 5.0	18.59 ± 3.82
	Fall	49 ± 19	2.74 ± 0.12	0.16 ± 0.01	12.9 ± 2.6	18.35 ± 1.63
	Winter	18 ± 8	2.98 ± 0.38	0.17 ± 0.02	12.5 ± 3.2	16.73 ± 3.13
	Spring	16 ± 15	3.00 ± 1.50	0.17 ± 0.02	12.6 ± 1.4	16.90 ± 4.09
OWB Pasture	Summer	7 ± 4	2.64 ± 0.33	0.15 ± 0.02	12.0 ± 2.4	11.92 ± 3.16
	Fall	10 ± 3	3.15 ± 0.63	0.17 ± 0.03	16.7 ± 3.0	14.66 ± 2.97
	Winter	5 ± 2	2.94 ± 0.37	0.16 ± 0.02	21.3 ± 5.9	16.41 ± 2.13
	Spring	8 ± 5	3.01 ± 0.80	0.16 ± 0.03	22.7 ± 12.7	16.45 ± 5.08
TGP	Summer	15 ± 9	3.81 ± 0.97	0.20 ± 0.04	16.1 ± 1.6	11.73 ± 2.74
	Fall	13 ± 2	3.63 ± 0.38	0.19 ± 0.02	22.4 ± 4.0	16.42 ± 1.80
	Winter	11 ± 4	3.52 ± 0.70	0.19 ± 0.03	24.2 ± 2.9	21.93 ± 2.63
	Spring	13 ± 6	3.71 ± 0.79	0.20 ± 0.04	25.4 ± 4.7	21.81 ± 2.22

Conventionally tilled (CT) wheat; no-till (NT) canola; old world bluestem (OWB) pasture; tallgrass prairie (TGP)

Table S2.2 Average relative abundance (%) of abundant phyla in soil from conventionally tilled (CT) winter wheat, no-till (NT) canola, Old World Bluestem (OWB) pasture, and native tallgrass prairie (TGP) systems from August 2016 to July 2017.

	CT	NT	OWB	TGP
Acidobacteria	11.75%	11.32%	13.53%	9.20%
Actinobacteria	24.67%	23.55%	21.45%	20.16%
Bacteroidetes	2.92%	2.81%	2.74%	2.54%
Chloroflexi	9.90%	4.23%	4.78%	4.73%
Cyanobacteria	2.35%	0.76%	1.29%	0.32%
Firmicutes	9.68%	15.95%	18.03%	17.51%
Gemmatimonadetes	1.00%	1.57%	1.04%	1.40%
Nitrospirae	0.51%	0.76%	0.26%	0.73%
Planctomycetes	2.57%	2.79%	2.99%	3.47%
Proteobacteria	21.20%	22.56%	20.84%	24.36%
Verrucomicrobia	1.78%	3.77%	3.67%	6.35%
Archaea Phyla	0.09%	0.17%	0.06%	0.14%

Table S2.3 Pairwise comparison p-values for relative abundance of phyla significantly different between land use types from August 2016 to July 2017. P-values FDR corrected. Land types include conventionally tilled (CT) winter wheat, no-till (NT) canola, Old World Bluestem (OWB) pasture, and native tallgrass prairie (TGP). All phyla used in pairwise comparisons previously found to be significant by field using PERMANOVA with 999 permutations and Bray-Curtis dissimilarity metric.

	OWB:NT	**OWB:TGP**	**OWB:CT**	**NT:TGP**	**NT:CT**	**TGP:CT**
Acidobacteria	0.002	0.002	0.006	0.002	0.375	0.002
Actinobacteria	0.027	0.233	0.006	0.006	0.238	0.006
Chloroflexi	0.126	0.918	0.002	0.172	0.002	0.002
Cyanobacteria	0.001	0.001	0.001	0.001	0.001	0.001
Firmicutes	0.338	0.950	0.002	0.338	0.002	0.002
Gemmatimonadetes	0.002	0.002	0.411	0.193	0.002	0.002
Proteobacteria	0.075	0.003	0.639	0.050	0.127	0.003
Verrucomicrobia[a]	0.613	0.001	0.001	0.001	0.001	0.001

[a]Verrucomicrobia found to have significant difference in dispersion within group based off of multivariate homogeneity of groups dispersions test. Potentially contributes to significant differences observed between land use types.

Table S2.4 F-values of phyla relative abundance significantly different between seasons. The last timepoint in July was left out of the analysis because it stood alone for the summer 2017 season. All phyla individually compared across seasons using PERMANOVA with 999 permutations and Bray-Curtis dissimilarity metric. Asterisk indicates significant differences *indicate significant ($p < 0.05$) effects, ** indicate significant ($p < 0.01$) effects, *** indicate significant ($p < 0.001$) effects. Italicized values indicate a p-value of $0.05 < p < 0.1$.

	CT	**NT**	**OWB**	**TGP**
Acidobacteria	-	-	-	7.17**
Actinobacteria	-	-	-	5.10**
Bacteroidetes	6.65**	7.23***	-	-
Chloroflexi	-	-	-	3.80*
Cyanobacteria	3.73*	-	-	-
Firmicutes	*2.52*	3.62*	-	-
Gemmatimonadetes	-	-	-	-
Nitrospirae	4.98**	8.61**	4.86**	7.17**
Planctomycetes	3.13*	6.85***	20.00***	16.47***
Proteobacteria	18.77***	-	-	3.50*
Verrucomicrobia	3.17*	-	-	-
Archaea Phyla	-	3.70*	3.14*	9.27**

Conventionally tilled (CT) wheat; no-till (NT) canola; old world bluestem (OWB) pasture; tallgrass prairie (TGP)

Table S2.5 Spearman correlations between relatively abundant phyla and soil properties within land use types. Correlation coefficients with $p < 0.05$ are indicated in bold, coefficients with $0.05 < p < 0.1$ are indicated in italics, and data omitted where $p > 0.1$.

		TopN		OM		TN		NH_4^+		SWC	
	Phylum	Rho	*p*	Rho	*p*	Rho	*p*	Rho	*p*	Rho	*p*
CT Wheat	Acidobacteria	*0.406*	*0.076*	-	-	-	-	-	-	-	-
	Actinobacteria	-	-	-	-	-	-	-	-	-	-
	Bacteroidetes	**-0.602**	0.005	**0.467**	0.038	*0.382*	*0.096*	**-0.553**	0.013	-	-
	Chloroflexi	-	-	**0.523**	0.018	**0.567**	0.009	-	-	**-0.674**	0.002
	Cyanobacteria	-	-	**0.620**	0.004	**0.532**	0.016	-	-	-	-
	Firmicutes	-	-	-	-	-	-	*0.409*	*0.075*	-	-
	Gemmatimonadetes	-	-	-	-	-	-	-	-	-	-
	Nitrospirae	-	-	**-0.845**	0.000	**-0.732**	0.000	-	-	**0.550**	0.012
	Planctomycetes	**-0.469**	0.037	-	-	0.263	-	**-0.600**	0.006	-	-
	Proteobacteria	-	-	**-0.742**	0.000	-	-	-	-	-	-
	Verrucomicrobia	-	-	**-0.740**	0.000	**-0.715**	0.000	-	-	**0.612**	0.005
NT Canola	Acidobacteria	-	-	**0.715**	0.000	**0.682**	0.001	-	-	**-0.640**	0.002
	Actinobacteria	-	-	-	-	-	-	-	-	-	-
	Bacteroidetes	**-0.769**	0.000	**0.464**	0.039	**0.487**	0.029	-	-	-	-
	Chloroflexi	-	-	-	-	-	-	-	-	**-0.471**	0.036
	Cyanobacteria	-	-	**0.505**	0.023	**0.448**	0.048	-	-	**-0.688**	0.001
	Firmicutes	**0.470**	0.036	**-0.588**	0.006	**-0.576**	0.008	-	-	**0.576**	0.008
	Gemmatimonadetes	-	-	*-0.382*	*0.096*	*-0.391*	*0.088*	-	-	-	-
	Nitrospirae	**0.434**	0.056	**-0.809**	0.000	**-0.833**	0.000	-	-	**0.580**	0.007
	Planctomycetes	**-0.481**	0.032	**0.805**	0.000	**0.798**	0.000	-	-	**-0.648**	0.002
	Proteobacteria	-	-	-	-	-	-	-	-	-	-
	Verrucomicrobia	-	-	**-0.682**	0.001	**-0.700**	0.001	-	-	**0.489**	0.029
OWB Pasture	Acidobacteria	-	-	-	-	-	-	**0.567**	0.009	**0.520**	0.020
	Actinobacteria	-	-	-	-	*-0.409*	*0.073*	-	-	-	-
	Bacteroidetes	-	-	*0.439*	*0.053*	-	-	*0.420*	*0.065*	-	-
	Chloroflexi	-	-	-	-	**-0.469**	0.037	-	-	-	-
	Cyanobacteria	-	-	-	-	-	-	-	-	-	-
	Firmicutes	*0.432*	*0.057*	-	-	-	-	-	-	-	-
	Gemmatimonadetes	-	-	-	-	*-0.406*	*0.075*	-	-	-	-
	Nitrospirae	-	-	**-0.605**	0.005	**-0.447**	0.048	**-0.467**	0.038	-	-
	Planctomycetes	-	-	-	-	-	-	-	-	-	-
	Proteobacteria	-	-	-	-	-	-	-	-	-	-
	Verrucomicrobia	**-0.570**	0.009	**-0.462**	0.042	**-0.477**	0.033	-	-	-	-
TGP	Acidobacteria	-	-	-	-	-	-	*-0.381*	*0.098*	**-0.660**	0.002
	Actinobacteria	-	-	-	-	-	-	-	-	*0.441*	*0.053*
	Bacteroidetes	**0.471**	0.036	**0.508**	0.022	**0.461**	0.041	-	-	-	-
	Chloroflexi	-	-	-	-	-	-	-	-	*0.442*	*0.052*
	Cyanobacteria	-	-	-	-	-	-	-	-	-	-
	Firmicutes	-	-	-	-	-	-	-	-	-	-
	Gemmatimonadetes	-	-	-	-	-	-	-	-	-	-
	Nitrospirae	-	-	-	-	-	-	**-0.486**	0.030	-	-
	Planctomycetes	-	-	-	-	-	-	-	-	*0.388*	*0.092*
	Proteobacteria	-	-	-	-	-	-	-	-	**-0.480**	0.034
	Verrucomicrobia	**-0.631**	0.003	**-0.590**	0.006	**-0.661**	0.002	-	-	-	-

Table S2.6 OTUs significantly correlated ($p \leq 0.05$) to functional gene groups present in both the tallgrass prairie (TGP) and conventionally tilled (CT) wheat field. Correlations based on Spearman correlations with direction of relationship presented as positive (+) and negative (-).

Category	OTU	Phylum	Class	Order	Family	Genus	Correlation	
							TGP	CT
Carbon cyc.[a]	OTU 193246	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Strepto-myces	-	+
Carbon deg.[b]	OUT 188347	Chloroflexi	Chloroflexi	Chloroflexales	Chloroflexaceae	Rosei-flexus	-	+
	OTU 160059	Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	Kineo-sporia	-	+
Carbon fixation	OTU 193246	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Strepto-myces	-	+
	OTU 77827	Gemmatimon-adetes	Gemmatimon-adetes	Gemmatimo-nadales	Gemmatimon-adaceae		+	+
Methane	OTU 156892	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	Frankia	-	+
	OTU 319788	Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	Kineo-sporia	-	+
Sulfur	OTU 456750	Proteobacteria	Deltaproteo-bacteria	Desulfuro-monadales	AKYG597		+	+
Nitrifi-cation	OTU 376259	Firmicutes	Bacilli	Bacillales	Thermoactino-mycetaceae	Shimaz-uella	+	-
N fixation[c]	OTU 19539	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	+	-
	OTU 345627	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	+	+
	OTU 478718	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	+	-
Metal homeo-stasis	OTU 188347	Chloroflexi	Chloroflexi	Chloroflexales	Chloroflexaceae	Rosei-flexus	-	+
	OTU 242252	Proteobacteria	Betaproteo-bacteria	Burkholderiales	Oxalobacteraceae	Massilia	+	+
	OTU 353029	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	+	+
Organic remed-iation	OTU 242252	Proteobacteria	Betaproteo-bacteria	Burkholderiales	Oxalobacteraceae	Massilia	+	+

[a] carbon cycling (cyc)
[b] carbon degradation (deg)
[c] nitrogen (N) fixation

Table S3.1 Topological properties of Empirical MENs of converted cropland (CT wheat) and tallgrass prairie (TGP) control. All networks constructed using a threshold value (St) of 0.96.

	Sample time	No. of ASVs*	Nodes	Link	R^2	Avg K	GD	Avg CC	Con	M	Total mod-ules	No. large module **	No. nodes in large module **	% nodes in large module **
CT Wheat	Jun-17	2153	688	875	0.795	2.544	5.871	0.085	0.412	0.784	106	11	456	66.3
	Jul-17	1974	1082	3809	0.795	7.041	3.868	0.279	0.734	0.452	76	10	919	84.9
	Aug-17	2145	748	942	0.879	2.519	5.169	0.099	0.189	0.828	115	13	482	64.4
	Sep-17	2000	600	679	0.822	2.263	5.310	0.078	0.192	0.841	116	11	329	54.8
	Oct-17	2122	1172	5237	0.774	8.937	3.773	0.254	0.825	0.426	54	10	1046	89.2
	Nov-17	2092	942	3243	0.846	6.885	3.994	0.235	0.702	0.439	74	12	776	82.4
	Dec-17	1861	860	3589	0.814	8.347	3.293	0.164	0.537	0.432	91	4	642	74.7
	Jan-18	1946	959	3350	0.785	6.986	3.878	0.271	0.697	0.480	70	9	783	81.6
	Feb-18	1944	861	1893	0.795	4.397	4.813	0.152	0.599	0.591	81	9	672	78.0
	Mar-18	1873	653	1087	0.747	3.329	4.855	0.178	0.464	0.735	68	10	492	75.3
	Apr-18	1479	308	278	0.952	1.805	4.777	0.095	0.054	0.901	85	6	106	34.4
	May-18	2009	930	3402	0.787	7.316	4.115	0.223	0.700	0.484	72	9	764	82.2
	Jun-18	2205	1564	8454	0.808	10.811	4.041	0.278	0.720	0.501	90	11	1337	85.5
	Jul-18	2058	1094	5344	0.750	9.770	3.669	0.307	0.774	0.375	68	12	954	87.2
	Aug-18	1690	708	1472	0.796	4.158	4.732	0.205	0.709	0.585	62	11	577	81.5
	Sep-18	2080	626	579	0.927	1.850	8.736	0.057	0.154	0.917	132	10	310	49.5
	Oct-18	1991	510	484	0.884	1.898	4.208	0.090	0.068	0.940	121	8	217	42.5
	Nov-18	2123	716	866	0.879	2.419	8.273	0.106	0.324	0.863	103	13	477	66.6
	Dec-18	1992	1166	5899	0.754	10.118	3.556	0.315	0.871	0.348	55	10	1063	91.2
TGP	Jun-17	3192	1044	1263	0.746	2.420	7.660	0.153	0.053	0.929	163	19	689	66.0
	Jul-17	3388	1081	1183	0.749	2.189	4.469	0.164	0.056	0.945	183	20	681	63.0
	Aug-17	3287	1212	1454	0.843	2.399	5.613	0.117	0.155	0.959	179	28	838	69.1
	Sep-17	3357	1241	1430	0.844	2.305	6.333	0.096	0.113	0.951	193	26	812	65.4
	Oct-17	3368	1141	1238	0.783	2.170	3.747	0.104	0.052	0.953	191	25	743	65.1
	Nov-17	2982	1126	1270	0.815	2.256	5.126	0.127	0.088	0.925	186	22	723	64.2
	Dec-17	3200	1078	1229	0.765	2.280	4.179	0.142	0.058	0.932	193	21	666	61.8
	Jan-18	3320	1060	1079	0.745	2.036	3.708	0.081	0.038	0.959	192	31	692	65.3
	Feb-18	3016	920	969	0.818	2.107	3.890	0.127	0.044	0.940	196	17	482	52.4
	Mar-18	2987	897	1082	0.740	2.412	3.179	0.104	0.041	0.931	172	18	525	58.5
	Apr-18	3110	980	957	0.723	1.953	6.088	0.072	0.084	0.951	179	21	581	59.3
	May-18	3448	1180	1465	0.814	2.483	3.315	0.151	0.039	0.947	193	19	748	63.4
	Jun-18	3457	1124	1333	0.771	2.372	3.329	0.101	0.037	0.952	186	21	696	61.9
	Jul-18	3488	1739	2167	0.780	2.492	10.44	0.116	0.143	0.958	234	32	1236	71.1
	Aug-18	3324	1047	1165	0.730	2.225	4.772	0.115	0.051	0.954	215	20	570	54.4
	Sep-18	3420	1200	1436	0.848	2.393	3.443	0.137	0.030	0.958	194	25	777	64.8
	Oct-18	2946	797	829	0.831	2.080	3.717	0.131	0.064	0.947	170	17	432	54.2
	Nov-18	3312	1122	1342	0.846	2.392	4.949	0.115	0.101	0.931	189	24	711	63.4
	Dec-18	3511	1275	1318	0.783	2.067	4.170	0.112	0.046	0.952	203	24	843	66.1

*The total numbers of ASVs with non-zero sequence numbers in at least 6 of the 8 samples used in each network.
**Large modules contain 10 or more nodes.

Table S3.2 Topological properties of Random MENs of converted cropland (CT wheat) and tallgrass prairie (TGP) control

Land use	Sample time	GD +/- SD	avgCC +/- SD	Modularity +/- SD	HD +/- SD	Con +/- SD
CT Wheat	Jun-17	4.447 +/- 0.080	0.025 +/- 0.005	0.689 +/- 0.005	3.987 +/- 0.053	0.718 +/- 0.028
	Jul-17	3.003 +/- 0.017	0.202 +/- 0.006	0.307 +/- 0.002	2.798 +/- 0.011	0.961 +/- 0.011
	Aug-17	4.720 +/- 0.080	0.013 +/- 0.004	0.701 +/- 0.006	4.240 +/- 0.056	0.704 +/- 0.030
	Sep-17	4.852 +/- 0.096	0.012 +/- 0.003	0.749 +/- 0.006	4.307 +/- 0.067	0.620 +/- 0.032
	Oct-17	2.933 +/- 0.014	0.196 +/- 0.007	0.263 +/- 0.002	2.740 +/- 0.009	0.980 +/- 0.006
	Nov-17	3.100 +/- 0.018	0.147 +/- 0.007	0.313 +/- 0.003	2.871 +/- 0.013	0.952 +/- 0.012
	Dec-17	2.997 +/- 0.018	0.157 +/- 0.007	0.274 +/- 0.003	2.778 +/- 0.013	0.941 +/- 0.016
	Jan-18	3.027 +/- 0.015	0.187 +/- 0.008	0.309 +/- 0.003	2.815 +/- 0.010	0.956 +/- 0.011
	Feb-18	3.486 +/- 0.029	0.085 +/- 0.007	0.450 +/- 0.004	3.212 +/- 0.020	0.916 +/- 0.020
	Mar-18	3.824 +/- 0.048	0.063 +/- 0.007	0.560 +/- 0.004	3.475 +/- 0.031	0.882 +/- 0.023
	Apr-18	6.118 +/- 0.499	0.004 +/- 0.004	0.849 +/- 0.010	4.950 +/- 0.299	0.369 +/- 0.040
	May-18	3.084 +/- 0.017	0.152 +/- 0.007	0.304 +/- 0.003	2.867 +/- 0.012	0.955 +/- 0.013
	Jun-18	2.978 +/- 0.011	0.133 +/- 0.004	0.240 +/- 0.002	2.782 +/- 0.007	0.977 +/- 0.006
	Jul-18	2.787 +/- 0.014	0.275 +/- 0.008	0.236 +/- 0.002	2.601 +/- 0.009	0.973 +/- 0.009
	Aug-18	3.399 +/- 0.028	0.110 +/- 0.008	0.461 +/- 0.004	3.132 +/- 0.019	0.911 +/- 0.020
	Sep-18	6.166 +/- 0.263	0.003 +/- 0.002	0.866 +/- 0.007	5.262 +/- 0.189	0.410 +/- 0.029
	Oct-18	5.178 +/- 0.222	0.008 +/- 0.004	0.830 +/- 0.007	4.450 +/- 0.146	0.415 +/- 0.032
	Nov-18	4.853 +/- 0.082	0.011 +/- 0.003	0.723 +/- 0.006	4.354 +/- 0.057	0.676 +/- 0.029
	Dec-18	2.745 +/- 0.012	0.330 +/- 0.009	0.224 +/- 0.002	2.567 +/- 0.008	0.977 +/- 0.007
TGP	Jun-17	4.610 +/- 0.060	0.014 +/- 0.003	0.722 +/- 0.005	4.188 +/- 0.039	0.650 +/- 0.023
	Jul-17	4.679 +/- 0.071	0.012 +/- 0.003	0.770 +/- 0.006	4.235 +/- 0.050	0.553 +/- 0.026
	Aug-17	4.793 +/- 0.063	0.009 +/- 0.002	0.731 +/- 0.005	4.382 +/- 0.044	0.631 +/- 0.023
	Sep-17	4.638 +/- 0.069	0.010 +/- 0.002	0.745 +/- 0.005	4.239 +/- 0.047	0.584 +/- 0.023
	Oct-17	4.759 +/- 0.070	0.009 +/- 0.002	0.776 +/- 0.006	4.322 +/- 0.049	0.537 +/- 0.022
	Nov-17	4.911 +/- 0.075	0.011 +/- 0.003	0.763 +/- 0.006	4.432 +/- 0.056	0.594 +/- 0.027
	Dec-17	4.733 +/- 0.066	0.013 +/- 0.003	0.752 +/- 0.005	4.279 +/- 0.047	0.596 +/- 0.023
	Jan-18	4.853 +/- 0.080	0.007 +/- 0.002	0.807 +/- 0.006	4.384 +/- 0.058	0.463 +/- 0.021
	Feb-18	4.881 +/- 0.093	0.011 +/- 0.003	0.790 +/- 0.006	4.363 +/- 0.065	0.518 +/- 0.026
	Mar-18	4.609 +/- 0.065	0.013 +/- 0.003	0.717 +/- 0.005	4.179 +/- 0.044	0.631 +/- 0.024
	Apr-18	4.752 +/- 0.090	0.008 +/- 0.002	0.823 +/- 0.006	4.275 +/- 0.064	0.424 +/- 0.021
	May-18	4.801 +/- 0.061	0.011 +/- 0.002	0.715 +/- 0.004	4.367 +/- 0.040	0.669 +/- 0.020
	Jun-18	4.672 +/- 0.056	0.010 +/- 0.002	0.732 +/- 0.005	4.270 +/- 0.041	0.612 +/- 0.025
	Jul-18	4.933 +/- 0.053	0.009 +/- 0.002	0.722 +/- 0.004	4.523 +/- 0.038	0.680 +/- 0.020
	Aug-18	4.775 +/- 0.070	0.011 +/- 0.003	0.762 +/- 0.005	4.325 +/- 0.047	0.561 +/- 0.021
	Sep-18	4.947 +/- 0.059	0.009 +/- 0.002	0.736 +/- 0.005	4.501 +/- 0.041	0.643 +/- 0.022
	Oct-18	4.694 +/- 0.085	0.010 +/- 0.003	0.788 +/- 0.006	4.211 +/- 0.060	0.493 +/- 0.024
	Nov-18	4.687 +/- 0.055	0.012 +/- 0.002	0.728 +/- 0.005	4.264 +/- 0.041	0.627 +/- 0.022
	Dec-18	4.828 +/- 0.078	0.008 +/- 0.002	0.803 +/- 0.006	4.377 +/- 0.057	0.489 +/- 0.021

*100 random networks were generated by randomly rewiring all the links of a corresponding empirical network with the identical numbers of nodes and links. Values shown are the mean values and standard derivations from 100 random networks.

Table S3.3 Correlations between soil and climatic properties detected using canonical correspondence analysis (CCA).

	CT and TGP *		CT Wheat ┼		TGP *	
	F	*p*	F	*p*	F	*p*
Total N	-	-	2.30	0.006**	-	-
$NH4^+$	2.02	0.012*	2.74	0.007**	1.41	0.015*
Topsoil NO_3^-	2.83	0.001***	3.32	0.001***	2.04	0.001***
Organic matter	6.54	0.001***	2.32	0.004**	2.70	0.001***
Available P	14.35	0.001***	3.14	0.001***	4.61	0.001***
Soil pH	5.84	0.001***	6.59	0.001***	2.04	0.001***
Soil temperature	1.71	0.020*	-	-	1.19	0.103
Soil water content	4.34	0.001***	3.47	0.002**	1.42	0.018*
Average rainfall	2.05	0.006**	1.78	0.014*	1.52	0.008**
Average air temperature	1.78	0.012*	1.70	0.035*	1.17	0.113
Distance between replicates	2.22	0.003**	2.49	0.004**	1.42	0.011*

All CCA models significant based on anova ($p \leq 0.01$).
P-values are followed by "*" for $p \leq 0.05$, "**" for $p \leq 0.01$, and "***" for $p \leq 0.001$.
*Redundant variables removed (VIF >= 20) which included total N, max daily air temperature, and min daily air temperature.
┼ Redundant variables removed (VIF >= 20) which included soil temperature, max daily air temperature, and min daily air temperature.

Table S3.4 Mann-Whitney U tests on relative abundances of highly abundant microbial phyla and classes between CT wheat and TGP.

		Mann-Whitney U test*		
Phylum/Class		Mann-Whitney W	p-value	Effect of land conversion
Acidobacteria		8945954	< 2.20E-16	↓
	Acidobacteria_Gp1	805112	1.39E-08	↑
	Acidobacteria_Gp3	315256	2.14E-11	↑
	Acidobacteria_Gp4	278258	< 2.20E-16	↓
	Acidobacteria_Gp5	25425	< 2.20E-16	↓
	Acidobacteria_Gp6	251511	< 2.20E-16	↓
	Acidobacteria_Gp7	28974	4.46E-08	↓
	Acidobacteria_Gp16	28592	2.34E-03	↓
	Acidobacteria_Gp17	2535	< 2.20E-16	↓
Actinobacteria		12447030	< 2.20E-16	↑
	Actinobacteria	11329842	< 2.20E-16	↑
	Thermoleophilia	25116	< 2.20E-16	↓
Armatimonadetes		275936	1.72E-04	↑
Bacteroidetes		14609563	< 2.20E-16	↑
	Bacteroidia	130016	< 2.20E-16	↓
	Cytophagia	867636	3.25E-01	NS
	Sphingobacteriia	4547038	< 2.20E-16	↑
Candidate_division_WPS-1		365526	6.17E-01	NS
Candidate_division_WPS-2		10431	< 2.20E-16	↑
Chloroflexi		4848430	8.34E-13	↑
	Anaerolineae	524084	< 2.20E-16	↓
	Ardenticatenia	6708	6.73E-01	NS
	Thermomicrobia	171814	2.02E-02	↓
	Ktedonobacteria	275000	1.48E-02	↑
Cyanobacteria		60414	< 2.20E-16	↑
Firmicutes		3576756	9.85E-01	NS
	Clostridia	947141	3.18E-05	↑
	Bacilli	455786	< 2.20E-16	↑
Gemmatimonadetes		1766812	1.14E-03	↑
	Gemmatimonadetes	1766812	1.14E-03	↑
Planctomycetes		5527590	< 2.20E-16	↓
	Planctomycetia	4986834	< 2.20E-16	↓
Proteobacteria		130708090	< 2.20E-16	↑
	Alphaproteobacteria	10862558	5.21E-03	↑
	Betaproteobacteria	919741	< 2.20E-16	↑
	Deltaproteobacteria	15550195	< 2.20E-16	↓
	Gammaproteobacteria	6510492	3.32E-07	↑
Thaumarchaeota		5670	2.89E-02	↓
Verrucomicrobia		3147920	< 2.20E-16	↓
	Opitutae	54836	9.04E-07	↓
	Spartobacteria	192696	< 2.20E-16	↓
	Subdivision3	840356	< 2.20E-16	↓

*In Mann-Whitney U test results, "NS" denotes not significant.
All phyla and classes were represented by five of more keystone nodes from networks

Table S3.5 List of preserved module pairs, the number of overlapping and non-overlapping nodes in paired modules.

Pairs*	Overlapping nodes	Nodes only in module1	Nodes only in module2	Nodes absent from both modules	Fisher exact test p-value	Adjusted p-value**
July17_CT_M1 - Aug18_CT_M0	36	207	74	1125	1.59E-05	0.003
July17_CT_M1 - Dec18_CT_M0	52	191	159	1350	5.05E-06	0.001
July17_CT_M1 - Feb18_CT_M2	39	204	75	1169	8.27E-07	0.000
July17_CT_M1 - Jan18_CT_M3	54	189	158	1186	2.28E-05	0.005
July17_CT_M1 - July18_CT_M0	79	164	165	1207	4.55E-14	0.000
July17_CT_M1 - June18_CT_M1	56	187	197	1591	6.30E-07	0.000
July17_CT_M1 - Mar18_CT_M5	22	221	29	1135	9.23E-06	0.002
July17_CT_M1 - Nov17_CT_M2	49	194	135	1201	1.87E-05	0.006
July17_CT_M1 - Oct17_CT_M0	71	172	236	1247	1.38E-06	0.000
July17_CT_M2 - July18_CT_M2	91	209	255	1060	3.59E-05	0.009
July17_CT_M2 - June18_CT_M2	107	193	355	1376	2.17E-08	0.000
Aug17_CT_M14 - Nov18_CT_M16	3	3	3	1265	1.16E-06	0.001
Aug17_CT_M5 - Dec17_CT_M0	15	16	214	1122	3.58E-05	0.007
Aug17_CT_M5 - Jan18_CT_M3	16	15	196	1205	1.20E-06	0.000
Oct17_CT_M0 - Dec17_CT_M0	66	241	163	1082	2.29E-04	0.037
Oct17_CT_M0 - Dec18_CT_M0	60	247	151	1281	2.31E-05	0.005
Oct17_CT_M0 - Jan18_CT_M3	65	242	147	1130	1.49E-05	0.004
Oct17_CT_M0 - July18_CT_M0	71	236	173	1239	1.93E-06	0.001
Oct17_CT_M1 - Jan18_CT_M1	45	222	108	1209	3.26E-05	0.009
Oct17_CT_M3 - Dec18_CT_M1	74	156	281	1228	4.15E-06	0.001
Oct17_CT_M3 - Feb18_CT_M1	43	187	124	1219	4.37E-05	0.011
Oct17_CT_M3 - July18_CT_M2	79	151	267	1222	3.48E-08	0.000
Oct17_CT_M3 - June18_CT_M2	74	156	388	1519	5.27E-05	0.021
Oct17_CT_M3 - May18_CT_M0	55	175	156	1265	3.40E-07	0.000
Nov17_CT_M1 - Aug18_CT_M1	30	148	90	1083	1.70E-04	0.042
Nov17_CT_M1 - Dec18_CT_M1	68	110	287	1152	1.21E-07	0.000
Nov17_CT_M2 - Dec17_CT_M0	49	135	180	1021	1.24E-04	0.021
Nov17_CT_M2 - Dec18_CT_M0	57	127	154	1279	6.27E-12	0.000
Nov17_CT_M2 - Jan18_CT_M3	55	129	157	1112	6.68E-09	0.000
Nov17_CT_M2 - July18_CT_M0	46	138	198	1228	1.37E-04	0.042
Nov17_CT_M2 - Mar18_CT_M5	20	164	31	1080	5.88E-06	0.002
Nov17_CT_M2 - May18_CT_M0	47	137	164	1161	5.59E-06	0.001
Dec17_CT_M0 - Aug18_CT_M0	34	195	76	965	4.08E-04	0.048
Dec17_CT_M0 - Dec18_CT_M0	53	176	158	1188	8.26E-06	0.001
Dec17_CT_M0 - May18_CT_M0	52	177	159	1062	1.96E-04	0.023
Dec17_CT_M2 - Dec18_CT_M1	68	101	287	1119	3.09E-08	0.000
Jan18_CT_M3 - Dec18_CT_M0	47	165	164	1221	7.28E-05	0.014
Jan18_CT_M3 - Feb18_CT_M2	33	179	81	1136	3.91E-05	0.008
Jan18_CT_M3 - July18_CT_M0	55	157	189	1209	7.41E-06	0.002
Jan18_CT_M3 - June18_CT_M1	53	159	200	1574	1.64E-07	0.000
Jan18_CT_M3 - June18_CT_M2	73	139	389	1385	6.05E-05	0.020
Jan18_CT_M3 - Mar18_CT_M5	25	187	26	1045	3.60E-08	0.000
Jan18_CT_M3 - May18_CT_M0	59	153	152	1119	1.24E-08	0.000
Jan18_CT_M5 - Nov18_CT_M22	3	25	2	1395	6.63E-05	0.022
Feb18_CT_M1 - June18_CT_M2	65	102	397	1375	3.75E-06	0.001
Feb18_CT_M2 - Mar18_CT_M5	15	99	36	1050	3.20E-05	0.007
Mar18_CT_M4 - May18_CT_M0	15	15	196	1068	1.51E-05	0.003

Mar18_CT_M4 - Sept18_CT_M8	4	26	6	1093	7.64E-05	0.024
Mar18_CT_M5 - May18_CT_M0	22	29	189	1054	3.22E-06	0.001
Apr18_CT_M0 - Dec18_CT_M1	21	14	334	986	1.97E-05	0.003
Apr18_CT_M0 - June18_CT_M2	22	13	440	1221	8.90E-06	0.002
May18_CT_M0 - Dec18_CT_M0	45	166	166	1289	1.04E-04	0.018
May18_CT_M0 - June18_CT_M1	46	165	207	1497	1.69E-04	0.048
May18_CT_M0 - June18_CT_M2	92	119	370	1334	2.83E-11	0.000
May18_CT_M0 - Sept18_CT_M8	7	204	3	1151	1.52E-04	0.042
May18_CT_M0 - Sept18_CT_M81	7	204	3	1151	1.52E-04	0.042
May18_CT_M1 - June18_CT_M2	67	108	395	1345	8.19E-06	0.002
June18_CT_M1 - Aug18_CT_M0	32	221	78	1507	1.12E-05	0.003
June18_CT_M1 - Dec18_CT_M0	46	207	165	1697	1.32E-05	0.004
June18_CT_M1 - July18_CT_M0	56	197	188	1549	1.45E-06	0.001
June18_CT_M2 - Aug18_CT_M3	50	412	56	1320	3.38E-07	0.000
June18_CT_M2 - Dec18_CT_M1	126	336	229	1424	5.23E-11	0.000
June18_CT_M2 - July18_CT_M2	138	324	208	1320	1.05E-14	0.000
July18_CT_M0 - Dec18_CT_M0	59	185	152	1314	1.78E-08	0.000
July18_CT_M2 - Dec18_CT_M1	103	243	252	1112	5.05E-06	0.001
Oct18_CT_M11 - Nov18_CT_M22	2	3	3	1095	1.64E-04	0.050
Oct18_CT_M11 - Nov18_CT_M221	2	3	3	1095	1.64E-04	0.050

No significantly preserved module pairs in TGP or between TGP and CT wheat based on Bonferroni adjusted p-values.
*The identified module pairs are shown as “network_ID of the first module -- network_ID of the second module”.
**P-values from Fisher exact test were adjusted by the Bonferroni procedure based on the total number of Fisher exact test.

Table S3.6 Number of keystone nodes in each MENs

Land use	Network	No. of network hubs	No. of module hubs	No. of connectors
CT Wheat	Jun-17	1	22	5
	Jul-17	3	25	47
	Aug-17	0	15	0
	Sep-17	0	17	1
	Oct-17	6	26	76
	Nov-17	8	23	87
	Dec-17	0	24	15
	Jan-18	2	32	35
	Feb-18	4	19	48
	Mar-18	0	20	2
	Apr-18	0	4	0
	May-18	2	26	25
	Jun-18	0	50	11
	Jul-18	5	30	37
	Aug-18	2	21	28
	Sep-18	0	17	0
	Oct-18	0	9	0
	Nov-18	0	19	2
	Dec-18	5	34	37
TGP	Jun-17	0	31	0
	Jul-17	0	28	0
	Aug-17	0	41	0
	Sep-17	0	36	0
	Oct-17	0	32	0
	Nov-17	0	32	1
	Dec-17	0	30	0
	Jan-18	0	37	0
	Feb-18	0	23	0
	Mar-18	0	28	0
	Apr-18	0	27	0
	May-18	0	34	0
	Jun-18	0	42	0
	Jul-18	0	53	0
	Aug-18	0	30	0
	Sep-18	0	42	0
	Oct-18	0	22	0
	Nov-18	0	34	0
	Dec-18	0	35	0

Table 3.7 List of network hubs, their taxonomic information, and relative abundance.

ASV	Network	Mod-ule	Phylum	Class	Order	Relative abund.(%)
148	CT_June17	0	Acidobacteria (1.00)	Acidobacteria_Gp1 (1.00)	Unclassified	0.155
299	CT_July17	1	Actinobacteria (1.00)	Actinobacteria (1.00)	Actinomycetales (1.00)	0.141
15	CT_July17	3	Actinobacteria (1.00)	Actinobacteria (1.00)	Actinomycetales (1.00)	0.567
1475	CT_July17	3	Chloroflexi (0.86)	Ktedonobacteria (0.86)	Ktedonobacterales (0.86)	0.035
150	CT_Oct17	0	Chloroflexi (0.82)	Ktedonobacteria (0.81)	Ktedonobacterales (0.80)	0.096
14	CT_Oct17	3	Proteobacteria (1.00)	Alphaproteobacteria (1.00)	Sphingomonadales (0.99)	0.580
195	CT_Oct17	1	Acidobacteria (1.00)	Acidobacteria_Gp1 (1.00)	Unclassified	0.256
105	CT_Oct17	1	Proteobacteria (1.00)	Alphaproteobacteria (1.00)	Rhizobiales (0.99)	0.195
311	CT_Oct17	0	Actinobacteria (1.00)	Actinobacteria (1.00)	Acidimicrobiales (0.98)	0.090
270	CT_Oct17	3	Bacteroidetes (1.00)	Sphingobacteriia (1.00)	Sphingobacteriales (1.00)	0.075
153	CT_Nov17	1	Actinobacteria (1.00)	Actinobacteria (0.99)	Solirubrobacterales (0.92)	0.085
336	CT_Nov17	0	Actinobacteria (1.00)	Actinobacteria (0.99)	Solirubrobacterales (0.98)	0.053
38	CT_Nov17	1	Proteobacteria (0.97)	Alphaproteobacteria (0.97)	Alphaproteobacteria_incertae_sedis (0.60)	0.376
117	CT_Nov17	4	Actinobacteria (0.99)	Actinobacteria (0.98)	Gaiellales (0.95)	0.148
972	CT_Nov17	0	Proteobacteria (1.00)	Deltaproteobacteria (0.98)	Myxococcales (0.96)	0.061
57	CT_Nov17	0	Acidobacteria (1.00)	Acidobacteria_Gp1 (1.00)	Unclassified	0.273
80	CT_Nov17	2	Firmicutes (0.69)	Clostridia (0.32)	Clostridiales (0.26)	0.162
105	CT_Nov17	1	Proteobacteria (1.00)	Alphaproteobacteria (1.00)	Rhizobiales (0.99)	0.163
57	CT_Jan18	2	Acidobacteria (1.00)	Acidobacteria_Gp1 (1.00)	Unclassified	0.276
110	CT_Jan18	4	Actinobacteria (1.00)	Actinobacteria (1.00)	Actinomycetales (1.00)	0.136
60	CT_Feb18	0	Acidobacteria (1.00)	Acidobacteria_Gp1 (1.00)	Unclassified	0.155
294	CT_Feb18	1	Actinobacteria (1.00)	Actinobacteria (0.79)	Solirubrobacterales (0.68)	0.073
303	CT_Feb18	2	Actinobacteria (1.00)	Actinobacteria (1.00)	Actinomycetales (1.00)	0.060
1266	CT_Feb18	8	Actinobacteria (1.00)	Actinobacteria (1.00)	Actinomycetales (1.00)	0.027
171	CT_May18	3	Actinobacteria (1.00)	Actinobacteria (1.00)	Actinomycetales (1.00)	0.131
2778	CT_May18	3	Proteobacteria (1.00)	Betaproteobacteria (1.00)	Burkholderiales (0.99)	0.125
617	CT_July18	7	Actinobacteria (1.00)	Actinobacteria (0.99)	Gaiellales (0.98)	0.038
161	CT_July18	9	Proteobacteria (0.97)	Deltaproteobacteria (0.92)	Myxococcales (0.76)	0.056
853	CT_July18	6	Acidobacteria (1.00)	Acidobacteria_Gp3 (1.00)	Unclassified	0.024
136	CT_July18	7	Actinobacteria (1.00)	Actinobacteria (1.00)	Actinomycetales (1.00)	0.286
345	CT_July18	1	Actinobacteria (1.00)	Actinobacteria (1.00)	Actinomycetales (1.00)	0.061
15	CT_Aug18	3	Actinobacteria (1.00)	Actinobacteria (1.00)	Actinomycetales (1.00)	0.701
105	CT_Aug18	1	Proteobacteria (1.00)	Alphaproteobacteria (1.00)	Rhizobiales (0.99)	0.113
65	CT_Dec18	4	Thermotogae (0.14)	Thermotogae (0.14)	Petrotogales (0.14)	0.213
183	CT_Dec18	3	Bacteroidetes (0.33)	Cytophagia (0.13)	Cytophagales (0.13)	0.137
131	CT_Dec18	3	Gemmatimonadetes (0.92)	Gemmatimonadetes (0.92)	Gemmatimonadales (0.92)	0.069
181	CT_Dec18	0	Nitrospirae (0.96)	Nitrospira (0.96)	Nitrospirales (0.96)	0.166
196	CT_Dec18	6	Chloroflexi (0.35)	Ktedonobacteria (0.30)	Ktedonobacterales (0.30)	0.215

Network hubs have Zi greater than 2.5 and Pi greater than 0.62.
No TGP networks had networks hubs.

Table S3.8 Indexes for determining network stability

Index	Process	Formula	Details
Robustness	simulated	$\mathrm{wMIS}_i = \frac{\sum_{j \neq i} b_j \, sij}{\sum_{j \neq i} bj}$	The abundance-weighted mean interaction strength (wMIS) of node *i* where b_j is the relative abundance of species *j* and s_{ij} is the association strength between species *i* and *j*, which is measured by Pearson correlation coefficient. After removing the selected nodes from the MEN, if wMIS*i* = 0 (all the links to species *i* have been removed) or wMIS*i* < 0 (not enough mutualistic association between species *i* and other species for its survival), node *i* was considered extinct/isolated and thus removed from the network. This process continued until all species had positive wMISs.
Vulnerability	simulated	$\max\left(\frac{E - E_i}{E}\right)$ $E = \frac{1}{n(n-1)} \sum_{i \neq j} \frac{1}{d(i,j)}$	The vulnerability of a network is indicated by the maximal vulnerability of nodes in the network where *E* is the global efficiency and E_i is the global efficiency after removing node *i* and its entire links. Next, the global efficiency of a graph was calculated as the average of the efficiencies over all pairs of nodes where *d*(*i*,*j*) is the number of edges in the shortest path between node *i* and *j*.
Node constancy	empirical	$\frac{\mu_i}{\sigma_i}$	Constancy measures temporal stability of species and is defined as μ/σ, where μ is the mean of abundance over time and σ is the standard deviation. Here, we constancy was calculated as the constancy of node *i* when μ_i is the mean and σ_i is the standard deviation of abundances of species *i* in the networks from different timepoints. The abundance of species *i* at a certain time point was positive only if species *i* was in the MEN at that time point. Otherwise, the abundance of species *i* was considered zero for that time point. For $\sigma_i = 0$, the constancy was not finite and thus removed from subsequent analyses.
Link constancy	empirical	$l_{ij+} = \frac{\mu_{l_{ij+}}}{\sigma_{l_{ij+}}} \quad l_{ij-} = \frac{\mu_{l_{ij-}}}{\sigma_{l_{ij-}}}$	Link constancy was adapted from node constancy and calculated in a similar manner. l_{ij+}=1 if nodes *i* and *j* were positively linked in a network, l_{ij-}=1 if nodes *i* and *j* were negatively linked in the network, and $l_{ij+} = l_{ij-} = 0$ is there was no link between *i* and *j*. For example, μ_{lij+} is the mean and σ_{lij+} is the standard deviation of l_{ij+} in all the networks from different time points, and μ_{lij-} and σ_{lij-} are the mean and standard deviation of l_{ij-} in all the networks from different timepoints. For σ_{lij+}, the constancy of l_{ij+} was not finite and thus removed from subsequent analyses, and similarly for l_{ij-}.
Node persistence	empirical	$\frac{\sum_{k=1}^{S} \prod_{i=1}^{v} \delta_{i,k}}{S}$	The node persistence is defined as the proportion of coexisting species (over the total number of species) at an ecological regime. It was calculated as the he percentage of nodes present in the network in multiple consecutive months where *v* is the number of samples taken from the same field plot at multiple consecutive time points. *S* is the total ASV number in the networks, and $\delta_{i,k}$ is a Dirac delta function with $\delta_{i,k}$=1 if the abundance of ASV *k* in sample *i* is larger than 0 and $\delta_{i,k}$=0 if ASV *k* is not present in sample *i*.
Compositional stability	empirical	$\sqrt{\frac{\sum_{k=1}^{S} v(\min_i y_{i,k})}{\sum_{k=1}^{S} \sum_{i=1}^{v} y_{i,k}}}$	The compositional stability evaluates the change in community structure over time. Compositional stability was calculated for a microbial community in the networks using the sample x ASV matrix where *v* is the number of samples taken from the same field site/plot at multiple consecutive timepoints. *S* is the total number of ASVs in the network. $yi_{,k}$ is the abundance of ASV *k* in sample *i*. If community structure has no change ($y_{i,k} = \min(i)\ y_{i,k}$) the stability index is equal to 1. If community structure is completely different among timepoints (min(*i*) yi,k=0) the stability index is 0.

Appendix B : Supplementary Figures

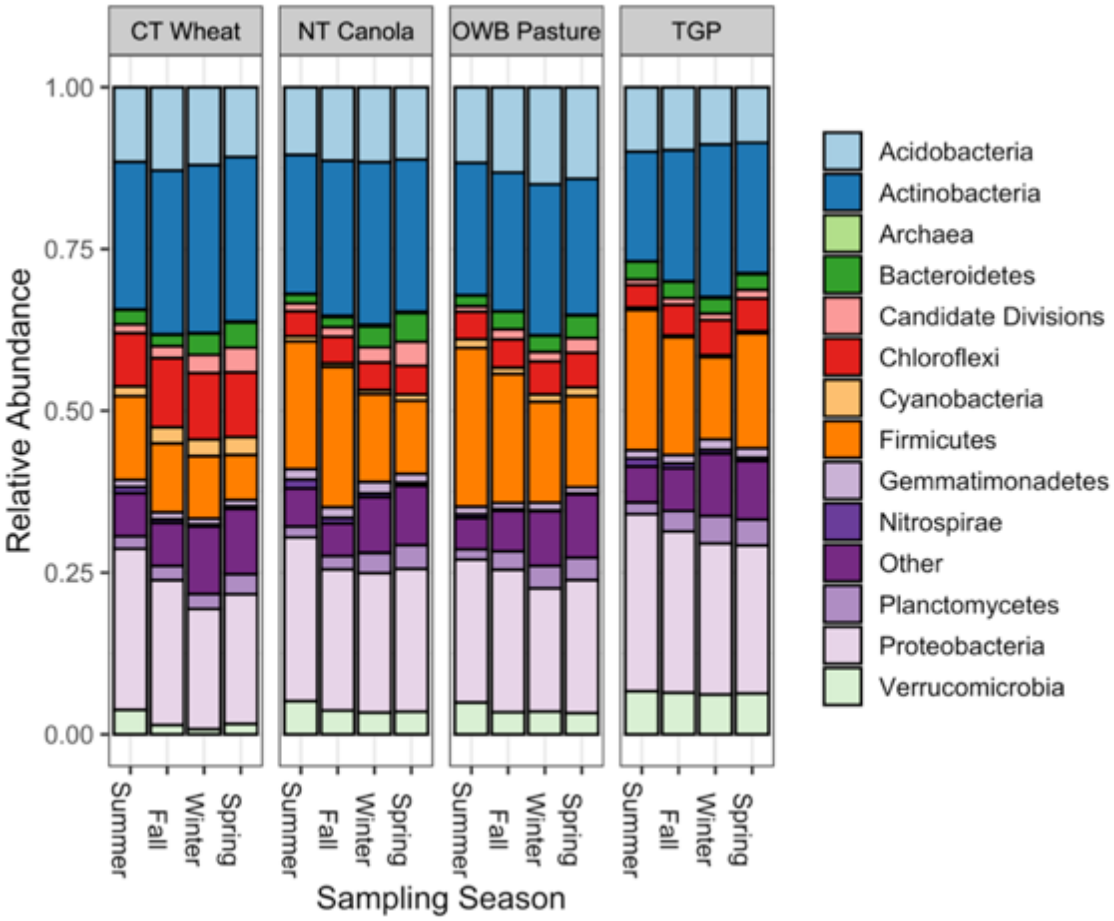

Figure S2.1 Relative abundance of dominant bacterial phyla in soils separated by land use and season. Based on proportional frequencies of DNA sequences classified at the phylum level. Fields include conventionally tilled (CT) wheat, no-till (NT) canola, Old World Bluestem (OWB) pasture, and native tallgrass prairie (TGP).

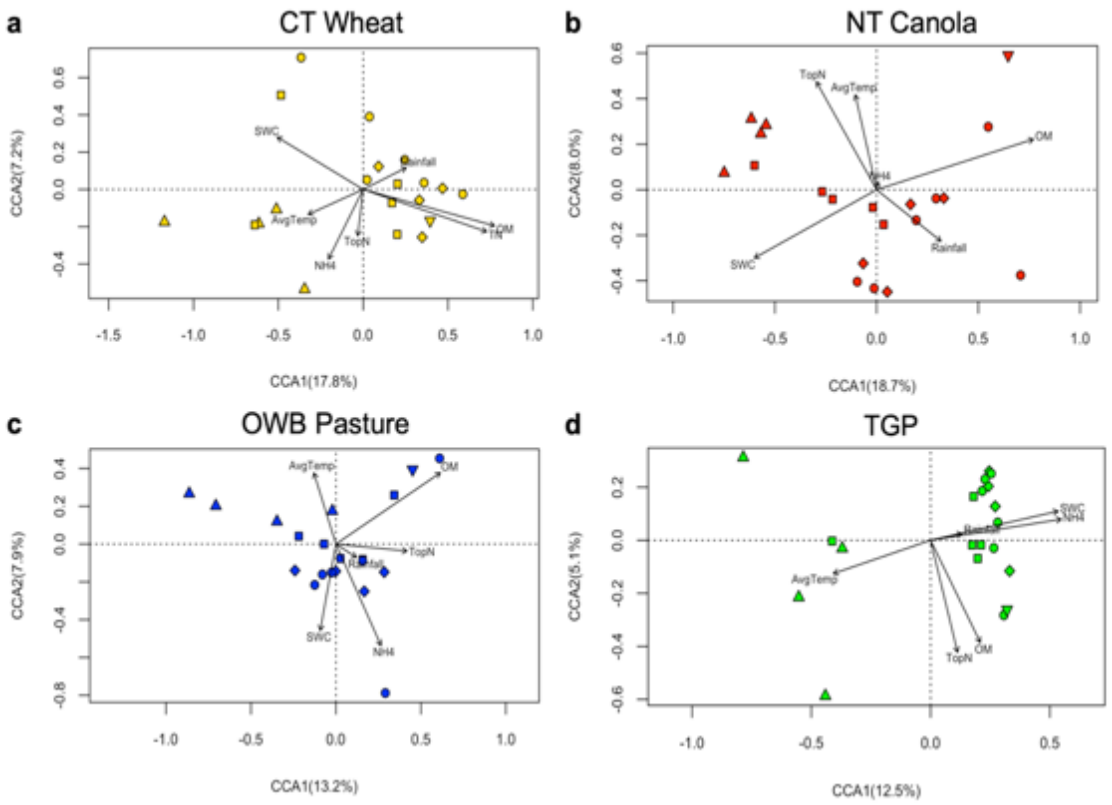

Figure S2.2 Canonical correspondence analysis (CCA) for each land use type. Redundant variables were removed if present (VIF > 15). a) conventionally tilled (CT) wheat, b) no-till (NT) canola, c) Old World Bluestem (OWB) pasture, d) native tallgrass prairie (TGP). All CCA formula significant ($p < 0.05$).

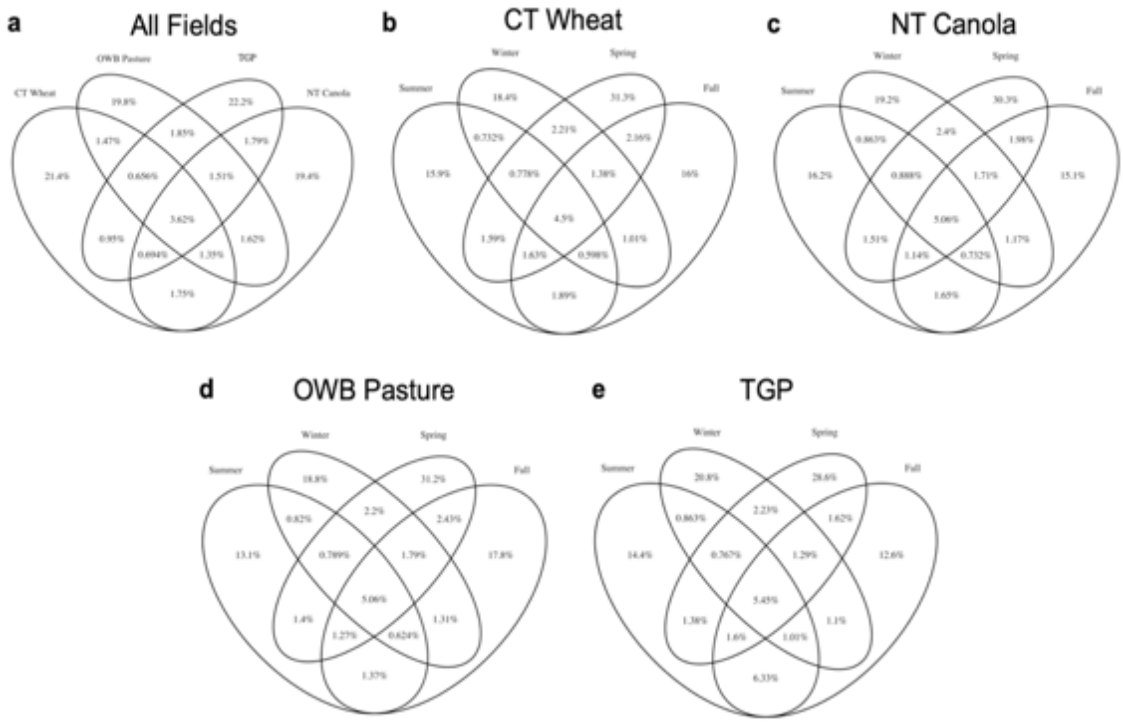

Figure S2.3 Unique OTUs across different land uses (a) and seasons (b-e). The percentage of unique OTUs found in each season presented using a Venn diagram.

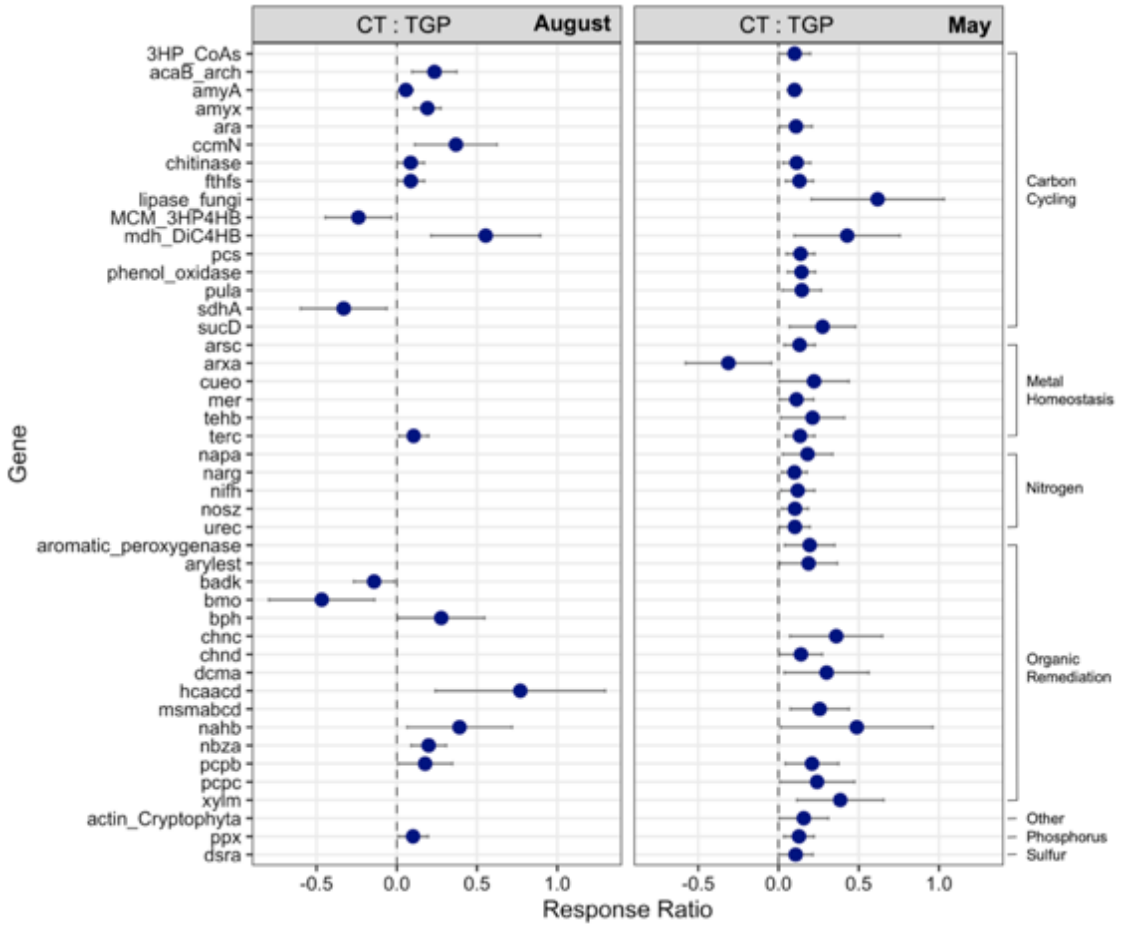

Figure S2.4 Differences in relative gene abundance during August and May between the tallgrass prairie (TGP) and CT wheat function community based on response ratio. All genes greater than 0.0 were greater in the prairie community. All genes present significantly different by 90% confidence interval, * 95% confidence interval, and ** 99% confidence interval.

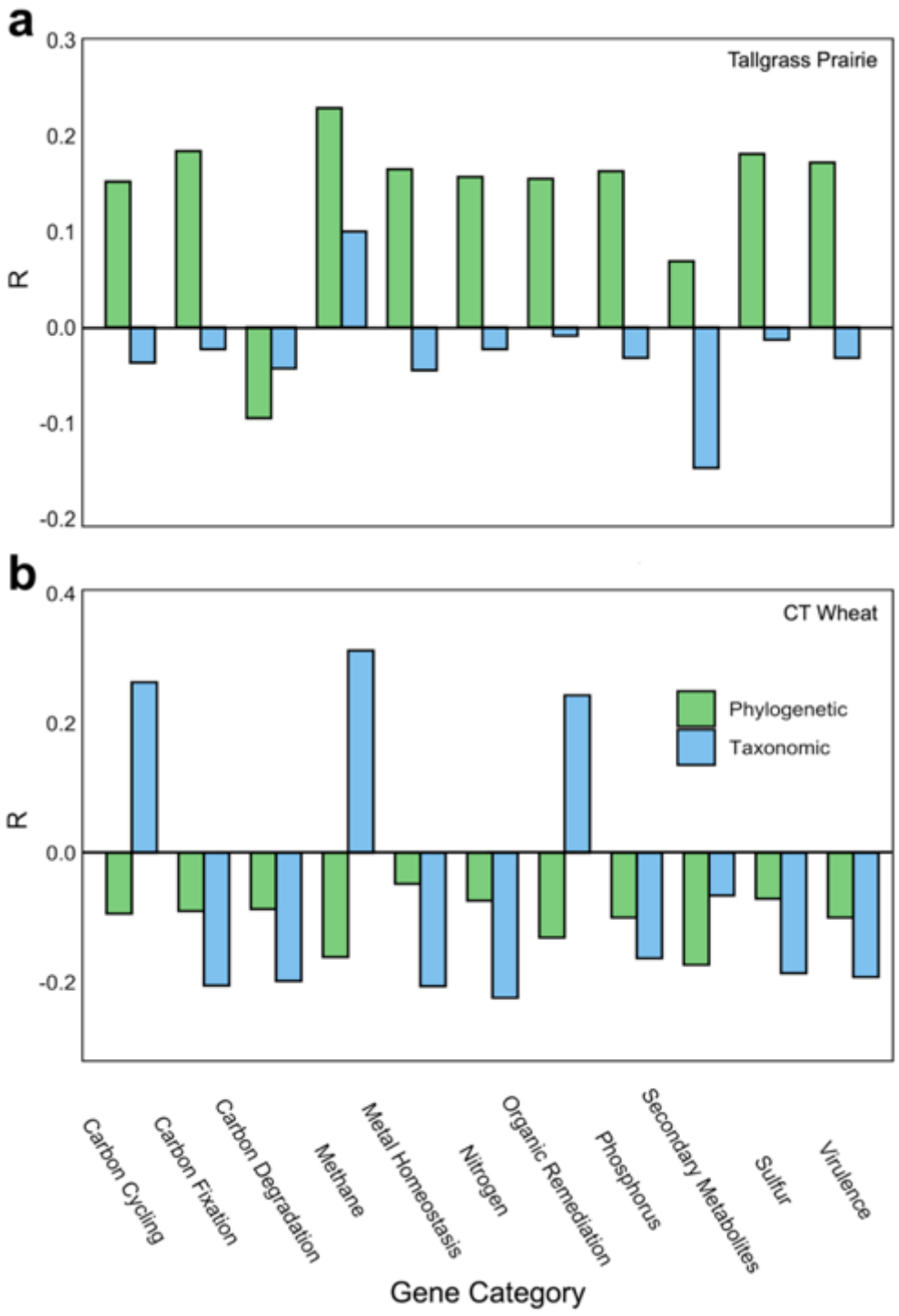

Figure S2.5 Relationship between functions diversity, taxonomic diversity, and phylogenetic diversity for tallgrass prairie (TGP) and CT wheat based on Mantel test. Mantel test used Pearson correlations. Taxonomic diversity was based on Bray-Curtis distance metric and UniFrac weighted distance metric was used for phylogenetic diversity. GeoChip functional data was grouped by probes into main gene categories.

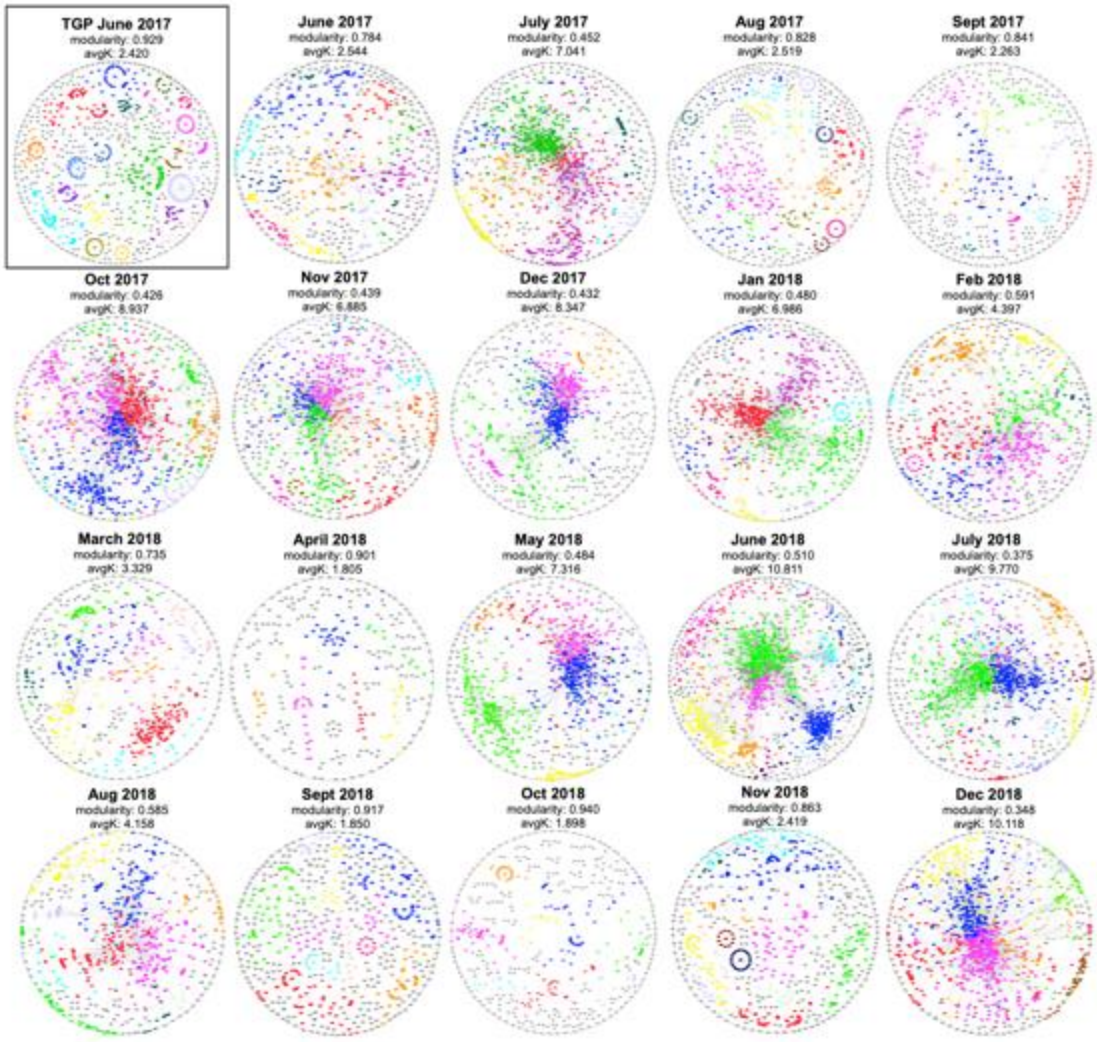

Figure S3.1 Visualization of soil microbial networks over time. MENs were constructed for nineteen sampling months from June 2017 – December 2018. As networks on the native TGP system remained relatively similar over, a single visual representation of the control site is outlined in a black square. The other networks depict the temporal differences of the nineteen MENs in the CT wheat land use. Large module with ≥10 node are shown in different colors, and smaller modules are shown in gray. The average K and modularity is shown above each network. Detailed network topological attributes are listed in Supplementary Table 3.1.

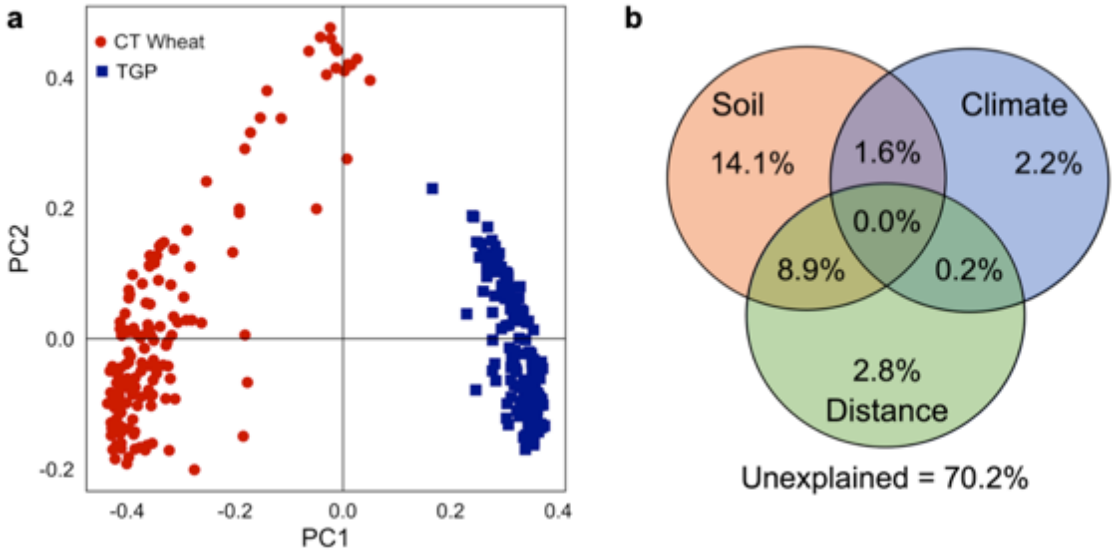

Figure S3.2 Networked community structure differences and environmental drivers. a) Principal coordinate analysis (PCoA) of the structure of the networked communities based on Bray-Curtis distance. Networked communities were significantly different ($p \leq 0.05$) based on land use and sampling month according to three non-parametric permutations tests found in Table 1. Red circles represent networks under CT wheat land use and blue squares represent native TGP (control) land use. b) Variation partitioning analysis (VPA) based on significant ($p \leq 0.01$) CCA model. Soil category includes soil temperature, soil water content (SWC), soil pH, topsoil nitrate (NO_3^-), ammonia (NH_4^+), soil organic matter (OM), and available phosphorus (P). Climate category includes average rainfall and average air temperature. Details of the CCA model can be found is Supplementary Table 3.3.

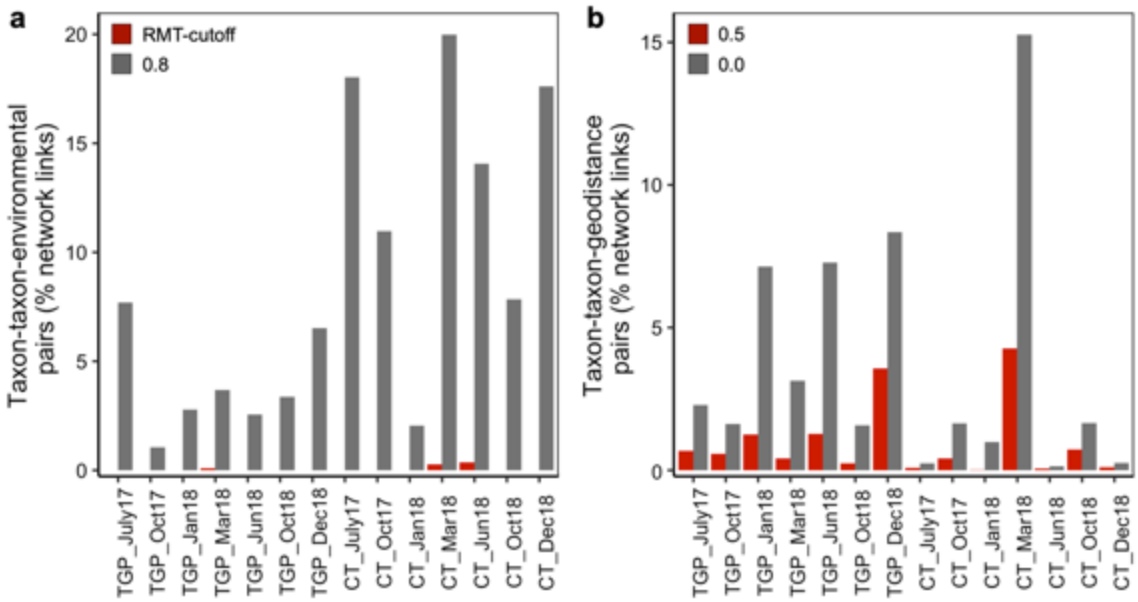

Figure S3.3 Detecting the contributions of environmental filtering or dispersal limitation to the observed network links using the Link Test for Environmental filtering or Dispersal limitation (LTED). a) The links in the MENs were tested with the 12 soil and climatic variables at the network correlation cutoff (St = 0.96) and a lower correlation threshold cutoff of $|r| \geq 0.8$. In short, if a link between two taxa was caused by their covariation with environmental conditions, strong correlations between each taxon and the responsible environmental variable should be observed. b) If dispersal limitation simultaneously affects the abundance distribution of two species across space, the abundances of both species are expected to covary with spatial distance. Therefore, assuming dispersal limitation was the only factor governing community assembly, the farther away the sampling locations, the larger difference in the observed species abundances. For a pair of linked nodes in a network, it was tested whether significant ($p \leq 0.05$, $r > 0$) and significant strong positive ($p \leq 0.05$, $r \geq 0.5$) correlations were observed simultaneously between the pairwise distance among sampling locations, and the difference in their relative abundance among samples based on Pearson correlation.

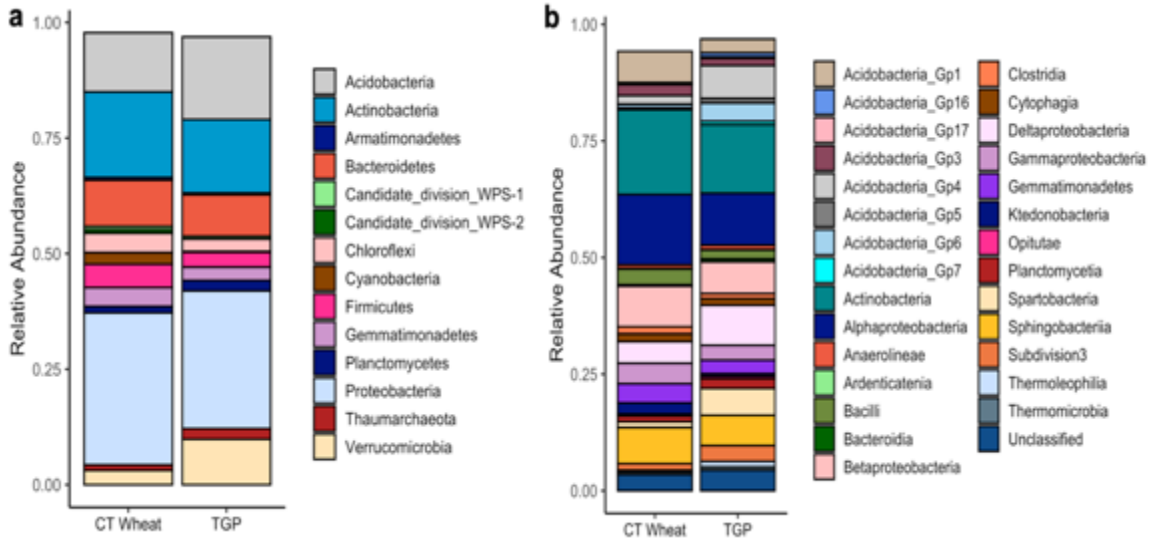

Figure S3.4 Taxonomic composition of networked microbial communities under CT wheat cropland use and native prairie land use**.** Microbial community relative abundance at the a) phylum level and b) class level. Detailed changes between taxa based on land use conversion for long-term CT wheat land use in Supplementary Table 2. Based on Mann-Whitney U test, Acidobacteria, Thaumarchaeota, and Verrucomicrobia significant decreased on average under cropland use. While Proteobacteria, Cyanobacteria, Chloroflexi, Bacteroidetes, and Actinobacteria significantly increased on average under cropland use. Overall, the relative abundance of eight phyla and eleven classes that significant increased ($p \leq 0.05$) under cropland use.

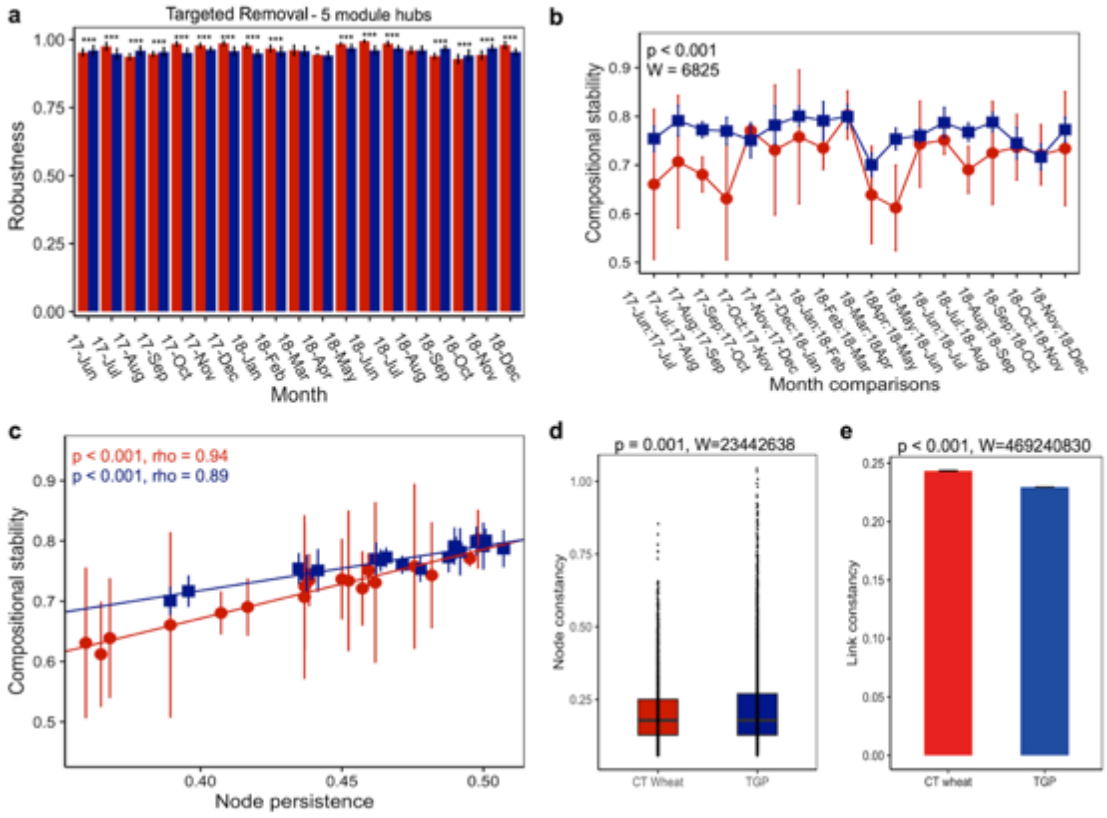

Figure S3.5 Additional temporal dynamics of network stability. a) Robustness measured by removing 5 module hubs from each of the empirical MENs. For April 2018, only 4 module hubs removed as that was the total module hubs in CT wheat for that network. CT wheat shown in red and native TGP shown in blue for all graphs. Error bars represent the standard deviation of 100 repetitions of the simulation. Robustness for each timepoint was compared between CT wheat and native TGP land use using a two-sided t-test. Significant differences are indicated by "*" for $p \leq 0.05$, "**" for $p \leq 0.01$, and "***" for $p \leq 0.001$. b) Compositional stability of the networked community over time shown as consecutive monthly comparisons. Overall compositional stability between CT wheat and native TGP land use compared using Mann-Whitney U Test. c) Spearman correlation of compositional stability and node persistence for CT wheat and native TGP land use. Spearman's correlation coefficient and p-value shown in graph. d) Network node constancy. Each box shows the constancy distribution of all node, averaged between land uses. e) Unweighted network link constancy. Each box shows the constancy distribution of the links in the networks under each land use. For c and d, Mann-Whitney U Test was used to compare differences in constancy between the CT wheat and native TGP land use.

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