Aboveground-belowground interactions: Investigating the role of plant communities in structuring soil bacterial communities

BY

Cheryl A. Murphy

Submitted to the graduate degree program in Ecology and Evolutionary Biology and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

_____
Chairperson: Dr. Bryan Foster

_____
Dr. Helen Alexander

_____
Dr. Sharon Billings

_____
Dr. David Fowle

_____
Dr. Jennifer Roberts

Date Defended: August 22, 2012

The Dissertation Committee for Cheryl A. Murphy
certifies that this is the approved version of the following dissertation:

Aboveground-belowground interactions: Investigating the role of plant communities in structuring soil bacterial communities

_____
Chairperson: Dr. Bryan Foster

Date approved: August 22, 2012

ABSTRACT

Environmental heterogeneity is a traditional explanation for the biodiversity observed in nature and could be key in structuring soil microbial communities. From the soil microbe perspective, heterogeneity can be generated by both edaphic properties and by plant communities through litter inputs and root exudation. This dissertation focuses on investigating the role that plant communities play in potentially creating environmental heterogeneity within the soil and how that impacts soil bacterial community structure.

Soil biota can be influenced at both local and regional scales. In Chapter 1, within a restoration context, I not only determined if plant restoration resulted in soil bacterial community restoration, I compared the relative importance of soil properties, plant communities and regional processes within an old-field and remnant prairie. I found that the capacity of the plant community to influence soil bacteria varied depending upon restoration age and land-use history.

In Chapter 2, I tested the hypotheses that an increase in plant richness could promote a more diverse rhizosphere bacterial community and if any response was a result of differing plant species harboring distinct bacterial compositions (plant identity effect). Results indicated that plant identity and plant presence were more important for structuring rhizosphere bacterial communities than plant richness, potentially because not enough environmental heterogeneity was generated within the overall rhizospheres to elicit a response by the bacteria.

Rhizodeposition can vary temporally in response to plant phenology, potentially influencing the detection of a plant identity effect over time. Further, rhizosphere bacterial compositions could display seasonal patterns by responding to root exudation patterns generated by plant phenology. In Chapter 3, rhizosphere bacterial communities of a forb species exhibited seasonal changes potentially associated with plant phenology, whereas those of two grass species

changed over time, but not seasonally. These different temporal patterns generated conditions in which the plant identity effect of the forb was not permanent.

Overall, my results show that resource heterogeneity promoted by plant communities, both spatially and temporally, can be an important, but not exclusive component, in shaping soil bacterial communities. Further, the influence of the plant community can vary depending upon species, plant phenology, and community composition.

ACKNOWLEDGEMENTS

Despite finishing my Ph.D. in Tennessee, the 10 years that I spent in Lawrence working on both my Masters and Doctorate degrees were wonderful. The research that I have been able to do has been fun, sometimes frustrating and ultimately a very rewarding experience. But one of the best parts has been the people that I have met along the way. Many have helped me throughout this journey and, below, I briefly take a moment to give my thanks.

First and foremost, I would like to thank my advisor, Dr. Bryan Foster. I could not have chosen a better advisor for my time at KU and I appreciate everything he has taught me. At the beginning of my Ph.D., he was very willing to support my desire to explore soil ecology, even as we were both venturing into a field that was new to both of us. I would also like to thank him for all of his guidance and patience while finishing my degree long-distance. It's always harder to finish away from the lab, but his understanding about my situation made it that much easier.

I would also like to thank the members of my committee for all of their support and helpful suggestions during the course of my degree: Drs. Helen Alexander, Sharon Billings, David Fowle and Jennifer Roberts. I am happy to say that I have been able to work one-on-one with each of them in some capacity while I was in Lawrence. I was a TA for both Helen Alexander and Sharon Billings and I learned a lot about teaching from them both. Helen was kind enough to allow me to plow up one of her past experimental sites to use for my rhizosphere study. Further, I was able to work in Sharon's lab to analyze part of my soil samples, which I am very thankful for. I would not have been able to do this research without the help of David Fowle and Jennifer Roberts. They both loaned out lab space and use of their equipment for the majority of my research. I cannot say how much I appreciate their generosity.

My office mates have been an invaluable part of my life. I would be hard pressed to find a better group of people to be my friends and work with at the same time. They have always been willing to talk, go over research ideas, edit manuscripts, help with fieldwork, let me crash at their place and especially have a good time. Through the course of the past six years, we have all moved on to other places to do other things, but have still kept in touch and I hope that we will continue to do so in the future. To Todd Aschenbach, Cathy Collins, Tim Dickson, Sarah Hinman, Irene Khavin and Erin Questad: thank you so much for everything!!

I want to give a special thank you to Lisa Tiemann, my fellow graduate student. You gave me a place to stay for many months when I was in town to finish my lab work. You were a kindred spirit and a sounding board as we were struggling to use DNA techniques with Kansas soils. Your friendship has been priceless.

To Karla Leslie and Arne Sturm within the Fowle and Roberts Lab: thank you for sharing your lab space with me. It was not something that you had to do and I greatly appreciate your generosity throughout the past several years. Especially to Karla, we were able to commiserate together about our PCR results, or lack there of, and getting PLFA glassware cleaned. It has been a pleasure to work with you both.

Many people helped me learn and fine-tune the molecular techniques for this project, something I was doing from scratch. Andrew O'Neill got me started learning how to extract DNA, run a PCR and a gel, things I had not done before. Tim West gave me some lab space at the beginning of my adventure so I could iron out the kinks of DNA extraction and also process the soils I had collected. Dr. Belinda McSwain Sturm helped me get through a rough patch with my PCRs – they were not amplifying and she helped me figure out why. I also took up a lot of freezer space with my soil samples and Drs. Mark Richter and Paulyn Cartwright were kind to

donate some of their freezer space for my cause. Michael Grose helped me through the DNA sequencing at the Molecular Phylogenetics Lab in the Natural History Museum. Lastly, I would like to thank the Department of Biological and Environmental Sciences at the University of Tennessee-Chattanooga for lab and freezer space that allowed me to process soils while living in Tennessee.

I would not have been able to set up my experiment without the help of those that work at NESA and KBS: Dean Kettle, Bruce Johanning (for plowing and mowing my field site), Galen Pittman (ongoing field support) and Scott Campbell (helped me find my field site). There are so many people that have helped me with the soil processing and data collection used for this dissertation. Without their help, I think I would still be trying to collect rhizosphere soil from all of my plots. Thanks! Grant Adams, Alex Bittel, Paul Crangle, Ariana Jones, Kane Keller, Dan Lygisse, Omar Malik, Becky Peters, Sara Pittman, Diane Slocum, Lynn Swafford, Jackie Wilson, and Andrea Wolfe.

Funding for my research has come from various sources. Much of the molecular work was funded through the University of Kansas General Research Fund. Support for Research Assistantships and supplies were provided by the grants NSF 614488 and NSF 950100. The University of Kansas Field Station and Ecological Reserves Small Grants Program and EEB Student Endowment Funds helped fund many soil property and molecular analyses. My results were presented at national meetings and were funded, in part, by the Graduate Student Paper Presentation Travel Awards from EEB. I was able to attend a Soil Ecology Course in The Netherlands partly through the financial support of KU Women 4 KU Women Fund and the Graduate Student Research Training Travel Award.

My family has been great throughout this whole thing. I am sure that when they heard I was going to stay in school and work towards a Doctorate degree they thought I was a bit crazy, but they supported me none-the-less. I would also like to thank my sister, Christine, and my parents for their help with Norah during my defense week. Without their assistance, I would not have been able to defend this early in the semester.

Last, but definitely not least, I would like to thank my other half, Stylianos Chatzimanolis for all of his support, love and maybe not so gentle pushing to get finished over the past eight years. It was not easy for either of us to be living apart while I was doing the bulk of my research in Lawrence or when I was driving back and forth from Tennessee and Kansas. He donated some of his lab space so I could finish processing the soils and so I could escape from the house and do some writing undisturbed. He was wonderful after Norah was born, giving up a lot of his time to make sure that I got time to write, something that he did not have to do: my deepest love and thanks. And to Norah – you were the best motivation to finish my dissertation. I hope I am and will be a positive female role model for you as you grow up and that this dissertation is an example that you are capable of doing anything you set your mind to.

TABLE OF CONTENTS

GENERAL INTRODUCTION

Soil is a vital component of ecosystems: it regulates plant productivity and sustains a vast array of biogeochemical processes such as carbon and nitrogen cycling (Zak et al. 2003). The majority of these soil processes (up to 90%) are reactions mediated by soil microorganisms. Thus, soil microbes are essential to ecosystem functioning (Spehn et al. 2000; Nannipieri et al. 2003). A basic assumption has been that soil microbial communities are functionally redundant. In other words, regardless of the microbial community composition, when placed in similar environments the communities will function in an identical manner (van Elsas et al. 2012). However, more and more we are finding that soil microbial composition and diversity can be important for ecosystem processes such as decomposition rates (Strickland et al. 2009).

Even with the potential significance of soil microbial community composition and diversity for ecosystem processes, relative to plants and animals, we know very little about which microorganisms inhabit the soil and the factors that shape microbial communities (Broughton and Gross 2000; Horner-Devine et al. 2004a). This is largely because it has been very difficult to observe and describe soil microbes, the majority of which cannot be cultured in the laboratory (Kent and Triplett 2002; Prosser et al. 2007). But with the recent development of DNA-based techniques, a more complete picture is slowly developing. For example, it has been estimated that the diversity of soil microbes, especially bacteria, is tremendous with thousands of distinct genomes within one gram of soil (Torsvik et al. 1990). Yet, we are just beginning to discover what lies beneath our feet and determining the key factors that structure soil microbial communities will only enhance our understanding of their impact on ecosystem processes.

For over a century, ecologists have been describing the distributions, abundances and diversity of organisms to better understand the mechanisms that regulate biodiversity across

spatial and temporal scales (Tilman and Pacala 1993; van der Gast et al. 2008; Hovatter et al. 2011). To this end, several hypotheses have been proposed that provide insight into the factors that structure communities (e.g. keystone species hypothesis, intermediate disturbance hypothesis, productivity-diversity relationships, neutral theory) (Paine 1966; Connell 1978; Rosenzweig 1995; Hubbell 2001). However, most ecological theory has been developed for macroorganisms with little application to soil microbial ecology until recently (Nannipieri et al. 2003; Smith et al. 2005). For instance, Fierer et al. (2007) applied the concept of r- and K-selected species to soil bacteria. Using observational, experimental and meta-analytical approaches, they found that bacteria such as beta-Proteobacteria corresponded to the r-selected species classification (copiotrophs) because they are fast growing and quickly take advantage of high nutrient supplies. In contrast, Acidobacteria were classified as K-selected (oligotrophs) due to their higher abundance in low C environments and slow growth rates. Horner-Devine et al. (2004b) found that soil bacterial communities display a taxa-area relationship similar to plants and animals: the number of taxa encountered increases asymptotically with increasing area due to factors such as environmental heterogeneity. Still, there is a lot we do not know concerning the patterns of soil microbial diversity and the factors that structure their communities. Incorporating current ecological theory into microbial ecology will not only increase our basic understanding of how soil microbial communities are structured but will aid in our efforts to conserve biodiversity within a world that is increasingly being altered by human activity (Nannipieri et al. 2003; Prosser et al. 2007; van der Gast et al. 2008).

My research focuses on investigating the role that environmental heterogeneity and plant communities play in governing soil bacterial diversity and in shaping the compositions of soil bacterial communities. Niche-based hypotheses have been traditionally applied to try and

account for the diversity we observe in nature (Leibold et al. 2004). The idea is that an increase in resource diversity can result in greater species coexistence by increasing opportunities for resource partitioning. This niche-based view assumes that available species differ in their ecological traits and exhibit trade-offs in their ecological requirements such that each species is limited by a different resource (MacArthur 1958; Harner and Harper 1976; Tilman 2004). Resource niches not only vary spatially, but also temporally, increasing the breadth of heterogeneity within a habitat and potentially influencing community compositions (Chesson and Huntly 1997).

Soil is perhaps the most spatially and temporally heterogeneous environments on Earth, composed of a complex array of physical structures (e.g. soil aggregates and pores) and chemical compounds, with significant fluctuations in temperature, moisture and nutrient availabilities (Schmidt et al. 2007). Many soil ecologists, focusing upon these soil edaphic properties, have found that soil pH, texture, moisture, organic matter and nutrient availability (among others) can all be important in explaining soil bacterial community compositions (Fierer et al. 2003; Fierer and Jackson 2006; Dimitriu and Grayston 2010; Harrison and Bardgett 2010).

Garnering less attention is how soil bacterial communities are impacted by the environmental heterogeneity promoted by the co-occurring plant communities (Hooper et al. 2000; Berg and Smalla et al. 2009). Heterotrophic bacteria rely upon decomposing plant material and plant root exudates for survival (Zak et al. 2003). All else being equal, a more diverse plant community could provide a greater variety of resources through differences in the amount, nature and timing of litter inputs and root exudation, thereby supporting a more diverse assemblage of soil bacteria (Kowalchuk et al. 2002; Innes et al. 2004; Eisenhauer et al. 2010; Lamb et al. 2011). The work encompassed within this dissertation centers around the hypothesis that the plant

community provides an additional means of soil environmental heterogeneity and is an avenue for influencing the soil bacterial community. Throughout this dissertation, I examine the impacts of the plant community on soil bacterial communities in grassland habitats from both an applied (Chapter 1) and theoretical perspective (Chapters 2 and 3), within contrasting soil environments (bulk soil, rhizosphere) and across differing spatial (field landscape, rhizosphere) and temporal scales (one growing season or multiple samplings over two growing seasons). Below I briefly describe the context in which I investigate plant community effects upon soil bacterial communities, followed by a summary of the main objectives, for each chapter.

Chapter 1: Evaluating the roles of plant communities, soil properties and regional processes upon soil bacterial communities within a grassland restoration experiment.

Successful restoration is not only restoring plant communities, but also restoring the ecosystem processes that have been altered. Because soil bacteria are integral to ecosystem functioning, restoring bacterial compositions has attracted great interest. But, to do so, we need a better understanding of what factors govern these communities. Soil bacteria can potentially be influenced at the local scale through environmental heterogeneity generated by both soil properties and the plant community, as described above. Furthermore, restored plant communities could affect soil bacteria directly over the short-term through root exudation and easily decomposable litter or indirectly through longer-term litter inputs and improved soil quality. Simultaneously, regional processes such as dispersal limitations, stochastic assembly and historical legacies could be impacting soil bacterial community structure independent from local niche-based effects (metacommunity theory).

Using a tallgrass prairie restoration experiment located throughout an old-field landscape, I ask if tallgrass prairie plant restoration can produce changes in bulk soil bacterial communities six growing seasons after restoration. I hypothesized that restored tallgrass prairie could directly impact soil bacteria over the short-term if different bacterial communities were observed under the old-field vs. restored plant communities. Second, I determined the relative importance of the spatial heterogeneity of plant communities, soil properties and regional processes for the bulk soil bacterial communities found throughout the restoration experiment. In doing so, I could evaluate the metacommunity dynamics of the soil bacteria. Lastly, to gauge the potential success of the restoration further, both the soil bacterial communities and metacommunity dynamics of the restoration experiment were compared to those of a remnant prairie located nearby.

Chapter 2: Plant presence and species composition, but not plant diversity, influence rhizosphere bacterial communities within a Kansas experiment.

In Chapter 2, the focus shifts from bulk soil bacterial communities throughout an old-field landscape, to the rhizosphere within an experiment 1400m^2 in size. The rhizosphere is the portion of soil that is being directly influenced by the root. Within this area, soil microbes can obtain plant-derived carbon primarily through rhizodeposition, but also from decaying root biomass. The predominant component of rhizodeposition is root exudation in the forms of organic acids, sugars and amino acids. This creates an environment in which the rhizosphere selects for or against particular microbes, increasing microbial biomass, lowering microbial diversity and giving rise to distinct microbial community compositions compared to bulk soil (also known as the plant presence effect or rhizosphere effect). Further, the rhizosphere

communities can be very distinct among differing plant species because root exudation patterns can be very plant-specific (plant identity effect).

Currently, it is thought that with increasing plant species richness, soil bacteria can be positively influenced through an increase in the diversity of root exudates and litter inputs. These increases can potentially lead to a greater diversity of food resources and available niches to support a more diverse bacterial community, although evidence for this is limited. Many that have found a positive relationship between plant richness and soil bacterial diversity have attributed the results to an indirect effect of plant productivity, which is often increased with higher plant richness, and not plant richness *per se*. Further, most of these studies have focused on bulk soil communities with only a few examining rhizosphere bacterial communities. It is possible that rhizosphere bacterial communities could show a stronger response to plant richness via the plant identity effect, because rhizosphere communities can be far more plant-driven than bulk soil communities.

For this study, I used three perennial grassland plant species and established plant communities that were composed of monocultures of all three species, a full combination of 2-species mixtures and a 3-species mixture. Using these treatments, I examined the effects of plant identity on rhizosphere bacterial diversity and composition and determined if increased plant richness (1-3) led to an increase in rhizosphere bacterial diversity. Lastly, by using plant biomass measurements, I established if any observed plant richness effects could be attributed to differences in plant richness *per se*, independent of any differences in plant production.

Chapter 3: Temporal dynamics of soil bacterial communities in the rhizospheres of three perennial grassland species.

As stated above, it is hypothesized that different plant species can harbor distinct microbial communities within their rhizospheres through differences in the compositions and amounts of root exudates (plant identity effect). However, unique rhizosphere microbial communities associated with different plant species are not always found within the literature potentially for several reasons, one being that detecting a plant identity effect could depend upon sampling time. Because patterns of root exudation can vary due to plant phenology, it is possible that the plant identity effect could also vary in response to changing exudation patterns. Further, changes in rhizosphere microbial community structure responding to root exudation patterns associated with plant phenology could exhibit seasonal patterns.

For this study, the same experiment as described in Chapter 2 was used, but I focused on the monoculture plant communities. I sampled rhizosphere bacterial communities from the three perennial grassland plant species four times over two growing seasons, corresponding with the phenology of the plant species (active growth vs. flowering). From these samples, I determined the extent of the plant identity effect among the three plant species and if this effect was maintained over time. Secondly, I investigated if rhizosphere bacterial communities exhibited seasonal patterns corresponding to plant phenology. If a plant species' rhizosphere communities exhibited primarily seasonal patterns, I expected a consistent change in rhizosphere bacterial communities for each year sampled.

With this dissertation, my goal is to not only satisfy my own keen interest and curiosities about the world beneath our feet, but to also contribute to the developing field of soil ecology. By using an approach based upon current ecological theory, I hope to provide further insight into

the elements that shape our soil biota, while also advancing our ability to generalize ecological patterns across all types of organisms, including plants, animals and microorganisms. Given the large abundances, diversities and significance of microorganisms, “ecological theory that has been developed for plants and animals has limited value if it does not apply to microbial communities” (Prosser et al. 2007).

CHAPTER 1: **Evaluating the roles of plant communities, soil properties and regional processes upon soil bacterial communities within a grassland restoration experiment**

Abstract:

Restoration of terrestrial habitats is increasingly focused upon the restoration of both the plant communities and ecosystem function. However, successfully restoring ecosystem function requires knowledge of the factors that govern soil bacterial communities (SBC). These factors can include i) the resource heterogeneity produced by plant communities through short-term direct (root exudates) and/or long-term indirect (litter inputs) effects; ii) the heterogeneity of soil environmental properties; and iii) regional processes which could include dispersal limitations, stochastic assembly and historical legacies. Understanding the relative importance of these factors for SBC structure and function will increase our basic knowledge of soil communities and our ability to restore native ecosystems. I utilized an ongoing tallgrass prairie restoration experiment in northeastern Kansas to assess if seed sowing restored native tallgrass plant communities and produced changes in SBC over the short-term. I further examined the relative importance of the spatial heterogeneity of plant communities, soil environmental properties and regional processes for SBC structure. To evaluate the restoration, a nearby remnant prairie was used as a reference. After six growing seasons, seed sowing restored plant community diversity comparable to the remnant prairie. Within the restoration experiment, SBC were not influenced by plant restoration potentially due to insufficient time since restoration. Instead, the local heterogeneity of soil environmental properties and regional processes were important for structuring SBC. In contrast, plant communities were the primary factor influencing SBC within

the remnant prairie. Different SBC between the restoration experiment and the remnant prairie could reflect differing land-use histories and restoration age.

INTRODUCTION

Historically, the tallgrass prairie encompassed 63 million hectares within the Great Plains of North America. Agricultural activity, since the 1830's, has almost eliminated the tallgrass prairie leaving less than one percent intact (Samson and Knopf 1994). This generates conditions in which native prairie remnants are extremely isolated within a highly fragmented agricultural landscape, increasing the risk for local and regional extinctions of native prairie species and invasion by exotics (Parker et al. 1993; Kindscher and Tieszen 1998; Smith and Knapp 2003; Murphy et al. 2004). Furthermore, fire suppression has promoted the encroachment of woody species into these grasslands (Norris et al. 2001). These alterations have led to concern over the ability to effectively conserve and restore native tallgrass prairie (Kettle et al. 2000).

Restoration of native prairie in abandoned agricultural fields is of great interest because of the many ecosystem services that prairie vegetation provides (Baer et al. 2002). Restoring native grasslands can increase primary production, aid with sequestration of soil carbon, preserve biodiversity and decrease soil erosion as compared with crop agriculture (Metting et al. 2001; Foster et al. 2004). Prairies can also provide enhanced areas for livestock grazing, wildlife habitat, pollination services and recreation. The inherent aesthetic values that arise from the mere fact that the prairie resource exists can be very important as well (Risser 1996).

Restoration has typically been viewed as successful when the target plant communities have been re-established. But there is increasing awareness that restoration of ecosystem

function is critical to the success of tallgrass prairie restoration (Baer et al. 2002; Allison et al. 2005). Restored native plant communities aid in re-establishing ecosystem function through increased primary production and nutrient retention (Kindscher and Tieszen 1998; Foster et al. 2004). Nevertheless, numerous ecosystem functions are harbored within the soil, of which 80-90% are reactions mediated by soil microbial communities, including decomposition, regulation of nutrient availability and transformations of soil organic matter (Spehn et al. 2000; Nsabimana et al. 2004; Harris 2009). Yet, soil microbial communities, especially bacterial, are often overlooked and only recently have been a focus in restoration experimental studies (Potthoff et al. 2006). These studies have shown that the extent of bacterial restoration can be highly variable, dependent upon factors such as the plant community, soil pH, soil texture, time since restoration and land-use history (McKinley et al. 2005; Bach et al. 2010; Card and Quideau 2010). When the aim is to restore an ecosystem, such as tallgrass prairie, incorporating knowledge of soil biota and processes into management plans is important. This cannot be accomplished until we better understand which factors are most important in structuring soil microbial communities, in general, as well as their significance to restoration.

Currently, soil ecologists exploring the factors that govern soil bacterial community structure and diversity have focused upon two main processes. First, niche-based explanations have long been a cornerstone in ecology to account for the variation observed in communities (Leibold et al. 2004). Differences in soil microbial abundance and diversity could, therefore, be the result of the environmental heterogeneity of soil properties, such as soil pH, texture, moisture and organic matter (Ramette and Tiedje 2007; Wang et al. 2008). Of potentially equal importance, but much less studied, is the role of resource heterogeneity fostered by the co-occurring plant communities (Hooper et al. 2000). A more diverse plant community could

potentially support a more diverse assemblage of soil bacteria by offering a greater variety of resource opportunities via increased root exudates and litter diversities (Kowalchuk et al. 2002). Second, spatial isolation of the soil microbial communities throughout the landscape arising from dispersal limitations, historical legacies and/or stochastic assembly could contribute to the patterns observed in soil microbial communities independent from niche-based processes (Ettema and Wardle 2002; King et al. 2010). However, most studies have either concentrated on heterogeneity of soil properties or spatial isolation, while concurrently ignoring plant communities, making it difficult to determine their relative importance to microbial community structure.

When focusing on environmental heterogeneity of soil properties to best explain variation in soil bacterial community structure, many studies have found soil pH to be a primary correlate (Kennedy et al. 2004; Fierer and Jackson 2006; Harrison and Bardgett 2010). However, other soil properties, such as texture, moisture, organic matter, nutrient availability and microbial biomass have all been found to be significant (Fierer et al. 2003; DeGrood et al. 2005; Dimitriu and Grayston 2010; Harrison and Bardgett 2010). Continued research of these soil environmental properties will be needed as more areas are subject to land-use change and soil qualities are dramatically altered (e.g. land degradation can lead to decreased soil organic matter, weakened soil structure and decreased soil pH) (Tiessen et al. 1982; Hazra and Tripathi 1986; Haland 1992). In order to restore soil bacterial communities, knowledge of which soil environmental factors are vital to the target communities will increase our ability to construct and carry out restoration management plans. For example, the time and effort required for increasing soil organic matter levels to restore soil bacterial communities could be very different than if the trait of importance is soil texture.

The restoration of native plant communities and diversity on degraded agricultural land may potentially lead to changes in the soil microbial community through a variety of different direct and indirect mechanisms. Over the short-term, restored plant communities could directly shape soil bacterial communities via increased resource heterogeneity, as a consequence of differences in the quantities and qualities of root exudation (rhizodeposition) and decomposability of litter and roots (Rovira 1969; Nannipieri et al. 2003; Innes et al. 2004; Bezemer et al. 2006; Gruter et al. 2006). Rhizodeposition has been shown to impact soil biota by increasing microbial biomass and changing the dominant microbes (Cheng and Gershenson 2007; Hawkes et al. 2007). For example, the accumulation of species-specific soil pathogens can develop in corn agricultural fields and necessitate crop rotation (Reynolds et al. 2003). Different plant species can vary in their chemical composition (e.g. C/N ratio) and subsequently alter the types of microbes in the soil based upon the microbes' ability to decompose the available litter. Hossain et al. (2010) examined how litter from 15 herbaceous plant species influenced soil microbial communities and found that litter with higher N contents had a greater abundance of Gram-negative bacteria and lower abundances of actinomycetes and fungi. These direct and more immediate effects of plant species and communities could hypothetically occur over a period of a few years. Observing a change in soil bacterial communities and diversity shortly after plant restoration could lend support to the idea that plants can influence soil bacterial communities directly over the short-term.

In contrast, plant community restoration could indirectly impact soil bacterial communities by influencing the soil-building processes that occur over the long-term. Restoring native plant communities has been shown to significantly increase primary production, both above- and belowground (Foster et al. 2007). Over time, these inputs can improve soil quality,

leading to increased soil bacterial diversity and altered communities (Karlen et al. 1999; Ettema and Wardle 2002; Allison et al. 2005; Bach et al. 2010). McKinley et al. (2005) found, when examining a tallgrass prairie restoration chronosequence in Illinois that soil quality indicators and soil bacterial communities of restored sites were shifted towards the values of a native prairie remnant, as compared to an agricultural field. In addition, sites under restoration for longer periods of time (24 years) were more similar to native prairie than those in restoration for a shorter period (7 years). This suggests that over time, through the indirect effects of restoring native plant communities, and therefore improving soil quality, soil bacterial communities can be restored or at least approach that of native remnants.

In addition to the local niche-based effects, microorganisms can have spatial patterns. Horner-Devine et al. (2004b) found that soil bacteria display a taxa-area relationship similar to that of macroorganisms. Others have also found soil bacterial communities to be spatially auto-correlated (Ramette and Tiedje 2007; King et al. 2010; Hovatter et al. 2011). The reasons underlying these spatial patterns are many and difficult to tease apart, but could be crucial to understanding soil biodiversity (Ettema and Wardle 2002). For instance, some proportion of spatial autocorrelation observed in soil bacterial communities could reflect selective effects of the environment that are spatially structured such as the spatial variation of soil environmental properties and/or the spatial distribution of plant communities. Spatial autocorrelation in soil bacterial communities could also potentially arise as a result of spatial isolation through dispersal limitations, historical legacies and/or stochastic assembly that have acted largely independent from the selective effects of the soil environment or the plant community (Ramette and Tiedje 2007). These three factors (soil environmental properties, plant communities and spatial isolation) could explain a certain amount of variability in soil bacterial composition and

diversity. However, each can potentially interact and co-vary and researchers are only starting to develop a means of partitioning them out (Ramette and Tiedje 2007; Mummey et al. 2010). Even though considerable work has been done to gain a better understanding of the relative contribution of niche-based explanations vs. purely spatial effects in metacommunities of macroorganisms (Leibold et al. 2004; Cottenie 2005), the metacommunity dynamics of microorganisms is largely unknown (Ramette and Tiedje 2007). Understanding the relative importance of soil environmental properties, plant communities, and spatial isolation will improve our basic knowledge of the factors structuring soil bacterial communities and will only aid in our ability to restore native tallgrass prairie ecosystems.

This study utilized an ongoing tallgrass prairie restoration experiment in which I assessed the effects of restoration after six growing seasons on both plant and soil bacterial communities within an abandoned old-field landscape in northeastern Kansas. I had four objectives for this study: 1) Determine the extent to which native species seed sowing restored native tallgrass prairie plant community composition and diversity; 2) Examine if plant restoration produced changes in soil bacterial communities, and in doing so, assess potential direct and short-term influences of changes in plant species composition and diversity on soil bacterial communities; 3) Examine the extent to which the bacterial communities approached that observed in a nearby undisturbed native prairie remnant located on similar soil; and 4) Investigate the relative importance of the spatial heterogeneity of soil environmental variables, plant communities, and spatial isolation to the variation observed in the soil bacterial communities across this grassland landscape. Answering these objectives will help elucidate which factors structure soil bacterial communities in this system.

METHODS

Study Sites

The restoration experiment and reference prairie (Dogleg Prairie) are located at the University of Kansas Field Station (KUFS) and managed by the Kansas Biological Survey. These sites are within the eastern deciduous forest-tallgrass prairie ecotone of Northeastern Kansas (Lat 39°03′ N, Long 95°12′ W) and comprised of undulating hills and swales. Soils of both the restoration experiment and Dogleg Prairie are Pawnee clay loams (montmorillonitic, mesic Aquic Argiudolls) formed under glacial deposits of till and loess with weathering of interbedded limestones and shales (U.S. Department of Agriculture Soil Conservation Service 1977). The region has a mean annual temperature of 12.9°C and mean annual precipitation of 930 mm (Atmospheric Science Library 1990).

Restoration Experiment

Experimental Design: The restoration experiment was initiated to explore the effects of restoring native tallgrass plant species diversity on ecosystem processes within a degraded former agricultural landscape (Foster et al. 2004). The 20ha old-field grassland was historically tallgrass prairie with some trees along draws (Kettle and Whittemore 1991). In the early 1900's, the area was plowed for agriculture and later seeded with introduced cool-season grasses and converted into pasture/hay management. The University of Kansas acquired KUFS in 1970 and the experimental study site has not been used for grazing or hay production for at least 30 years. The field has been managed as open grassland with periodic mowing to prevent woody plant encroachment. Fire has not been used as a management tool prior to the establishment of the experiment. Currently, perennial grasses such as *Bromus inermis* (introduced C_3-grass),

Schedonorus phoenix (introduced C_3-grass), *Poa pratensis* (introduced C_3-grass) and *Andropogon virginicus* (native C_4-grass) are dominant throughout the experimental site (Foster et al. 2007).

In 1999, forty-2.5m x 2.5m blocks were established, scattered throughout the old-field grassland in areas indicative of the topographic variation. In each block, four-1m^2 quadrats were established containing one of four randomly assigned treatments: control (Control); seed addition of 24 native prairie species (Restored); annual disturbance (Dist.); and seed addition plus annual disturbance (Restored+Dist.) (Foster et al. 2004). Buffer strips (0.5m) separated each quadrat. For this study, I present results from 18 of the 40 blocks located primarily on upland sites (3.5ha). Only Control and Restored treatments were sampled.

In January 2000, 400 seeds from each of the 24 selected native tallgrass prairie species were sown into the Restored treatment quadrats by hand. The tallgrass prairie species were chosen based upon a range of functional group traits. Further, these plant species occurred naturally within prairie habitats less than two kilometers from the experimental study site (Foster et al. 2004). Table 1 lists the sown native tallgrass plant species.

Sampling Procedures: During the sixth year of the restoration experiment, plants and soils were sampled twice within the growing season which corresponded with peak biomass of the cool- and warm-season plant species that dominated the Control and Restored treatments, respectively: 6-9 June 2005 and 9 September 2005. In order to obtain a representative sample of the plant and soil communities and still have space to allow for both of these sampling dates, sampling strips were used within each quadrat (described below). All plant and soil samples taken during these two time periods were processed separately and later combined to characterize the growing

season as a whole. A more detailed description of combining the seasonal data is presented in the "Data Analyses" section.

Soil bacterial communities were sampled by taking three soil cores (15cm depth) evenly spaced throughout a 0.1m x 1m strip located on the east side of each quadrat (June), which were pooled and immediately frozen at -80°C until further processing. A strip from the west side of each quadrat was sampled in September in the same manner.

After soils were taken to assess bacterial communities, the plant community was sampled by clipping a 0.1m x 1m strip over the same location as the soil samples to optimize the potential association of soil bacterial communities with the plant communities. Plants were clipped at ground level and sorted to live and litter fractions. Live biomass was further sorted to species. All plant materials were dried to constant weight at 74°C. Plant abundance values (based on live biomass) were converted to proportions to allow for comparison with the reference prairie.

Several soil environmental properties were sampled to use as potential predictors for soil bacterial community structure, some of which are described here and some are described below (soil microbial biomass and soil pH). For each sampling period, two additional soil cores (15cm depth) were taken to measure available nitrogen and soil moisture. The cores were sampled within a strip located through the center of each quadrat and pooled, from which two subsamples were taken: 10g of soil was used for the extractable nitrogen procedure (Robertson et al. 1999) and 100g to assess soil moisture and standardize inorganic nitrogen values. Total soil carbon (C), nitrogen (N), and organic matter (OM) were sampled from a single core in the center of each quadrat, only in June. The samples were air-dried and measured using a LECO CN dry combustion analyzer (C and N). Percent OM was determined using the Walkley-Black procedure (Walkley and Black 1934). Estimates of soil texture and cation exchange capacity (CEC) were

acquired from soil sampled in October 2004 as part of a different study (Collins and Foster 2008). From this earlier study, I also obtained estimates of topographic position (elevation) and spatial location (Latitude and Longitude via GPS) for the study quadrats. Elevations within this site ranged between 324.3m and 332.3m (Mean = 328.5m, SD = 2.39, Coefficient of Variation = 0.007).

Reference Prairie

A common approach to studying restoration, and restoration of soil microbes in particular, is to compare the restored site to a reference site (Harris 2009). In this way, one can better evaluate if the restoration is moving towards a target ecosystem. The Dogleg Prairie (DLP), a tallgrass prairie remnant located within 250m of the restoration experimental field site was used as a reference prairie. It should be noted that the native tallgrass prairie species sown in the restoration experiment were representative of prairie remnants throughout the region, coinciding with the original goals of the experiment in 2000, and were not specifically aimed at representing the plant species of the Dogleg Prairie. The Dogleg Prairie was chosen as a reference site due to its similar soils and locality next to the restoration experiment. The two-hectare remnant, which has never been cultivated, is dominated by native warm-season tallgrass prairie grasses such as *Andropogon gerardii*, *Sorghastrum nutans*, *Panicum virgatum* and *Sporobolus heterolepis*. Management since 1990 has consisted of burning the entire remnant approximately every year including a burn on March 31, 2005, three months prior to sampling.

Sampling Procedures: Plant and soil communities were sampled within the Dogleg Prairie on 1 July 2005 and 10 September 2005. Seasonal data were later combined as described below.

Eighteen sampling locations were chosen at random, making certain that locations encompassed the entire remnant (2ha) and represented the topographical variation at this site.

Ocular estimates of percent cover were used to assess the plant communities using the same area as in the restoration experiment (0.1m x 1m strip). Plant aboveground biomass was not sampled at these locations. Soil bacterial communities were sampled in the same manner as the restoration experiment: three equally spaced soil cores (15cm depth) were taken within the 1m-strip area and pooled. Soils were stored at -80°C until further processing.

Similar to the restoration experiment, elevation and spatial location for each sampling location was recorded using GPS. Dogleg Prairie elevations ranged between 315.0m and 332.0m (Mean = 327.6m, SD = 4.16, Coefficient of Variation = 0.013).

Soil Bacterial Communities

T-RFLP Procedure: Roots were removed from the soil and dried to a constant weight (74°C) for root biomass measurements. Soils were sieved to 4mm, homogenized and sub-sampled three times: bacterial community analyses, microbial biomass and soil pH measurements.

Terminal-Restriction Fragment Length Polymorphism (T-RFLP) was used to assess soil bacterial communities. Despite its inability to estimate diversity and structure at fine taxonomic levels, T-RFLP is a reproducible technique having sufficient resolution to detect differences in soil bacterial community structure (as described below) (Fierer et al. 2003).

The following steps were undertaken for the T-RFLP procedure. First, soil DNA was extracted in triplicate for each quadrat and location (restoration experiment and Dogleg Prairie, respectively) using the MoBio PowerSoil DNA extraction kit. The extractions were pooled and

DNA was quantified by gel electrophoresis. Extractions were either concentrated or diluted to obtain a final concentration of 10ng DNA/uL extract.

16S rDNA from the extracted DNA samples was PCR-amplified using the universal eubacterial primers 6-FAM 8-27F, a fluorescently labeled forward primer (5′-AGRGTTTGATCMTGGCTCAG-3′), and 1389R, a non-labeled reverse primer (5′-ACGGGCGGTGTGTACAAG-3′) (Fierer et al. 2003) (Eurofins MWG Operon). For each quadrat/location, PCR reactions were performed in triplicate. Each 50μL PCR reaction contained 50ng DNA, 0.5μM of each primer, and 1x HotStar Taq Master Mix Kit (Qiagen), which included a final concentration of 2.5 units HotStar Taq DNA Polymerase, 1.5nM $MgCl_2$ and 200μM of each dNTP. Reaction mixtures were held for 15min at 95°C for activation. Reactions were cycled 32 times through three steps: 45s of denaturation at 94°C, 45s of annealing at 58°C and 90s for primer extension at 72°C. The final extension lasted 10min at 72°C. The triplicate PCR reactions were pooled, purified using the MoBio UltraClean PCR Clean-Up Kit, and yields quantified using gel electrophoresis.

Products of PCR reactions were initially digested using both *Rsa*I and *Msp*I restriction enzymes (New England Biolabs). However, only the *Rsa*I enzyme completely digested PCR product. Therefore, analyses of soil bacterial communities only included digestions using *Rsa*I. Each 20μL reaction contained 100ng of purified PCR product, 10 units of restriction enzyme and 2μL of buffer. Each reaction was digested for 3h at 37°C with an inactivation period of 20min at 65°C. The fluorescently labeled fragments were analyzed using an Applied Biosystems Instrument 3730 genetic analyzer. Terminal restriction fragment sizes between 25 and 875 bp, with peak heights > 25 fluorescent units were measured using Peak Scanner 1.0 analytical software (Applied Biosystems).

T-RFLP Profile Analysis: The bacterial community profiles were analyzed as described by Dunbar et al. (2001) and Fierer et al. (2003). Standardization of the terminal restriction fragment (TRF) patterns was performed with the following methodology. For each community profile, total peak area was calculated. For each TRF within the community profile, the proportion of total peak area was calculated (individual peak area/total peak area). Peak areas with a proportion < 0.5% were removed.

TRFs were aligned manually such that fragment sizes differing by ≤ 0.5bp were considered identical and clustered. To avoid clustering 'distinct close peaks', the maximum number of fragments assigned to a cluster was limited to the number of profiles being aligned. Once the maximum number was reached, a new cluster was created. For each cluster of TRFs, the average size was calculated and used to distinguish TRFs. In order to compare soil bacterial community structure, both the proportional peak area (abundance) and TRF size were used. Since different organisms can produce TRFs of similar size, TRFs of different lengths were assumed to represent different operational taxonomic units (OTUs) and not necessarily distinct bacterial species.

Soil Microbial Biomass

For both sampling periods, soil microbial biomass was measured from the restoration experiment and Dogleg Prairie using the procedure of Findlay et al. (1989). Briefly, for each quadrat/location, and using 5g of soil, phospholipids were dissolved in a chloroform phase and filtered using Na_2SO_4 until 10mL of chloroform was collected. Phospholipids were then concentrated to 1mL of chloroform. After digestion with 25μL of the chloroform solvent and 5% acid potassium persulfate, malachite green solution was used to allow measurement of

absorbance with a spectrometer (610nm). Using glycerophosphate standards, nmol of lipid bound phosphate was calculated for each sample and converted into cells per gram of soil (4 x 10^9 cells per 100nmol P).

Soil pH

Soil pH was measured using only samples collected in September and was based off the procedure of Robertson et al. (1999). Briefly, 30mL of de-ionized water was added to 15g soil in duplicate. This mixture was shaken and left to stand for 30min, after which time, soil pH was measured using a pH meter from Denver Instrument Company.

Data Analyses

The Dogleg Prairie reference site is used in this study to contrast plant and soil bacterial communities in the restoration experiment to communities found within the closest representative undisturbed prairie. This allowed me to qualitatively evaluate the effectiveness of restoration in establishing plant and soil bacterial communities. I was also able to compare the factors important for structuring soil bacterial communities in the restoration experiment with that of the reference site.

Because sampling locations in the Dogleg Prairie are spatially separated from the restoration experiment, analyses between the Dogleg Prairie and restoration experiment could not be done using all three 'treatments' (Control, Restored, DLP) simultaneously. Therefore, any analyses contrasting the Dogleg Prairie with the restoration experiment were done using each treatment of the restoration experiment on its own. For example, when analyzing plant communities, those from the Dogleg Prairie were compared to the Control treatment plant

communities. Then a separate analysis was done between plant communities of the Dogleg Prairie and the Restored treatment.

Furthermore, this study uses several sampling locations within on reference prairie to compare with the restoration experiment. Because I am only using one reference prairie, I cannot make extrapolations of the data to draw general inferences about all remnant tallgrass prairie. However, using the Dogleg Prairie as a reference does allow a gauge to evaluate restoration within the context of this study.

In order to portray and gain a better understanding of plant and soil bacterial communities over the entire growing season, all seasonal data were combined. To do this, I used the maximum value (e.g. maximum abundance values for each OTU, maximum available N, etc.) obtained from either season and compiled a new data set. These maximum values were used for all analyses.

Univariate Analyses: A 2-Way ANOVA without Replication was used to analyze alpha diversity indices (richness and Shannon Diversity, H´) and environmental variables of the restoration experiment. This ANOVA design is analogous to a paired t-test for the treatment factor, but also allows for testing the block factor (Sokol and Rohlf 1995). A 1-Way ANOVA was used for analyses between the treatments of the restoration experiment and Dogleg. The block factor was not included in these analyses because sampling locations of the Dogleg Prairie do not statistically correspond to the blocks of the restoration experiment.

Alpha diversity was calculated for both plant and soil bacterial communities in PC-Ord 4.14 (McCune and Mefford 1999). Diversity indices and environmental variables were analyzed

in PASW Statistics 18.0. Live aboveground biomass, root biomass, microbial biomass and available N required log transformation to meet ANOVA assumptions.

Multivariate Analyses: All multivariate analyses used relativized abundance matrices unless otherwise noted. Plant and soil bacterial communities were initially analyzed using Non-metric Multidimensional Scaling (NMS) within PC-Ord 4.14 using a Bray-Curtis similarity matrix. Scores generated from these analyses were used to graphically display the communities as ordinations.

To examine differences in plant and bacterial community composition between sowing treatments of the restoration experiment and between the restoration experiment and Dogleg Prairie, I used Permutational Multivariate Analysis of Variance (PERMANOVA) employed by PERMANOVA+ for Primer; Primer v6 Software (Clarke and Gorley 2006; Anderson 2001). PERMANOVA tests for differences among community matrices and calculates *p*-values through permutations of observations. The software also allows for the analysis of multivariate data using ANOVA designs such as 2-Way ANOVA without Replication (restoration experiment only) and 1-Way ANOVA (Control vs. DLP; Restored vs. DLP). All PERMANOVA analyses used 9999 permutations and the Bray-Curtis similarity distance measure.

Plant communities were assessed using three approaches: using plant species, grouping plant species into one of five functional groups (C_3-grass; C_4-grass; Legume; Forb; and Woody) and grouping plant species as either a tallgrass prairie species or not according to Freeman et al. (1991) (Prairie; Non-prairie: plant community composition analyses only). The native plant species sown into the restoration experiment were not necessarily species representative of the

DLP. In order to assess plant communities between the restoration experiment and DLP on a more commensurate level, both functional groups and prairie groups were used.

I also used Permutational Analaysis of Multivariate Dispersions (PERMDISP) in PERMANOVA+ for Primer (Anderson 2006; Clarke and Gorley 2006) to evaluate differences in multivariate dispersions of the plant and soil bacterial communities among treatments. This allowed me to measure beta-diversity (species turnover) among replicate plots of a given treatment or site. PERMDISP tests for differences in the dispersion of multivariate data by measuring distances from observations to their group centroid. Using a Bray-Curtis similarity matrix of presence/absence data, the results are directly interpretable as a test for similar beta-diversities among groups (Anderson 2001).

In addition to PERMANOVA and PERMDISP, I employed a regression approach to explore additional variables and underlying gradients that may be important in structuring the spatial variation of the soil bacterial communities sampled in the restoration experiment and Dogleg Prairie. In these analyses, variation in bacterial composition was explored as a function of three categories of explanatory variables: plant community composition, soil environmental variables and spatial location using Distance-Based Linear Models (DISTLM) within PERMANOVA+ for Primer (Anderson 2006; Clarke and Gorley 2006). DISTLM allows predictor variables to be fit individually or in groups and allows for model building by choosing from a suite of selection procedures and criterion. Analyses of the predictor variables/groups in the restoration experiment, using all quadrats together, were done separately from those of the Dogleg Prairie.

To determine if plant communities significantly predicted soil bacterial communities, the plant communities were organized using three different approaches. First, using the entire plant

species matrix could lead to variance inflation of the regression models. To reduce this potential problem, NMS scores from the first two axes were used to represent the plant species communities and analyzed as a group (Plant). The second approach involved using plant functional groups in place of plant species as the predictor variables. The categories are as described above and grouped together to use as one predictor group (Funct). The third approach used the Shannon diversity index (H´) as a predictor variable (Shannon). Species richness was not included to reduce multi-collinearities within the regression models. Only one of these plant predictor groups were included in any regression model at a time to determine which dimension of the plant community might best predict soil bacterial communities.

The environmental variables used to predict soil bacterial communities within the restoration experiment included: live aboveground biomass; root biomass; microbial biomass; soil pH; total soil C; % silt; % sand; C/N ratio; soil moisture, available N and elevation. Organic matter, total soil N, CEC and % clay were highly correlated with the included environmental variables and excluded from analyses to reduce multi-collinearities. Environmental variables used for the Dogleg Prairie analyses included: root biomass, microbial biomass, soil pH and elevation. Both of these sets of environmental variables were grouped and labeled 'Env'. In addition, a stepwise regression was run to determine which of the variables in Env were significantly correlated with soil bacterial communities on their own. Variables retained from this analysis formed the group Env2.

For both the restoration experiment and Dogleg Prairie, the spatial location of the quadrats/sampling locations was represented by their Latitude (x) and Longitude (y) positions. In order to account for more complex spatial patterns, such as patches and gaps, all terms for the third-order polynomials were calculated (Borcard et al. 1992; Cottenie et al. 2003). A stepwise

regression (DISTLM) was performed to narrow down the spatial variables to only those that were significant and placed in the predictor group, 'Spatial'. For the restoration experiment, 'Spatial' contained the x^3 and x^2y terms, whereas only the x term was included for DLP.

Analyses to determine which factors were important for the spatial variation found in the soil bacterial communities consisted of three main steps. First, bacterial composition was regressed on each predictor group separately to determine if there was a significant relationship with soil bacterial communities, ignoring all other groups. Subsequently, a regression including the three main groups was run ([Plant Env Spatial], [Funct Env Spatial] and [Shannon Env Spatial]). Third, using the groups Env2, Plant/Funct/Shannon and Spatial, another regression was run to determine if Env2 improved the model to a greater extent compared to the group Env. For all regressions, a Bray-Curtis similarity matrix, stepwise selection procedure, adjusted R^2 selection criterion and 9999 permutations were used. The model with the highest F-value after all terms were included was deemed the 'best' model and reported.

To examine the relative contributions of each predictor group to the total explained variation in the 'best' model, a distance-based Redundancy Analysis (dbRDA) with PERMANOVA+ for Primer (McArdle and Anderson 2001) was run. In this way, partial regression coefficients were calculated and I was able to partition out pure plant, pure environmental and pure spatial effects from any explained variation that was shared by these predictor groups. Analyses used a Bray-Curtis similarity matrix, R^2 selection criterion and 9999 permutations.

RESULTS

Plant Community Structure

In the restoration experiment, both plant species and functional group alpha diversity (richness and Shannon diversity, H´) was higher in Restored quadrats as compared to the Controls (Table 2 and 3). Analyses among the treatments of the restoration experiment and Dogleg Prairie indicated that the Control treatment had significantly lower plant species richness and Shannon diversity than the Dogleg Prairie. However, sowing native tallgrass prairie species increased plant species richness and Shannon diversity in the Restored quadrats to similar levels as the Dogleg Prairie (Table 2). When examined on a plant functional group level, richness values between the Control treatment and DLP were not significantly different, but Shannon diversity was significantly higher in the Dogleg Prairie. In addition, plant functional group richness and diversity were significantly lower in the Dogleg Prairie as compared to the Restored treatments (Table 3).

Control and Restored plant community compositions were significantly different when examined at the plant species, functional group and plant prairie group levels (Figure 1; Table 2, 3 and 4). *Bromus inermis* and *Poa pratensis*, both introduced cool-season grasses, dominated the Control quadrats (Table 5, 6 and 7). In contrast, Restored plant communities were dominated by a combination of sown native prairie warm-season grasses (*Tripsacum dactyloides*, *Sorghastrum nutans*), sown native prairie legumes (*Lespedeza capitata*) and introduced cool-season grasses (*Bromus inermis*). When comparing the plant community compositions of the restoration experiment with that of the Dogleg Prairie, I found that the remnant prairie plant communities were significantly different from both the Control and Restored treatments on both a plant species and functional group basis (Figure 1A and 1B, Table 2 and 3). However, plant prairie

group community compositions of the Dogleg Prairie were significantly different than those of the Controls but similar to the Restored treatments (Figure 1C, Table 4). Thus, native warm-season grasses and forbs dominated both the Dogleg Prairie and Restored quadrats. But these communities were comprised of different native tallgrass prairie species (DLP: *Sorghastrum nutans*, *Sporobolus heterolepis*, *Panicum virgatum* and *Antennaria neglecta*) (Table 4, 5, and 6).

When examining beta-diversities using plant species and plant functional groups, I found similar results. The Control treatment had higher beta-diversity than the Restored treatment (Figure 1A and 1B, Table 2 and 3). Plant beta-diversity of the Dogleg Prairie was not significantly different than the Controls, but higher than the Restored treatments.

Soil Bacterial Community Structure

As opposed to what was observed for the plant communities, soil bacterial OTU richness and Shannon diversity were not significantly different among Control, Restored and Dogleg Prairie soils (Table 8). PERMANOVA showed that Control and Restored treatments had similar soil bacterial community compositions and beta-diversities (Figure 2, Table 8). However, DLP soil bacterial OTU community compositions were different from the restoration experiment. Beta-diversities were significantly higher in the Dogleg Prairie than either treatment of the restoration experiment.

Environmental Variables

Analyses of soil environmental variables sampled from only the restoration experiment indicated no significant differences between Control and Restored treatments for soil moisture, available soil N, total soil N and C/N ratio (Table 9). However, both total soil C and OM were

higher in Control soils than Restored. These results could be a statistical/sampling artifact. In a previous analysis using all four original treatments of the restoration experiment, no significant differences were found among the treatments for these variables (Foster et al. 2007). Total soil N, total soil C, OM and C/N ratio showed differences among blocks. These soil properties, therefore, varied significantly throughout the landscape. Plant aboveground biomass was found to be greater in Restored quadrats compared to Controls (Table 9).

Root biomass from Restored quadrats and Dogleg Prairie were not significantly different but were both significantly higher than Controls (Table 9). These results are in contrast to what I found for microbial biomass. Restored quadrats had the highest amounts of microbial biomass when compared to both Controls and Dogleg Prairie, which were similar to each other. Soil pH was not significantly different between the Control and Restored treatments, but significantly lower than the Dogleg Prairie locations.

DISTLM and dbRDA Modeling of Soil Bacterial Community Structure

DISTLM results from the restoration experiment, using both Control and Restored treatments simultaneously, showed that the bacterial community varied among quadrats independent of plant community composition (both species and functional group composition) and plant diversity (Table 10). However, the Env, Env2 and Spatial predictor groups correlated significantly with bacterial OTU community compositions. Env2 included microbial biomass, soil pH, % sand and elevation, which were all significant correlates of soil bacterial communities.

Stepwise regression analyses of the restoration experiment showed that the predictor groups Env2 and Spatial were important correlates for soil bacterial OTU communities (Table

11). No predictor group representing plant communities were included in the final regression model. Using this model and the included predictor groups, the total explained variation (32.2%) was partitioned using dbRDA. Results indicated that the environmental factors of microbial biomass, soil pH, % sand and elevation (grouped as Env2) significantly explained 16.9% of the variation found in the soil bacterial communities, indicating a significant purely environmental effect upon soil bacterial communities ($F_{7, 27} = 1.69$; $p = 0.001$) (Figure 3). Spatial effects, independent of the environment significantly explained 11.1% of the total explained variation ($F_{7, 27} = 2.21$; $p = 0.001$). Variation that was shared between both the environmental variables and spatial location encompassed 4.2% of the total explained variation.

When examining the potential factors important for Dogleg Prairie soil bacterial community structure, I found that the plant species community, represented by NMS scores, was significantly correlated with soil bacterial OTU community structure (Table 10). However, neither Funct nor Shannon predictor groups were significant. The environmental variable, soil pH (Env2) and the predictor group, Spatial, were also significantly correlated with Dogleg Prairie soil bacterial communities.

When considering the soil bacterial communities of the Dogleg Prairie, the predictor groups Plant, Spatial and Env2 were included in the final stepwise regression model, but only Plant and Spatial were significant (Table 11). Partitioning of the total explained variation (37.9%) showed that only pure plant effects explained a significant amount of that variation (15.3%; $F_{5, 13} = 1.60$; $p = 0.045$) (Figure 3). Pure environmental and pure spatial effects explained 7.1 and 7.3% of the variation, respectively, but were not significant ($F_{5, 13} = 1.49$; $p = 0.113$ and $F_{5, 13} = 1.52$; $p = 0.117$, respectively). The shared variation among these three predictor groups encompassed 8.2% of the total explained variation in the model.

Stepwise regressions and dbRDA analyses were also conducted on each treatment of the restoration experiment on its own (Controls, Restored). However, the results did not elucidate any new patterns concerning the structuring of soil bacterial OTU communities and are not reported.

DISCUSSION

Local plant communities influenced soil bacterial community compositions within the native prairie remnant but not within the tallgrass prairie restoration field-site, suggesting that a six-year period of restoration was insufficient for soil bacterial communities to be directly influenced by plant-generated resource heterogeneity over the short-term. These results were observed even though seed sowing overcame dispersal limitations for both local and field-level plant diversities and established restored native tallgrass prairie communities when compared to the remnant prairie. Instead, soil bacterial communities within the restoration experiment were significantly correlated with both the local environmental heterogeneity generated by soil environmental properties and regional processes such as dispersal limitations, disturbance history and stochastic assembly. The disparate factors governing the distinct soil bacterial communities between the restoration experiment and Dogleg Prairie could reflect the legacies of their land-use histories and restoration age.

Plant Community Structure

Using seed sowing to restore native tallgrass prairie plant communities increased plant diversity at the local scale ($1m^2$), but not at larger spatial scales (beta-diversity) in this tallgrass

prairie system. At the local scale, lower diversities are often observed in tallgrass prairie restorations as compared to remnant prairies, potentially due to factors such as management limitations, the absence of grazing ungulates and the time required for restoration (Thompson 1992; Sperry 1994; Polley et al. 2005). Propagule availability could also hinder the establishment of native grasses and forbs (Tilman 1997; Foster et al. 2004). For example, Kindscher and Tieszen (1998) found that as the distance from an adjacent tallgrass prairie remnant increased, plant diversity in a 35 year-old restoration decreased, showing that dispersal limitations can greatly hinder restoration success. As in this study, enhanced seed sowing of target native plant species could increase the probability of establishing local plant diversities similar to native tallgrass prairie remnants. Assuming that prairie remnants and restoration sites will become more fragmented in the future, dispersal of native plant species will be increasingly hindered. Management strategies that employ comprehensive seed sowing could help offset these dispersal limitations and improve tallgrass prairie restoration at the local level (Foster et al. 2007).

Seed sowing also overcame dispersal limitations at the field level (beta-diversity). However, the higher seed inputs homogenized plant communities of the Restored quadrats, leading to an increase in plant community similarities and lower beta-diversities than both Controls and DLP. Because dispersal limitations are not often reproduced during restoration, decreased plant community variability is typically observed in restored prairie sites, as in this study (Polley et al. 2005). Varying the native tallgrass species sown throughout a restoration site could help increase field level plant diversities to those approaching remnant prairies.

As seen by the plant prairie group analyses, seed sowing did restore native tallgrass plant community compositions, despite different species comprising the Restored quadrats and remnant prairie. The differing species are most likely due to the sown native plant species chosen

for the restoration experiment, which could theoretically represent a remnant prairie within the region, but were not necessarily species representative of the Dogleg Prairie.

Soil Bacterial Community Structure in the Restoration Experiment

Local, niche-based explanations for structuring soil bacterial communities could include the resource heterogeneity produced by the coinciding plant communities either through short-term direct or long-term indirect effects (Jangid et al. 2011). As of the sixth year of the restoration experiment, no changes in soil bacterial community compositions and diversities (local and field level) were observed as a result of plant restoration. Thus, there was no indication that native tallgrass prairie plant restoration directly influenced soil bacterial communities over the short-term.

The lack of a relationship between plant communities and soil bacterial communities in this study is further demonstrated in two ways. First, the multivariate regression analyses showed no correlation, despite using three approaches to assess the relationship (i.e. plant species, plant functional groups, Shannon diversity). Second, no relationship was found when comparing the restoration experiment and the remnant prairie. Plant species community compositions of the Control, Restored and DLP quadrats differed greatly. If there were direct impacts of the plant communities, the soil bacterial community compositions should have also differed. Although this comparison is qualitative, it provides support for the result that plant restoration did not directly impact soil bacterial communities over the short-term.

Studies have shown that plant communities can influence soil bacterial communities through differing qualities and quantities of root exudates and litter (Kowalchuk et al. 2002; Innes et al. 2004; Bever et al. 2010; Hovatter et al. 2011). However, there is conflicting evidence

that plants directly influence soil bacterial communities of the bulk soil with few explanations given why no correlation might be found (Brodie et al. 2002; Kennedy et al. 2004; Jangid et al. 2011).

Within this study, it is possible that plant mediated effects were transient. In such a scenario, any impacts upon the soil bacterial communities could have happened shortly after plant restoration, and therefore, not detected. If this was the case, it could indicate a soil bacterial community resilient to changes in plant communities (Marshall et al. 2011). Further, it is possible that any plant effects could have been limited to the rhizosphere and swamped out by sampling the bulk soil (Kuske et al. 2002; Jangid et al. 2011).

No response of soil bacterial communities to plant restoration could also be because only certain microbes responded to vegetation, resulting in low abundance in soils and, therefore, were below the detection limit for T-RFLP (Jangid et al. 2011). T-RFLP can exclude the numerous, but rare, OTUs within a community profile, placing a limit upon the number of OTUs used to distinguish soil bacterial communities (Bent and Forney 2008). T-RFLP also generates profiles in which the actual taxonomic resolution is assumed to be higher than species level. Using this technique could mask any subtle changes potentially occurring at lower taxonomic resolutions in the Restored quadrats (Danovaro et al. 2006). Despite these inherent biases, T-RFLP can be very robust in distinguishing amongst fundamentally different bacterial communities (Fierer and Jackson 2006).

Finally, restoration age could explain why soil bacterial communities did not respond to plant restoration within this permanent old-field grassland. If the primary influence of plant generated resource heterogeneity were through litter inputs (long-term indirect), any changes of the soil bacterial communities in response to plant restoration would take time to establish. Six

years of tallgrass prairie restoration may not have been long enough to 'erase' the previous plant communities' legacies (McKinley et al. 2005; Card and Quideau 2010; Eisenhauer et al. 2010; Kulmatiski et al. 2011; Marshall et al. 2011). For example, similar soil microbial communities were found when comparing an annual grassland (>60yrs) to restored perennial grassland (4yrs) in California (Potthoff et al. 2006) and attributed, in part, to the build-up of soil structure, root systems, and carbon inputs after the abandonment of agricultural practices. All of these changes were the indirect results of permanent plant communities over the long-term (Zak et al. 2003; Potthoff et al. 2006; Plassart et al. 2008). Four years of plant restoration may not have allowed time for the further build-up and/or change of carbon inputs into this system, and thus, did not alter the soil bacterial communities that had been under permanent grassland for several decades.

Further, there is some evidence that restoration age can impact how similar soil microbial communities are between restored and native remnant sites (Allison et al. 2005; Boyle et al. 2005; McKinley et al. 2005). Bach et al. (2010) found that soil microbial communities from older tallgrass prairie restorations (12-18yrs) were more similar to native tallgrass prairie than newer restorations (2-12yrs). They estimated 30-40yrs was needed for soil microbial communities analogous to native tallgrass prairie, potentially due to the time required for further soil-building processes to occur after restoration (Card and Quideau 2010). In this study, restoration age could be contributing to the similar soil bacterial communities observed in the restoration experiment and help explain why soil bacterial communities in the Restored quadrats did not approach those of the reference prairie. Although I found indications that restoration could impact ecosystem traits as plant above- and belowground biomass and microbial biomass were increased, six growing seasons may not have been long enough for any further litter inputs from restoration to be manifested in the soil bacterial community compositions.

Analyses showed that soil bacterial communities of the restoration experiment were significantly influenced by both niche-based local effects through the environmental heterogeneity of soil properties and by pure spatial effects potentially arising from processes such as dispersal limitations, historical legacies and demographic stochasticity. It is possible that the significant spatial component could be reflecting an unmeasured environmental variable important for soil bacterial communities and co-varies strongly with space. However, if this were the case, it would strengthen the importance of soil variables in structuring soil bacterial communities (Cottenie 2005).

Four soil properties were important to the variation in soil bacterial community structure of the restoration experiment: elevation, % sand (texture), microbial biomass and soil pH. Elevation is considered an integrative variable representing a suite of biotic and abiotic characteristics to which soil biota can respond (e.g. changes in soil texture, productivities, nutrients, erosion dynamics, etc.) (Broughton and Gross 2000). In this study, soils were sampled primarily from upland sites, leading to a range in elevation of only eight meters. Despite the relatively uniform topography, the resulting environmental heterogeneity was enough to impact the soil bacterial communities, showing that "macroscopically uniform conditions are not perceived as such by microscopic soil organisms" (Ettema and Wardle 2002).

Soil texture can play a key role in driving soil microbial communities (Bezemer et al. 2006). Sessitsch et al. (2001) found that silt and clay particles support more productive and diverse microbial communities than sand particles potentially through two routes. First, silt and clay can provide refugia for microorganisms because pore sizes are too small for predators such as protozoa (Sessitsch et al. 2001). Second, sand particles can be depleted in OM and microbial biomass as compared to finer particle sizes, leading to lower nutrient availabilities in sand.

Therefore, only those soil microbes adapted to limited nutrient conditions would be found in sand particles (Sessitsch et al. 2001). Soil pH can influence soil bacterial communities across spatial scales (rhizosphere to continents) and ecosystems (Fierer and Jackson 2006; Dimitriu and Grayston 2010; Mummey et al. 2010). It is thought that soil pH can both directly influence soil bacteria by changing biochemical structures and indirectly by altering nutrient soil solubility (Hovatter et al. 2011).

The significant spatial effects found in the restoration experiment suggest that soil bacterial communities in this old-field grassland were partly influenced by spatial processes acting independent of the environment, such as dispersal limitation, historical legacies, and demographic stochasticity. Dispersal limitations could allow for ecological drift of soil bacterial communities throughout the old-field. Over ecological time-scales, restricted movements of bacterial cells could create patches in community compositions that are further developed by demographic stochasticity (stochastic births and deaths), leading to the spatial variation observed (Martiny et al. 2011).

The significant pure spatial effect could also be due to the cultivation history (historical legacies) of this old-field experimental site as cultivation can bring about lasting changes in soil qualities and soil communities that could take decades to recover (Tiessen et al. 1994; Murphy et al. 2006; Plassart et al. 2008; Bach et al. 2010). For example, Jangid et al. (2011) concluded that land-use history was more important in determining soil microbial communities than either soil properties or vegetation. The authors found that soil microbial communities were similar between sites that were cultivated historically (cropland vs. converted grassland). However, soil microbial communities between converted and native grasslands were different, despite similar vegetation. In addition, when comparing native forests and grasslands, which differed in soil

properties and vegetation, soil microbial communities were similar. The legacy effects of past managements were influencing the observed soil microbial communities.

Despite the significant effects of the environmental soil properties and spatial isolation to the soil bacterial community variation observed in the restoration experiment, 67.8% of the variation remained unexplained. This could be partly due to any error associated with both measuring the environmental variables and assessing the bacterial communities. In addition, unmeasured environmental soil properties could contribute to the unexplained variation. These environmental properties would likely be uncorrelated with the environmental variables that were measured and simultaneously not spatially structured. Lastly, stochastic effects and/or disturbances that are not spatially structured could contribute to the unexplained variation.

Soil Bacterial Community Structure in the Dogleg Prairie

Within the Dogleg Prairie, multivariate analyses indicated that soil bacterial communities could be structured by the environmental heterogeneity provided by both the plant communities and soil properties (soil pH). There was also evidence of spatial autocorrelation among the soil bacterial communities. However, after variance partitioning of these three factors, only the plant species communities were significant in shaping soil bacterial communities in this remnant prairie. This finding suggests two things. First, soil bacterial communities within the Dogleg Prairie are associated most strongly with the environment determined by the plant communities and independent of the soil properties. Second, the spatial autocorrelation observed in the soil bacterial communities is primarily the result of spatial autocorrelation in the plant community and not due to pure regional processes.

The environment provided by the plant communities is most likely the result of both short-term direct influences by root exudation and long-term indirect plant legacy effects generating a resource gradient throughout the prairie and allowing for OTU sorting along that gradient (Ettema and Wardle 2002; Innes et al. 2004; Cottenie 2005; Gruter et al. 2006). However, there is a possibility that soil bacterial and plant communities share an environmental variable(s) important in shaping their compositions, but the two communities do not directly influence each other. This could result in a significant correlation between soil bacterial and plant communities, but the underlying causation is unknown (Ramette and Tiedje 2007). As with the restoration experiment, a large amount of variation in the Dogleg Prairie soil bacterial communities remained unexplained (62.1%). Potential reasons for this unexplained variation are as stated previously for the restoration experiment.

Restoration Experiment vs. Dogleg Prairie

In this study, soil bacterial communities under tallgrass prairie restoration did not approach those of the reference prairie, potentially due to several factors. First, as mentioned previously, the age of the restoration may not have allowed enough time for the restored plant communities to develop a link with and ultimately alter the soil bacterial communities. In the future, it would be interesting to compare these communities again to determine if, given enough time, plant restoration could change soil bacterial communities within the Restored quadrats. Given that the plant communities were comprised of different prairie species, one might expect that the soil bacterial communities under this plant restoration could also differ from both the old-field and the remnant prairie soil bacterial communities.

Second, the soil bacterial communities at these two sites were being influenced by different factors potentially resulting from their differing land-use histories. Studies examining the importance of local, niche-based vs. spatial effects upon metacommunities have found that the relative importance of these determinants can depend upon factors such as spatial scale (cm to continents), habitat type and land-use history (Cottenie 2005; Ramette and Tiedje 2007). Further, the relative importance of particular soil properties can be inconsistent depending upon agricultural legacies (Jangid et al. 2011; Martiny et al. 2011). The enduring impacts of cultivation within the restoration experimental field site could have brought about a change in the factors structuring soil bacterial communities, potentially increasing the importance of soil environmental properties and spatial isolation, as compared to the plant communities. For example, soil pH is an edaphic property typically altered with cultivation (Karlen et al. 1999; Knops and Tilman 2000; Buckley and Schmidt 2001; Allison et al. 2005; Murphy et al. 2006; Card and Quideau 2010). In this study, soil pH was significantly lower in the restoration experiment than the Dogleg Prairie and also an important correlate with soil bacterial communities for both sites. This suggests that if soil pH was altered due to prior cultivation in the restoration experiment, this potentially could have contributed to the different soil bacterial communities between the restoration experiment and Dogleg Prairie. After the variance partitioning, however, soil pH (as part of Env2) remained significant only in the restoration experiment. In contrast, the remnant soil bacterial communities were significantly influenced only by the environmental heterogeneity provided by the plant communities possibly because the link between the remnant plant and soil bacterial communities have had several centuries to establish. Cultivation potentially could have increased the importance of soil environmental

properties for structuring soil bacterial communities (Dimitriu and Grayston 2010). However, any direct observations regarding these possible alterations cannot be made within this study.

Lastly, the physical separation of the restoration experimental field site and Dogleg Prairie cannot be ruled out as a possible explanation for the observed differences in soil bacterial communities. Within each field site, a degree of spatial autocorrelation was exhibited. Different soil bacterial communities between these two sties could arise from the mere fact that they were spatially separated and communities in close proximity tend to be more similar than those located further apart (Horner-Devine et al. 2004b; Baker et al. 2009).

Implications for Restoration

Restoration of native ecosystems is an important, yet complicated task. Seed sowing can be a successful approach to restore local plant species richness and diversity, but restoring field level plant diversities could be harder to achieve without incorporating variation in the species sown throughout a site. Restoring plant communities aids in reestablishing ecosystem functioning on degraded agricultural land. But to fully restore ecosystem traits, soil biota and functioning also need to be restored. As seen within this study, restoring plant communities may not necessarily translate into restored soil bacterial communities over the short-term, but will likely take time (i.e. several decades) to accomplish. This is potentially due to the inherent changes that occur to the soil after cultivation and the time it takes for the re-establishment of soil properties. Assuming that the build-up of litter inputs is a main influence of the plant community upon soil bacterial communities, time may also be required for the previous plant communities' legacies to be replaced by the plant restorations'. Further, the factors driving soil

bacterial community structure could be altered due to previous land-use, making restoration more complex.

Table 1. The 24 native tallgrass plant species sown into the Restored quadrats within the restoration experiment. Taxonomy follows Great Plains Flora Association (1986) and USDA NRCS Plants Online Database.
[1] C_3-G = Cool-season grass; C_4-G = Warm-season grass; F = Forb; L = Legume.

Species	**Family**	**Functional Group**[1]
Achillea millefolium	Asteraeae	F
Amorpha canescens	Fabaceae	L
Andropogon gerardii	Poaceae	C_4-G
Asclepias tuberosa	Asclepiadaceae	F
Bouteloua curtipendula	Poaceae	C_4-G
Dalea candidum	Fabaceae	L
Dalea purpurea	Fabaceae	L
Desmanthus illinoensis	Fabaceae	L
Echinacea pallida	Asteraceae	F
Elymus canadensis	Poaceae	C_3-G
Eragrostis trichodes	Poaceae	C_3-G
Lespedeza capitata	Fabaceae	L
Liatris pycnostachya	Asteraceae	F
Monarda fistulosa	Lamiaceae	F
Panicum virgatum	Poaceae	C_4-G
Ratibida columnifera	Asteraceae	F
Ratibida pinnata	Asteraceae	F
Rudbeckia hirta	Asteraceae	F
Salvia azurea	Lamiaceae	F
Schizachyrium scoparium	Poaceae	C_4-G
Sorghastrum nutans	Poaceae	C_4-G
Sporobolus cryptandrus	Poaceae	C_4-G
Symphyotrichum novae-angliae	Asteraceae	F
Tripsacum dactyloides	Poaceae	C_4-G

Table 2. Plant species alpha diversity (richness, Shannon diversity: H′), community composition and beta-diversity. Results are presented for comparisons of the restoration experiment, Controls and DLP (Dogleg Prairie), and Restored and DLP.
[1] Means (SE) for each treatment and variable; Controls n=17; Restored n=17; DLP n=18.
[2] Analyses of the restoration experiment used 2-Way ANOVA without Replication model; analyses between Controls and DLP, and Restored and DLP used 1-Way ANOVA model.
[3] Plant community composition analyses were performed using a Bray-Curtis abundance matrix in PERMANOVA.
[4] Beta-diversity: Average distances (SE) from an observation to the treatment centroid as computed by PERMDISP with a Bray-Curtis P/A matrix. The block factor in the restoration experiment could not be tested because n=2 for each block.
[5] Pseudo-F: F-value generated from PERMANOVA and PERMDISP. Perm P: *p*-values generated from 9999 permutations of the data.
Significant effects at α=0.05 level are in bold.
NA: Factor was not included in the analyses or the information was not available.

		Plant Species Richness		**Plant H′**		**Plant Species Community Composition** [3, 5]		**Plant β-Diversity** [4, 5]	
	Control [1]	7.53 (0.681)		1.19 (0.090)		NA		35.77 (1.406)	
	Restored [1]	12.59 (0.665)		1.76 (0.077)		NA		26.66 (1.560)	
	DLP [1]	13.56 (0.933)		1.92 (0.093)		NA		39.76 (1.916)	
		F_{df}	**P**	**F_{df}**	**P**	**Pseudo-F_{df}**	**Perm P**	**Pseudo-F_{df}**	**Perm P**
Restoration Experiment [2]	**Block**	$5.40_{16,16}$	**0.001**	$2.63_{16,16}$	**0.031**	$1.41_{16,16}$	**0.036**	NA	NA
	Trt	$90.47_{1,16}$	**0.001**	$41.62_{1,16}$	**0.001**	$23.88_{1,16}$	**0.001**	$18.82_{1,32}$	**0.001**
Controls vs. DLP [2]	**Trt**	$26.68_{1,33}$	**0.001**	$30.99_{1,33}$	**0.001**	$25.55_{1,33}$	**0.001**	$2.76_{1,33}$	0.123
Restored vs. DLP [2]	**Trt**	$0.70_{1,33}$	0.409	$1.66_{1,33}$	0.206	$16.40_{1,33}$	**0.001**	$27.72_{1,33}$	**0.001**

Table 3. Plant functional group alpha diversity (richness, Shannon diversity: H′), community composition and beta-diversity. Results are presented for comparisons of the restoration experiment, Controls and DLP (Dogleg Prairie), and Restored and DLP.
[1] Means (SE) for each treatment and variable; Controls n=17; Restored n=17; DLP n=18.
[2] Analyses of the restoration experiment used 2-Way ANOVA without Replication model; analyses between Controls and DLP, and Restored and DLP used 1-Way ANOVA model.
[3] Plant functional group community composition analyses were performed using a Bray-Curtis abundance matrix in PERMANOVA.
[4] Beta-diversity: Average distances (SE) from an observation to the treatment centroid as computed by PERMDISP with a Bray-Curtis P/A matrix. The block factor in the restoration experiment could not be tested because n=2 for each block.
[5] Pseudo-F: F-value generated from PERMANOVA and PERMDISP. Perm P: *p*-values generated from 9999 permutations of the data.
Significant effects at α=0.05 level are in bold.
NA: Factor was not included in the analyses or the information was not available.

		Plant Functional Group Richness		**Plant Functional Group H′**		**Plant Functional Group Community Composition**[3, 5]		**Plant Functional Group β-Diversity**[4, 5]	
	Control[1]	3.18 (0.261)		0.52 (0.097)		NA		16.70 (2.26)	
	Restored[1]	4.00 (0.000)		1.06 (0.039)		NA		3.97×10^{-15} (2.61×10^{-15})	
	DLP[1]	3.44 (0.258)		0.81 (0.050)		NA		16.22 (1.18)	
		F_{df}	**P**	**F_{df}**	**P**	**Pseudo-F_{df}**	**Perm P**	**Pseudo-F_{df}**	**Perm P**
Restoration Experiment[2]	**Block**	$1.00_{16,16}$	0.500	$0.72_{16,16}$	0.742	$0.83_{16,16}$	0.681	NA	NA
	Trt	$9.99_{1,16}$	**0.006**	$23.36_{1,16}$	**0.001**	$48.61_{1,16}$	**0.001**	$54.48_{2.32}$	**0.001**
Controls vs. DLP[2]	**Trt**	$0.53_{1,33}$	0.471	$7.43_{1,33}$	**0.010**	$121.19_{1,33}$	**0.001**	$0.04_{1,33}$	0.837
Restored vs. DLP[2]	**Trt**	$4.36_{1,33}$	**0.045**	$15.53_{1,33}$	**0.001**	$40.93_{1,33}$	**0.001**	$177.95_{1,33}$	**0.001**

Table 4. Plant prairie group community compositions. Results are presented for comparisons of the restoration experiment, Controls and DLP (Dogleg Prairie), and Restored and DLP.
[1] Controls n=17; Restored n=17; DLP n=18.
[2] Analyses of the restoration experiment used 2-Way ANOVA without Replication model; analyses between Controls and DLP, and Restored and DLP used 1-Way ANOVA model.
[3] Plant prairie group community composition analyses were performed using a Bray-Curtis abundance matrix in PERMANOVA.
[4] Pseudo-F: F-value generated from PERMANOVA and PERMDISP. Perm P: *p*-values generated from 9999 permutations of the data.
Significant effects at α=0.05 level are in bold.

		Plant Prairie Group Community Composition [3, 4]	
		Pseudo-F_{df}	**Perm P**
Restoration Experiment [1, 2]	**Block**	$1.41_{16,16}$	0.200
	Trt	$146.82_{1,16}$	**0.001**
Controls vs. DLP [1, 2]	**Trt**	$153.21_{1,33}$	**0.001**
Restored vs. DLP [1, 2]	**Trt**	$2.18_{1,33}$	0.127

Table 5. Average percent cover (SE) of the 10 most abundant plant species in the restoration experiment (n=17) and DLP (Dogleg Prairie; n=18). Habitat refers to the habitat type(s) in the surrounding landscape in which a given species has been recorded. Origin and Habitat classification follow Freeman et al. (1991). Taxonomy follows Great Plains Flora Association (1986) and USDA NRCS Plants Online Database.
[1] C_3-G = Cool-season grass; C_4-G = Warm-season grass; F = Forb; L = Legume.
[2] Origin: N = native; I = introduced.
[3] Habitat: G = Cool-season grasslands; O = successional/disturbed site; P = native tallgrass prairie; F = forest.

Species	Family	Functional Group[1]	Origin[2] and Habitat[3]	Control	Restored	DLP
Acalypha virginica	Euphorbiaceae	F	N-GOP	-		7.0 (1.9)
Andropogon gerardii	Poaceae	C4-G	N-GP	-	9.8 (2.8)	3.8 (3.7)
Andropogon virginicus	Poaceae	C4-G	N-GO	16.9 (5.4)	-	4.7 (1.9)
Antennaria neglecta	Asteraceae	F	N-GOP	-	-	25.1 (5.2)
Aristida oligantha	Poaceae	C4-G	N-OP	1.1 (0.9)	-	-
Asclepias verticillata	Asclepiadaceae	F	N-GOP	1.3 (0.4)	-	-
Asclepias viridis	Asclepiadaceae	F	N-GO	3.0 (0.3)	-	-
Bromus inermis	Poaceae	C3-G	I-GO	53.5 (6.9)	17.8 (3.2)	-
Euthamia graminifolia	Asteraceae	F	N-G	-	-	4.1 (2.4)
Leptoloma cognatum	Poaceae	C4-G	N-G	4.0 (2.4)	-	-
Lespedeza capitata	Fabaceae	L	N-P	-	26.5 (5.6)	-
Panicum virgatum	Poaceae	C4-G	N-GP	-	-	13.1 (4.1)
Poa pratensis	Poaceae	C3-G	I-GOP	30.5 (3.3)	11.6 (1.8)	-
Pycnanthemum tenuifolium	Lamiaceae	F	N-P	-	-	4.4 (3.7)
Salvia azurea	Lamiaceae	F	N-P	-	5.3 (1.4)	-
Schedonorus phoenix	Poaceae	C3-G	I-G	15.3 (5.9)	2.9 (1.4)	-
Schizachyrium scoparium	Poaceae	C4-G	N-GP	2.1 (2.1)	6.0 (2.1)	-
Solidago canadensis	Asteraceae	F	N-OP	5.4 (2.9)	-	14.8 (3.1)
Sorghastrum nutans	Poaceae	C4-G	N-P	-	23.5 (4.3)	22.6 (5.7)
Sporobolus heterolepis	Poaceae	C4-G	N-P	-	7.2 (3.3)	18.7 (4.4)
Tripsacum dactyloides	Poaceae	C4-G	N-OP	-	31.9 (7.5)	-

Table 6. Average percent cover (SE) of plant functional groups in the restoration experiment (n=17) and DLP (Dogleg Prairie; n=18).
[1] C_3-G = Cool-season grass; C_4-G = Warm-season grass; F = Forb; L = Legume; W= Woody.

Functional Group[1]	**Control**	**Restored**	**DLP**
C_3-G	91.7 (3.3)	31.5 (3.0)	2.9 (1.0)
C_4-G	25.1 (6.7)	62.7 (5.8)	58.2 (5.4)
F	11.0 (3.9)	9.1 (1.5)	59.8 (4.8)
L	0.3 (0.1)	27.2 (5.6)	2.3 (0.9)
W	0.0 (0.0)	0.0 (0.0)	2.0 (0.9)

Table 7. Average percent cover (SE) of plant prairie groups in the restoration experiment (n=17) and DLP (Dogleg Prairie; n=18). Plant habitat designations follow Freeman et al. (1991).

Prairie Group	Control	Restored	DLP
Non-prairie	90.3 (6.6)	23.0 (3.1)	12.9 (4.1)
Prairie	43.5 (6.1)	131.2 (4.9)	138.3 (4.5)

Table 8. Soil bacterial alpha diversity (richness, Shannon diversity: H′), community composition and beta-diversity. Results are presented for comparisons of the restoration experiment, Controls and DLP (Dogleg Prairie), and Restored and DLP.
[1] Means (SE) for each treatment and variable; n=18 for each treatment.
[2] Analyses of the restoration experiment used 2-Way ANOVA without Replication model; analyses between Controls and DLP, and Restored and DLP used 1-Way ANOVA model.
[3] Bacterial community composition analyses were performed using a Bray-Curtis abundance matrix in PERMANOVA.
[4] Beta-diversity: Average distances (SE) from an observation to the treatment centroid as computed by PERMDISP with a Bray-Curtis P/A matrix. The block factor in the restoration experiment could not be tested because n=2 for each block.
[5] Pseudo-F: F-value generated from PERMANOVA and PERMDISP. Perm P: *p*-values generated from 9999 permutations of the data.
Significant effects at α=0.05 level are in bold.
NA: Factor was not included in the analyses or the information was not available.

		OTU Richness		**OTU H′**		**OTU Community Composition[3, 5]**		**OTU β-Diversity[4, 5]**	
	Control[1]	61.78 (1.803)		3.63 (0.064)		NA		28.71 (0.961)	
	Restored[1]	65.06 (1.971)		3.71 (0.045)		NA		30.20 (1.197)	
	DLP[1]	62.11 (2.390)		3.61 (0.039)		NA		35.32 (1.493)	
		F_{df}	**P**	**F_{df}**	**P**	**Pseudo-F_{df}**	**Perm P**	**Pseudo-F_{df}**	**Perm P**
Restoration Experiment[2]	**Block**	$0.89_{17,17}$	0.594	$2.35_{17,17}$	**0.044**	$2.54_{17,17}$	**0.001**	NA	NA
	Trt	$1.42_{1,17}$	0.249	$1.96_{1,17}$	0.180	$1.03_{1,17}$	0.421	$0.94_{1,34}$	0.361
Controls vs. DLP[2]	**Trt**	$0.01_{1,34}$	0.912	$0.02_{1,34}$	0.880	$4.63_{1,34}$	**0.001**	$13.89_{1,34}$	**0.001**
Restored vs. DLP[2]	**Trt**	$0.90_{1,34}$	0.349	$1.05_{1,34}$	0.313	$5.17_{1,34}$	**0.001**	$7.17_{1,34}$	**0.019**

Table 9. Environmental variables sampled from the restoration experiment and Dogleg Prairie (DLP). Significant effects at α=0.05 level are in bold. NA: Factor was not included in the analyses or the information was not available.

[1] Means (SE) for each treatment and variable; n=18 for each treatment.

[2] Analyses of restoration experiment used 2-Way ANOVA without Replication model; analyses between Controls and DLP, and Restored and DLP used 1-Way ANOVA model.

		Soil Moisture		Avail. Soil N (µg/g dry soil)		% N		% C	
	Control[1]	0.29 (0.006)		1.69 (0.093)		0.17 (0.006)		1.96 (0.080)	
	Restored[1]	0.30 (0.007)		2.28 (0.458)		0.16 (0.005)		1.81 (0.048)	
	DLP[1]	NA		NA		NA		NA	
		F_{df}	**P**	**F_{df}**	**P**	**F_{df}**	**P**	**F_{df}**	**P**
Restoration Experiment[2]	**Block**	$0.73_{17,17}$	0.734	$1.79_{17,17}$	0.121	$5.21_{17,17}$	**0.001**	$6.18_{17,17}$	**0.001**
	Trt	$1.32_{1,17}$	0.266	$3.14_{1,17}$	0.095	$4.35_{1,17}$	0.054	$4.87_{1,17}$	**0.043**
Controls vs. DLP[2]	**Trt**	NA	NA	NA	NA	NA	NA	NA	NA
Restored vs. DLP[2]	**Trt**	NA	NA	NA	NA	NA	NA	NA	NA

Table 9 continued.

		% OM		C/N Ratio		Live Above Biomass (g/m^2)		Root Biomass (mg/cm^3)		Microbial Biomass (Cells/g soil)		Soil pH	
	Control[1]	3.22 (0.125)		11.49 (0.079)		349.19 (33.469)		3.09 (0.267)		4.68×10^6 (1.828×10^5)		5.42 (0.034)	
	Restored[1]	2.99 (0.089)		11.46 (0.101)		1211.76 (132.27)		4.41 (0.527)		5.46×10^6 (3.391×10^5)		5.37 (0.042)	
	DLP[1]	NA		NA		NA		4.47 (0.347)		4.38×10^6 (1.974×10^5)		6.03 (0.065)	
		F_{df}	**P**	**F_{df}**	**P**	**F_{df}**	**P**	**F_{df}**	**P**	**F_{df}**	**P**	**F_{df}**	**P**
Restoration Experiment[2]	**Block**	$5.40_{16,15}$	**0.001**	$3.23_{16,15}$	**0.014**	$1.63_{17,17}$	0.161	$1.68_{17,17}$	0.146	$2.64_{17,17}$	**0.026**	$2.09_{17,17}$	0.070
	Trt	$4.53_{1,15}$	**0.050**	$0.036_{1,15}$	0.851	$98.82_{1,17}$	**0.001**	$8.17_{1,17}$	**0.011**	$6.42_{1,17}$	**0.021**	$1.18_{1,17}$	0.292
Controls vs. DLP[2]	**Trt**	NA	NA	NA	NA	NA	NA	$12.21_{1,34}$	**0.001**	$1.41_{1,34}$	0.243	$70.07_{1,34}$	**0.001**
Restored vs. DLP[2]	**Trt**	NA	NA	NA	NA	NA	NA	$0.24_{1,34}$	0.628	$7.32_{1,34}$	**0.011**	$72.83_{1,34}$	**0.001**

Table 10. Results from regression analyses between soil bacterial communities and each predictor group/environmental variable on its own using DISTLM with PERMANOVA+ for Primer. Analyses of the restoration experiment included all quadrats. For each analysis a Bray-Curtis abundance matrix was used.
[1] Plant = predictor group for plant communities represented as scores from the first two NMS axes.
[2] Funct = predictor group of plant communities represented as functional groups: C_3-grass; C_4-grass; Forb; Legume and Woody.
[3] Shannon = predictor group of plant communities represented as Shannon diversity (H′).
[4] Env = predictor group for environmental variables. Restoration Experiment: live aboveground biomass; root biomass; microbial biomass; soil pH; total soil C; % silt; % sand; C/N Ratio; soil moisture, available N and elevation. DLP: root biomass; microbial biomass, soil pH and elevation. Significant relationships with individual environmental variables are listed below Env.
[5] Env2 = predictor group using only the environmental variables found to be significant correlates with soil bacterial communities on their own. Restoration Experiment: microbial biomass, soil pH, % sand and elevation. DLP: soil pH.
[6] Spatial = predictor group representing the spatial location of the soil bacterial communities. Includes the third polynomial(s) that were significant after stepwise regression using DISTLM. Restoration Experiment: x^3, x^2y. DLP: x.
[7] Pseudo-F: F-value generated from DISTLM program. Perm P: *p*-values generated from 9999 permutations of the data. Significant effects at α=0.05 level are in bold.

	Restoration Experiment			**DLP**		
Group/Env. Variable	**Pseudo-F_{df}[7]**	**Perm P[7]**	**R^2**	**Pseudo-F_{df}**	**Perm P**	**R^2**
Plant[1]	$0.88_{3,31}$	0.667	0.054	$1.99_{3,15}$	**0.013**	0.209
Funct[2]	$0.94_{6,28}$	0.643	0.144	$1.39_{6,12}$	0.066	0.367
Shannon[3]	$0.66_{2,32}$	0.870	0.020	$1.63_{2,16}$	0.085	0.093
Env[4]	$1.39_{12,22}$	**0.003**	0.411	$1.36_{5,13}$	0.091	0.295
Microbial Biomass	$2.04_{2,32}$	**0.008**	0.060	-	-	-
Soil pH	$2.09_{2,32}$	**0.009**	0.061	$2.50_{2,16}$	**0.012**	0.135
% Sand	$1.71_{2,32}$	**0.040**	0.051	-	-	-
Elevation	$2.95_{2,32}$	**0.001**	0.084	-	-	-
Env2[5]	$1.95_{5,29}$	**0.001**	0.212	$2.50_{2,16}$	**0.012**	0.135
Spatial[6]	$2.81_{3,31}$	**0.001**	0.153	$2.89_{2,16}$	**0.004**	0.153

Table 11. Stepwise regression results between soil bacterial communities and the predictor groups using DISTLM with PERMANOVA+ for Primer. Analyses of the restoration experiment included all quadrats. For each analysis a Bray-Curtis abundance matrix was used; adjusted R^2 was the selection criterion. The model with the highest ending F-value represented the 'best' model and is shown. Predictor groups are listed in the order in which they were included in the regression model.
[1] Plant = predictor group for plant communities represented as scores from the first two NMS axes.
[2] Env2 = predictor group using only the environmental variables found to be significant correlates with soil bacterial communities on their own. Restoration Experiment: microbial biomass, soil pH, % sand and elevation. DLP: soil pH.
[3] Spatial = predictor group representing the spatial location of the soil bacterial communities. Includes the third polynomial(s) that were significant after stepwise regression using DISTLM. Restoration Experiment: x^3, x^2y. DLP: x.
[4] Pseudo-F: F-value generated from DISTLM program. Perm P: *p*-values generated from 9999 permutations of the data. Significant effects at α=0.05 level are in bold.

	Predictor Group	Pseudo-F_{df}[4]	Perm P[4]	Cumulative Adjusted R^2	Cumulative R^2
Restoration Experiment	Env2[2]	$1.95_{5,29}$	**0.001**	0.103	0.212
	Spatial[3]	$2.21_{7,27}$	**0.001**	0.172	0.322
DLP	Plant[1]	$1.99_{3,15}$	**0.011**	0.104	0.209
	Spatial[3]	$1.98_{4,14}$	**0.028**	0.159	0.307
	Env2[2]	$1.49_{5,13}$	0.107	0.187	0.379

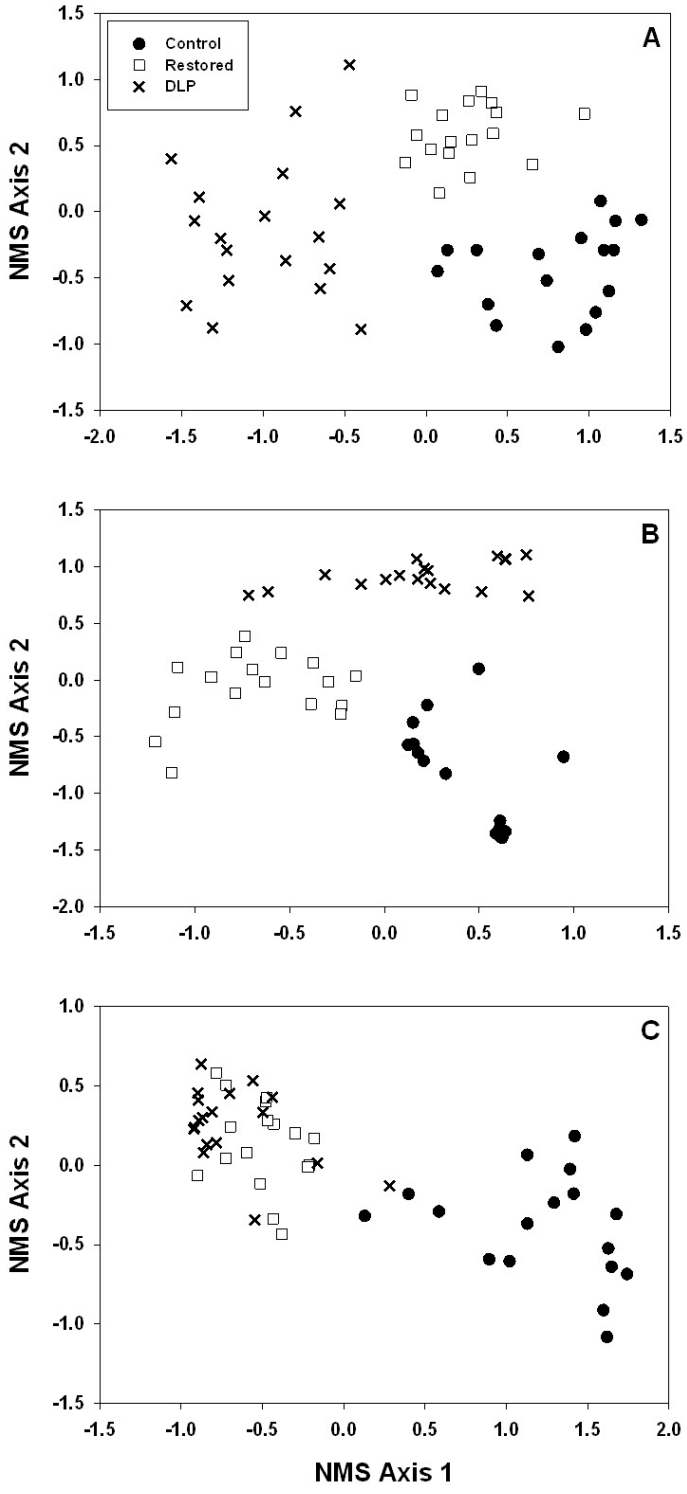

Figure 1. Non-metric Multidimensional Scaling plots for A) plant species community composition; B) plant functional group community composition; C) plant prairie group community composition.

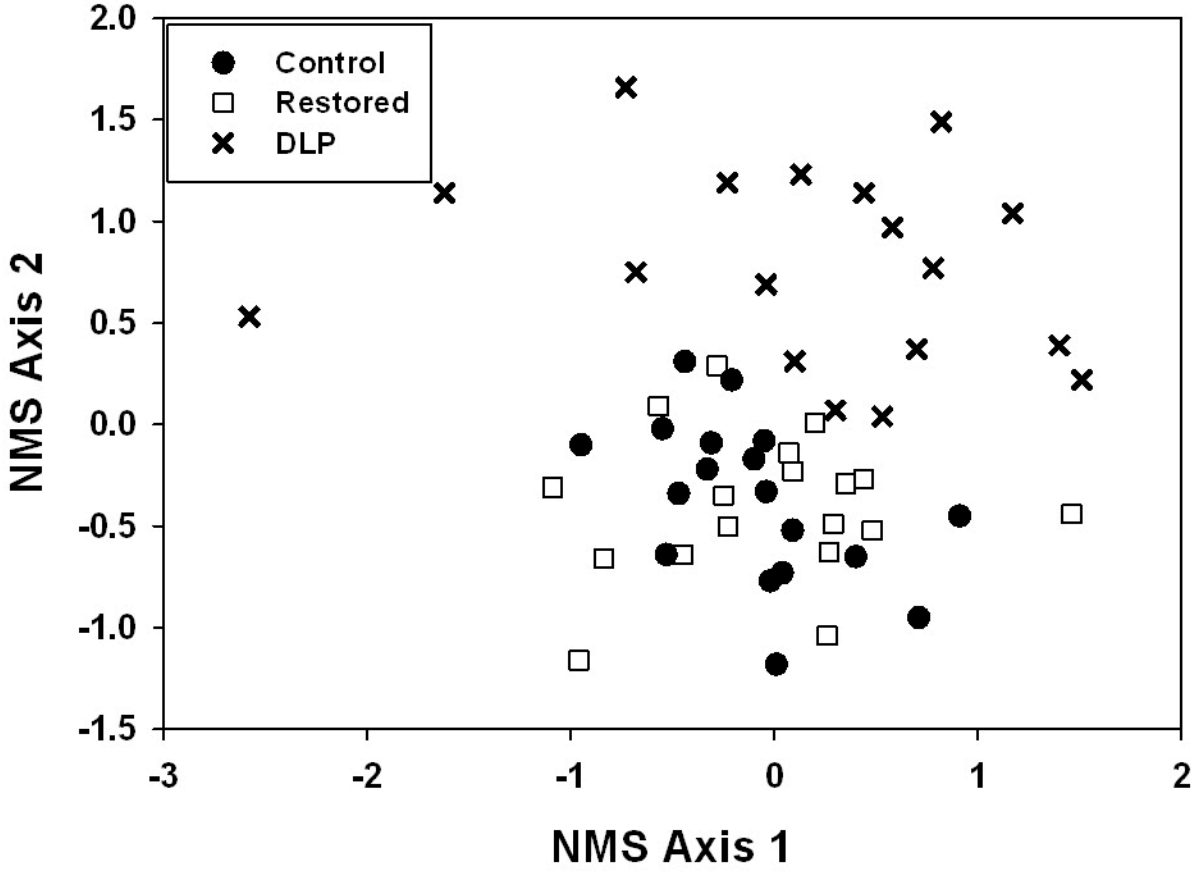

Figure 2. Non-metric Multidimensional Scaling plots for soil bacterial community composition.

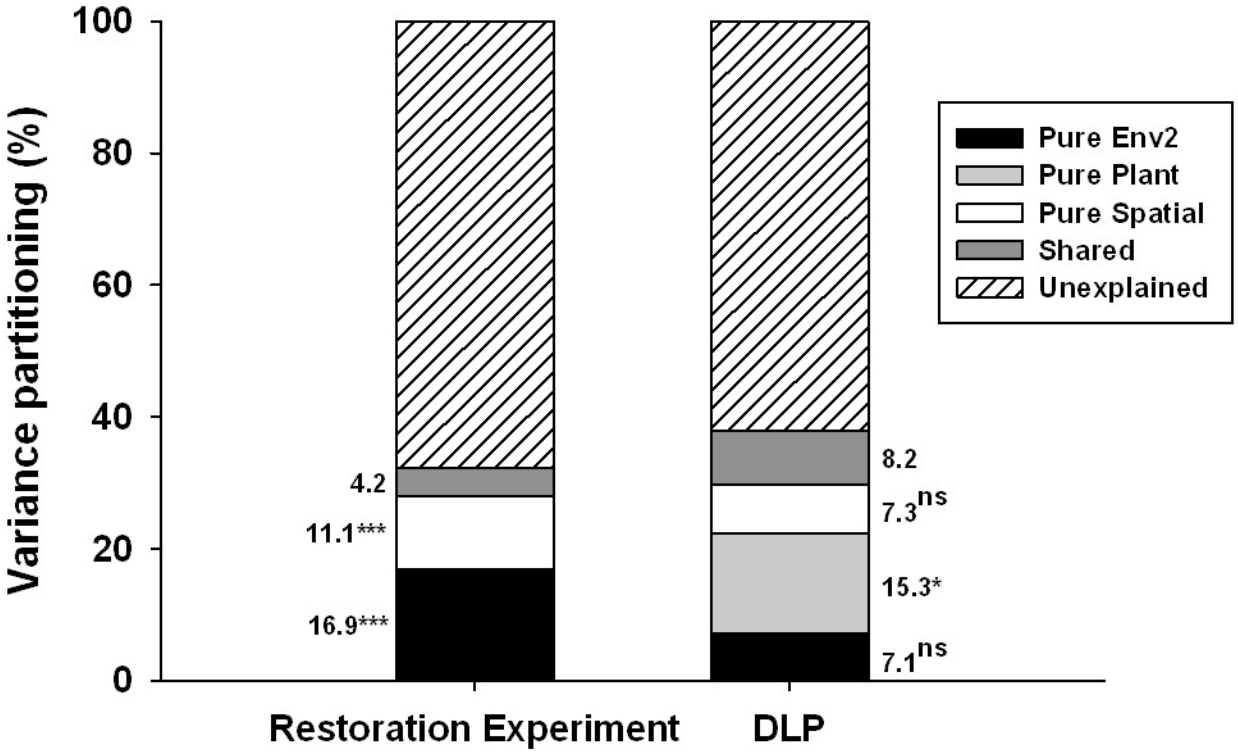

Figure 3. Variance partitioning (%) of the soil bacterial community compositions after DISTLM and dbRDA analyses. Values represent the amount of explained variation for each component. * $p < 0.050$, *** $p < 0.001$, ns = non-significant.

CHAPTER 2: **Plant presence and species composition, but not plant diversity, influence rhizosphere bacterial communities within a Kansas experiment**

Abstract:

Compared to plant communities with low richness, diverse plant communities could potentially foster greater soil environmental heterogeneity via greater diversities of litter inputs and root exudates. Because the majority of soil microbes use this plant material as food, increased plant richness could, therefore, promote a more diverse soil bacterial community. Often, however, increased primary production, not plant richness *per se*, has been the underlying cause of a positive relationship between plant richness and soil bacterial diversity in bulk soil. Further, few have examined this relationship within the rhizosphere, where interactions can be far more plant-driven than in bulk soil. Plant-specific root exudation, potentially resulting in unique rhizosphere communities associated with different plant species (plant identity effect) could provide the means for increased plant richness to promote rhizosphere bacterial diversity. Using the grassland species *Agrostis gigantea*, *Andropogon gerardii* and *Helianthus maximiliani*, I evaluated if an increase in plant richness (1-3) fostered increased rhizosphere bacterial diversity. I also assessed the degree of the plant identity effect by comparing rhizosphere bacterial compositions among the differing plant communities. Lastly, I determined if any effects upon rhizosphere bacterial communities were due to plant productivity, independent of plant richness or plant identity. My results indicated that the influence of plant identity, particularly *H. maximiliani*, and plant presence was more important for rhizosphere bacterial community structure than plant richness. Potentially, no relationship was found because of the small range in plant richness levels used, the dominant nature of *H. maximiliani*, and/or because additional environmental heterogeneity was

not promoted by *A. gigantea* and *A. gerardii*, which had similar rhizosphere communities. The plant identity effect of *H. maximiliani*, when compared to the other two plant species, was most likely due to different root exudation patterns and not plant productivity.

INTRODUCTION

The recognition that human activity has altered community compositions and accelerated the loss of diversity in ecosystems has stimulated research towards a better understanding of the links between biodiversity and ecosystem functioning (Vitousek et al. 1997). Through these studies, we are finding that a minimum number of species are essential for ecosystem functioning and greater diversity could be critical in maintaining ecosystem resilience, resistance and reliability to perturbations (Loreau et al. 2001; Milcu et al. 2010).

Soil microbial communities are integral to ecosystems because they mediate most soil processes such as carbon and nitrogen cycling (Zak et al. 2003). Because of the enormous diversity found in the soil, there has been a long-held assumption that soil microbial communities are functionally redundant (Torsvik et al. 1990; van Elsas et al. 2012). However, there is evidence showing that the diversity and composition of soil microbes can be significant to ecosystem performance (e.g. decomposition rates, resistance to invasion by bacterial pathogens) (Strickland et al. 2009; van Elsas et al. 2012). Despite the seeming importance of their diversities and compositions, the key factors shaping soil microbial community structure are only beginning to be explored (Horner-Devine et al. 2004a). In order to better recognize how soil microbial communities impact ecosystem processes, we need a greater understanding of how soil microbial communities are structured.

Environmental heterogeneity is often proposed as a mechanism for biological diversity. This hypothesis states that an increase in resource diversity can allow for higher species coexistence through greater resource partitioning. Species can divide the community such that each species is limited by a different resource, resulting in increased niches and greater diversity (MacArthur 1958; Tilman 2004). Soil bacterial communities can be shaped by the heterogeneity of edaphic soil properties such as soil pH, soil moisture, texture, and nutrient availability (Fierer and Jackson 2006; Bach et al. 2010; Harrison and Bardgett 2010). In addition, plant communities can provide a means to promote environmental heterogeneity within an ecosystem (Hooper et al. 2000; Berg and Smalla 2009). However, considerably less is known about the role that plant richness and compositions play in determining soil bacterial communities (Zak et al. 2003).

Because most soil bacteria are heterotrophic, they rely upon decomposing plant material and plant root exudates for most of their food, giving rise to two potential avenues linking plant richness to soil community structure (Zak et al. 2003; Loranger-Merciris et al. 2006). The present view is that with increasing plant richness, soil bacteria can be positively influenced through an increase in the diversities of root exudates and litter qualities and quantities. These increases can lead to a greater diversity of food resources and available niches to support a more diverse assemblage of microbes (Hooper et al. 2000; Porazinska et al. 2003; Eisenhauer et al. 2010; Lamb et al. 2011). If so, plant species loss from our ecosystems could result in a simplification of soil microbial communities, potentially impacting belowground processes (Milcu et al. 2010). However, whether increased plant richness increases the diversity of soil bacterial communities is still under debate.

A positive response between increased plant richness and soil bacterial communities has often been attributed to the indirect effect of plant productivity. An increase in plant richness

often yields increased net primary production, leading to greater quantities and diversities of resources entering the soil (Hooper et al. 2000; Tilman et al. 2001). These changes can ultimately increase microbial biomass and change microbial community compositions (Spehn et al. 2000; Zak et al. 2003; Bardgett and Wardle 2010). Just as frequently, however, no relationship between plant richness and soil bacterial communities has been observed (Broughton and Gross 2000; Brodie et al. 2002; Wardle et al. 2003; Bremer et al. 2009), potentially because the impacts of plant productivity could differ depending upon the group of soil biota examined, or the studies may not have been carried out long enough if soil bacterial communities have a delayed response to plant productivity (Eisenhauer et al. 2010; De Deyn et al. 2011). In addition, some have found that soil type can influence bacterial communities to a larger extent than plant richness (Kowalchuk et al. 2000). These studies have focused upon soil communities within the bulk soil (soil not specifically associated with plant roots). The degree of influence that the plant community exerts upon soil bacterial communities could depend upon the level of interaction between the two. Thus, bacterial communities within the rhizosphere may be far more plant-driven and immediate than those within the bulk soil (Kowalchuk et al. 2002). Very few have examined the relationship between increased plant richness and the associated rhizosphere bacterial communities (Kowalchuk et al. 2002; Kielak et al. 2008).

The rhizosphere is typically described as the portion of soil under the influence of the root system (Berg and Smalla 2009). The root system provides rhizosphere microbes with plant-derived carbon predominantly through two routes: root mass and rhizodeposition (Cheng and Gershenson 2007). As described above, decomposition of root biomass that may have increased as a result of increased plant richness could impact rhizosphere bacterial communities through larger quantities and more diverse carbon resources entering the rhizosphere.

Rhizodeposition, mainly consisting of root exudation, can supply between 5% and 44% of the carbon taken up during photosynthesis to the rhizosphere, providing a nutrient rich and distinctive environment for rhizosphere bacteria (Farrar et al. 2003; Nguyen 2005; Berg and Smalla 2009). Because of these exudates, primarily in the forms of organic acids, amino acids and sugars, the rhizosphere contains enhanced microbial biomass and activity when compared to bulk soil (Bardgett et al. 2005; Raaijmakers et al. 2009). Further, root exudates can select for or against particular soil bacteria, generally reducing the bacterial diversity within the rhizosphere (Kowalchuk et al. 2002). For example, *Pseudomonas* are often found in greater abundance in the rhizosphere than in bulk soil possibly because these bacteria have been categorized as R-strategists (copiotrophs) and quickly take advantage of the nutrient supply provided by the roots (Smit et al. 2001; Fierer et al. 2007). On the other hand, the rhizosphere can be relatively deficient in gram-positive bacteria and Acidobacteria (Olssen and Persson 1999; Kielak et al. 2008).

Despite a degree of commonality in the bacteria that live within the rhizosphere of different plant species, there is also a high degree of specificity because rhizodeposition can be very plant-specific (Hawkes et al. 2007). This can potentially lead to rhizosphere bacterial communities that are unique for each plant species (Berg and Small 2009; Bardgett and Wardle 2010). Through this plant identity effect, it is possible that a more diverse plant community could lead to a more diverse rhizosphere bacterial community (Kielak et al. 2008). Over time, a more diverse rhizosphere community could have important implications for plant growth, vegetation succession and nutrient cycling because these organisms can have both beneficial (e.g. produce plant growth hormones, suppress plant pathogens; fix nitrogen) and adverse (e.g. competition for nutrients, pathogens) effects upon their associated plant species (Westover et al. 1997; Nunan et al. 2005; Berg and Smalla 2009; Bever et al. 2010; Milcu et al. 2010). But whether or not plant

richness enhances rhizosphere bacterial communities is still ambiguous (Kielak et al. 2008). Further, most of our knowledge about plant identity effects is from economically important annual plant species (Hawkes et al. 2007). There is evidence that native vegetation can have very high plant specificity of rhizosphere bacterial communities (Berg and Smalla 2009). Very little is known about the impacts that native perennial species have upon their associated rhizosphere bacterial communities.

Here, I present results from a field experiment established in a Kansas grassland in which I examine the extent to which plant species richness, identity and community composition influences rhizosphere bacterial communities. My first objective was to determine if an increase in plant richness, generated by three perennial grassland species, corresponded with an increase in rhizosphere bacterial alpha diversity (richness and Shannon diversity, H′). Second, I examined the role of plant community composition upon rhizosphere bacterial alpha diversity and composition. In this way, the plant identity effect of the three plant species was evaluated. Third, I assessed if any observed effects of plant richness and plant identity upon the rhizosphere bacterial communities could be attributed to differences in plant productivity (especially root biomass) rather than differences in plant richness or plant identity *per se*. On the one hand, if any differences in rhizosphere bacterial communities became non-significant after accounting for variation in root biomass, this would suggest the differences were due to plant productivity and not necessarily plant richness or plant identity (De Deyn et al. 2011). On the other hand, if root biomass did not change the original outcome, this suggests something other than plant productivity could be influencing the relationship, potentially factors such as root exudation (Eisenhauer et al. 2010).

METHODS

Study Site

The study site chosen for this experiment was located within a former pasture/hay field at the University of Kansas Field Station (KUFS) (lat 39°03′ N, long 95°12′ W). Located along the eastern deciduous forest-tallgrass prairie ecotone of Northeastern Kansas, the area consists of undulating, ridge-to-swale topography. The study site was located on an upland ridgetop, gently sloping downwards from north to south. Soils are Pawnee clay loams (montmorillonitic, mesic Aquic Argiudolls) formed under glacial deposits of till and loess with weathering of interbedded limestones and shales (U.S. Department of Agriculture Soil Conservation Service 1977). The mean annual temperature is 12.9°C and mean annual precipitation is 930 mm (Atmospheric Science Library 1990).

The experiment was located within a 25m x 55m enclosure initially used between 1997-2000 to study changes in the frequency of crop-specific genetic markers in wild sunflower (*Helianthus annuus*) (Cummings et al. 2002). A 3m high fence surrounded the enclosure to exclude deer and other vertebrate herbivores. Since the conclusion of the wild sunflower experiment, the area has been periodically mown. Immediately prior to the current study, a survey showed the plant community was dominated by *Bromus inermis* (cool-season introduced grass). Also prevalent were saplings of *Ulmus rubra*, perennial native warm-season grasses (*Andropogon virginicus*, *Sporobolus* sp.) and forbs (*Solidago canadensis*) and the shrub, *Rubus occidentalis*.

Experiment Design and Establishment

In May 2006, soil within the enclosure was plowed, tilled and roto-tilled to homogenize the soil and diminish potential impacts of prior vegetation upon the soil bacterial community. The

experiment was set up as a randomized complete block design with ten blocks of nine treatments. Plots were 1.5m x 1.5m with alleyways between each. Two treatments were considered controls. One control did not contain plants, was continually weeded and used to measure the bulk soil and the effect of no plant inputs (NP). The other control treatment consisted of allowing plants to germinate from the seed bank and grow unchecked (SB). This treatment allowed me to measure rhizosphere soil from plant species emerging naturally from the seedbank and from communities with higher plant richness than the other treatments (SB control average plant richness = 31.2 ± 1.18). The remaining seven treatments were monocultures and mixtures (2-species; 3-species) of the three target plant species.

The target species were originally *Koeleria macrantha* (C_3-grass), *Andropogon gerardii* (C_4-grass) and *Helianthus maximiliani* (forb). All three are native tallgrass prairie species, perennials and relatively abundant in tallgrass prairie remnants throughout the area (Jog et al. 2006). After treatment application (seed sowing, described below), I observed that *K. macrantha* seed was contaminated with seeds of *Agrostis gigantea*. Although *A. gigantea* is not a native grass, it is a perennial, C_3-grass, with anthesis concurrent with *K. macrantha.* All monocultures targeting *K. macrantha* ultimately became monocultures of *A. gigantea* during the first growing season (2006) due to competitive exclusion. Therefore, this treatment used *A. gigantea* as the target species rather than *K. macrantha*. Seeded treatments included monocultures of each species (*Ag*, *An*, *He*), all combinations of 2-species mixtures (*AgAn*, *AgHe*, or *AnHe*) and a treatment comprising all three species (*AgAnHe*). This study used a more limited range of plant richness levels, which allowed for higher replication to test for the effects of plant identity on rhizosphere bacterial communities (Loranger-Merciris et al. 2006).

Seeds of the target species were purchased from Stock Seed Farms (Murdoch, NE) and were sown on 6 June 2006. For each plot, the amount sown was based upon the percent germination of each species (germination rates were provided by Stock Seed Farms) and a goal of 1000 seedlings per plot. Seeds were broadcast and the soil was raked to scrape the surface and cover the seeds. Alleyways and plots were weeded throughout the duration of the experiment.

Rhizosphere Bacterial Community Structure

To assess soil rhizosphere bacterial communities, in 2008, soils were sampled twice in all plots over the growing season (two years after seeding) coinciding with peak flowering of *A. gigantea* (11-14 June) and *A. gerardii* and *H. maximiliani* (21-22 August). For each sampling period, three randomly designated locations were chosen and the soil beneath the closest plants was sampled (2.5cm x 15cm) and placed into a ziplock bag. Soils were frozen at -80°C until further processing could occur. To collect rhizosphere soil, the soil cores were carefully broken apart to expose the roots. Any soil still attached to the root was considered rhizosphere soil and brushed into a collection tray. All roots were dried to a constant weight (74°C) for root biomass measurements. Further, roots from the three target species were easily distinguished from each other. Therefore, I was able to estimate root biomass for each target species in the mixture plots.

T-RFLP Procedure: Terminal-Restriction Fragment Length Polymorphism (T-RFLP) was used to assess rhizosphere bacterial communities. Even though T-RFLP does not allow for estimating diversity and structure at fine taxonomic levels, this technique is a reproducible method having sufficient resolution to detect differences in soil bacterial community structure (Fierer et al. 2003). Rhizosphere soil DNA was extracted in duplicate for each plot using the MoBio PowerSoil

DNA extraction kit as recommended by the manufacturer. The duplicate extractions were pooled and DNA was quantified by gel electrophoresis. Extractions were either concentrated or diluted to obtain a final concentration of 10ng DNA/uL extract.

16S rDNA from the extracted DNA samples was PCR-amplified using the universal eubacterial primers 6-FAM 8-27F, a fluorescently labeled forward primer (5′-AGRGTTTGATCMTGGCTCAG-3′), and 1389R, a non-labeled reverse primer (5′-ACGGGCGGTGTGTACAAG-3′) (Fierer et al. 2003). For each plot, PCR reactions were performed in triplicate. Each 50μL PCR reaction contained 50ng DNA, 0.5uM of each primer, and 1 x HotStar Taq Master Mix Kit (Qiagen), which included a final concentration of 2.5 units HotStarTaq DNA Polymerase, 1.5nM $MgCl_2$ and 200uM of each dNTP. Reaction mixtures were held for 15 minutes at 95°C for activation. Reactions were cycled 32 times through three steps: 45s of denaturation at 94°C, 45s of annealing at 58°C and 90s for primer extension at 72°C. The final extension lasted 10 minutes at 72°C. The triplicate PCR reactions were pooled, purified using the MoBio UltraClean PCR Clean-Up Kit, and yields quantified using gel electrophoresis.

Products of PCR reactions were initially digested using both *Rsa*I and *Msp*I restriction enzymes (New England Biolabs). However, only the *Rsa*I enzyme completely digested PCR product. Therefore, analyses of rhizosphere bacterial communities only included digestions using *Rsa*I. Each 20μL reaction contained 100ng of purified PCR product, 10 units of restriction enzyme and two μL of buffer. Each reaction was digested for three hours at 37°C with an inactivation period of 20 minutes at 65°C. The fluorescently labeled fragments were analyzed using an Applied Biosystems Instrument 3730 genetic analyzer. Terminal restriction fragment (TRF) sizes between 25 and 1000 bp, with peak heights > 25 fluorescent units were measured using Peak Scanner 1.0 analytical software (Applied Biosystems).

T-RFLP Profile Alignment: All rhizosphere bacterial community profiles were analyzed, standardized and aligned simultaneously using the program T-REX (Culman et al. 2009). T-REX is a free, online-based tool that was developed to aid in the alignment and analysis of T-RFLP datasets. Within T-REX, TRFs were first filtered to find 'true peaks' within the profiles, using peak area. TRFs were aligned such that fragment sizes differing by ≤ 0.5 bp were considered identical and clustered. To avoid clustering 'distinct close peaks', the maximum number of fragments assigned to a cluster was limited to the number of profiles being aligned. Once the maximum number was reached, a new cluster was created. For each cluster of TRFs, the average size was calculated and used to distinguish TRFs. The data matrix was exported and peak areas were relativized for each TRF within a community profile. Peak areas with a proportion <0.5% were removed (Fierer et al. 2003).

In order to compare rhizosphere bacterial community structure, both the proportional peak area (abundance) and TRF size were used. Because different organisms can produce TRFs of similar size, TRFs of different lengths were assumed to represent different operational taxonomic units (OTUs) and not necessarily distinct bacterial species.

Additional Measurements

In addition to sampling root biomass, as described above, aboveground productivity was sampled to further evaluate overall plant productivity. Aboveground productivity was estimated by clipping plant biomass (1m x 0.08m) to ground level after each soil sampling and dried at 74°C until constant weight. Inorganic N and soil moisture were measured because they are environmental variables that are frequently positively correlated with plant productivity and are important for microbial growth (Broughton and Gross 2000). Inorganic N was measured using

resin strip extractions according to Qian and Schoenau (1996). This was an integrative measure of available N spanning two weeks that overlapped with the soil rhizosphere sampling. Soil moisture was measured from the center of each plot immediately prior to soil sampling using a Trime-FM TDR instrument.

Soil microbial biomass was measured using the procedure of Findlay et al. (1989). Soil from the NP control and rhizosphere soil was pooled by treatment using soil remaining after the DNA extractions. Pooling allowed duplicate measurements for each treatment to be used. Briefly, phospholipids were dissolved in a chloroform phase and filtered using Na_2SO_4 until 10mL of chloroform was collected. Phospholipids were concentrated to 1mL of chloroform. After digestion with 5% acid potassium persulfate, malachite green solution was used to allow measurement of absorbance with a spectrometer (610nm). Using glycerophosphate standards, nmol of lipid bound phosphate was calculated for each sample and converted into cells per gram of soil ($4x10^9$ cells per 100nmol P).

Data Analyses

Rhizosphere bacterial diversities and compositions were assessed for the growing season as a whole. To do this, all data were combined using the maximum value obtained out of the two sampling periods (e.g. maximum proportion for each OTU; maximum root biomass). These maximum values were used for all analyses. Alpha diversity (richness and Shannon diversity, H′) was calculated for bacterial communities in PC-Ord 4.14 (McCune and Mefford 1999). Univariate analyses for OTU alpha diversity, aboveground and root biomass, inorganic N and soil moisture were performed in SPSS 19 using randomized complete block (RCB) ANOVAs, while 1-Way ANOVA was used to analyze soil microbial biomass. Least significance difference (LSD)

was used as post-hoc tests. Aboveground and root biomass were log-transformed to meet ANOVA assumptions.

Rhizosphere bacterial communities were assessed using two approaches. First, the effects of plant richness *per se* upon rhizosphere bacterial alpha diversities were analyzed by grouping the target species treatments by plant richness (1-3). Control treatments were included in these analyses. Second, to examine the effects of plant community composition, rhizosphere bacterial alpha diversities and compositions were analyzed by treatment. Rhizosphere bacterial community compositions were first analyzed using Non-Metric Multidimensional Scaling (NMS) within PC-Ord 4.14 using a Bray-Curtis similarity matrix. To graphically display differentiation in the rhizosphere bacterial communities, averages and standard errors of the first two NMS axis scores were calculated by treatment and displayed in an ordination diagram. Treatment differences among rhizosphere bacterial community compositions were tested with Permutational Multivariate Analysis of Variance (PERMANOVA) using PERMANOVA+ for Primer, Primer v6 Software (Anderson 2001; Clarke and Gorley 2006). PERMANOVA tests for differences among community matrices and calculates *p*-values through permutations of observations. The software also allows for the analysis of multivariate data using ANOVA designs such as RCB and ANCOVA and will perform *a posteriori* pair-wise comparisons among factor levels. All PERMANOVA analyses used relativised abundance matrices, 9999 permutations and the Bray-Curtis similarity distance measure.

To determine if the effects of plant community composition upon rhizosphere bacterial alpha diversities and compositions were potentially due to the root biomass of each plant species rather than root exudation, each species' root biomass was used as a covariate in ANCOVA analyses (within SPSS 19 and PERMANOVA), excluding the NP and SB controls. I inferred that

a particular species' root biomass was influencing rhizosphere bacterial communities if treatment differences were no longer found after the ANCOVA.

Further, to separate any plant richness or plant compositional effects from that of total root biomass, I analyzed rhizosphere bacterial diversities and compositions using ANCOVAs with total root biomass for each plot as a covariate. Live aboveground biomass, microbial biomass, inorganic N and soil moisture were also used as covariates in separate ANCOVA analyses. This was done to evaluate if these variables could help explain any plant richness or plant compositional effects found in the RCB ANOVA analyses.

RESULTS

Effects of Plant Richness

Across the 1, 2, and 3 plant species richness plots, increased plant richness did not correspond with an increase in rhizosphere bacterial alpha diversity (Figure 1) (OTU Richness: Block factor $F_{9, 76} = 4.53$ $p = 0.001$; Plant Richness factor $F_{4, 76} = 3.30$ $p = 0.015$) (OTU H′: Block factor $F_{9, 76} = 5.83$ $p = 0.001$; Plant Richness factor $F_{4, 76} = 2.84$ $p = 0.030$). In fact, all levels of plant richness had the lowest and similar values of OTU richness and Shannon diversities. In contrast, the SB control not only had the highest plant species richness (average plant richness = 31.2 ± 1.181), but also the highest rhizosphere bacterial richness and Shannon diversity. Soil bacterial alpha diversity of the NP control was intermediate between the SB control and the three levels of plant richness.

RCB ANOVA analyses of aboveground biomass indicated that an increase in plant species richness from 1 to 3 plant species corresponded with a significant increase in

aboveground biomass, consistent with other plant diversity-productivity studies (Tilman et al. 1996) (Figure 2A) (Block factor $F_{9,76} = 1.13$ $p = 0.355$; Plant Richness factor $F_{4,76} = 104.15$ $p = 0.001$). However, root biomass was not significantly different among the plant richness levels (Figure 2B) (Block factor $F_{9,76} = 2.30$ $p = 0.026$; Plant Richness factor $F_{4,76} = 17.78$ $p = 0.001$). The SB control had aboveground and root biomass values significantly lower than the three plant richness levels, but significantly higher than the NP control, which had the lowest biomass.

The three plant richness levels had similar rhizosphere microbial biomass and inorganic N (Table 1). However, as plant richness increased from 1 to 3 plant species, soil moisture decreased. The SB control had similar rhizosphere microbial biomass and inorganic N as the three plant richness levels, while soil moisture was intermediate between the NP control and the three plant richness levels. The NP control had the lowest microbial biomass (bulk soil), but the highest measurements of inorganic N and relatively high soil moisture contents.

In order to determine if the differences found among rhizosphere bacterial alpha diversities could be attributed to other factors than plant richness *per se*, ANCOVAs were performed using total root biomass, aboveground biomass, microbial biomass, inorganic N and soil moisture as covariates. Results indicated that even after accounting for these five variables, bacterial OTU richness still differed among the controls and the three plant richness levels as described above (Figure 1) (Table 2). In contrast, after accounting for the effects of root biomass, aboveground biomass and inorganic N, significant differences among the controls and plant richness levels of bacterial OTU Shannon diversities were no longer found.

Effects of Plant Community Composition

When analyzing rhizosphere bacterial OTU richness by treatment, I found that monocultures of *Ag* and *An* were similar and tended to have higher richness than *He* monocultures (Figure 3A) (Block factor $F_{9,72} = 4.73$ $p = 0.001$; Trt factor $F_{8,72} = 2.66$ $p = 0.013$). The 2-species and the 3-species mixtures tended to have the lowest rhizosphere bacterial OTU richness but were statistically similar to the monoculture plots (exception: *AgHe* was significantly lower than *Ag* and *An*). The SB control was similar to the NP control, *Ag* and *An* monocultures, but was significantly higher than all other treatments. The NP control had similar bacterial OTU richness as all other treatments except *AgHe*.

Rhizosphere bacterial OTU Shannon diversities of *Ag* and *An* monocultures were similar and significantly higher than *He* monocultures, which had the lowest rhizosphere bacterial OTU diversity of all the treatments (Figure 3B) (Block factor $F_{9,72} = 6.79$ $p = 0.001$; Trt factor $F_{8,72} = 2.66$ $p = 0.001$). In addition, the treatments that contained *Helianthus* as part of the plant community (*He*, *AgHe*, *AnHe* and *AgAnHe*) tended to have lower Shannon diversities than the other treatments. The SB and NP controls tended to have the highest rhizosphere bacterial OTU diversities.

PERMANOVA analyses indicated that the *Ag* and *An* monocultures had similar rhizosphere bacterial community compositions, which were both significantly different from the *He* monocultures (Figure 4; Table 3) (Block factor Pseudo-$F_{9,72} = 14.33$ $p = 0.001$; Trt factor Pseudo-$F_{8,72} = 3.16$ $p = 0.001$). *He* monoculture rhizosphere bacterial community compositions were also significantly different from all other treatments. The 2-species and 3-species mixtures had rhizosphere bacterial community compositions similar to each other, except between *AgAn* and *AnHe* treatments. The 2-species mixture of *AgAn* was similar to the rhizosphere bacterial

communities of both *Ag* and *An* monocultures. The mixtures of *AgHe*, *AnHe* and *AgAnHe* were statistically similar to *Ag* monocultures, but significantly different from *An* and *He* monocultures. Rhizosphere bacterial communities of the SB control were similar to only those of the *An* and *AgAn* treatments. The NP control had soil bacterial community compositions significantly different than all other treatments.

Treatments containing *Helianthus* within the plant community had the highest aboveground biomass (Figure 5A) (Block factor $F_{9,72} = 1.72$ $p = 0.101$; Trt factor $F_{8,72} = 84.73$ $p = 0.001$). Treatments containing *Agrostis* and *Andropogon* had similar aboveground biomass and were similar to the SB control. In contrast, the target species' treatments all had similar root biomass and were significantly higher than the SB control (Figure 5B) (Block factor $F_{9,72} = 2.42$ $p = 0.030$; Trt factor $F_{8,72} = 7.77$ $p = 0.001$). The NP control had the lowest aboveground and root biomass.

He monocultures and mixtures containing *Helianthus* had the highest rhizosphere microbial biomass and had lower soil moistures than *Ag* and *An* monocultures and the *AgAn* mixture (Table 4). The SB control had intermediate rhizosphere microbial biomass (similar to *AgHe* and *AgAnHe*) and soil moisture whereas the NP control had the lowest microbial biomass (bulk soil) and relatively high soil moisture. Inorganic N was similar across all treatments, except for the NP control, which was significantly higher.

Results of the ANCOVA analyses showed that even after accounting for differences in total root biomass, aboveground biomass, microbial biomass, inorganic N or soil moisture, treatment differences still remained for rhizosphere bacterial OTU alpha diversity and community compositions (Table 5). Further, I used ANCOVA to determine if a particular target plant species' root biomass could be underlying the treatment differences in rhizosphere bacterial

communities. From these analyses, I found that after accounting for *Agrostis* or *Helianthus* root biomass, rhizosphere bacterial OTU Shannon diversities no longer had treatment differences (Table 6). Treatment differences remained for all other ANCOVA analyses.

DISCUSSION

By using the three plant species *A. gigantea*, *A. gerardii* and *H. maximiliani*, I found that plant identity and plant presence were more important for structuring rhizosphere bacterial communities (alpha diversity and compositions) than plant richness. After increasing plant species richness from one to three plant species, there was no concurrent increase in rhizosphere bacterial diversity. To my knowledge, only two studies, both of which were conducted in the same field in The Netherlands, have explicitly examined the relationship between plant richness and rhizosphere bacterial diversity (Kowalchuk et al. 2002; Kielak et al. 2008). Kowalchuk et al. (2002) showed that higher plant diversity treatments (up to 15 plant species) maintained a higher level of bacterial diversity within the rhizosphere. However, four years later, Kielak et al. (2008) found no such relationship. Two explanations were given for these different outcomes: 1) different methods were used to determine bacterial communities (DGGE vs. cloning and sequencing) and/or 2) rhizosphere bacterial community compositions changed over the four years such that a relationship between plant richness and bacterial diversity was no longer found.

In this study, any transient effects of plant richness upon rhizosphere bacterial communities cannot be determined, and therefore, cannot be ruled out. T-RFLP could have been too coarse of a method to detect any plant richness effects, but this is probably not the case as results from the SB control suggest that higher plant richness could generate higher rhizosphere

bacterial diversity (Figure 1). This result also suggests that the range in plant richness levels used in this study may have been too small to detect any potential increases in bacterial diversity (Hedlund et al. 2003). Even when examining bulk soil microbial communities, many studies that used a larger range of plant richness levels (up to 32 or 60 species) exhibited a response from the bacterial community (Spehn et al. 2000; Gruter et al. 2006). This positive response, in many cases, was accredited to differences in plant productivity generated by the increases in plant richness, not the direct effects of plant richness *per se* (e.g. Zak et al. 2003). But others attributed the response potentially to rhizosphere effects that were large enough to impact the bulk soil community (Stephan et al. 2000; Eisenhauer et al. 2010). When using only eight or 15 plant species, bulk soil bacterial communities generally showed no response to plant richness, but were influenced primarily by edaphic soil properties (Brodie et al. 2002; Hedlund et al. 2003; Wardle et al. 2003; Zul et al. 2007; Bremer et al. 2009; Millard and Singh 2010).

A. gigantea and *A. gerardii* had similar rhizosphere bacterial alpha diversities and compositions. From these results, along with the fact that root biomass did not have a strong impact on the rhizosphere bacterial communities of these two plant species, the root exudation patterns could have been similar for these two grasses. Therefore, including *A. gigantea* and *A. gerardii* within the same plant community may not have enhanced the environmental heterogeneity of the overall rhizosphere habitat, did not support different bacterial populations, and contributed to the absence of a plant richness effect.

Selecting *H. maximiliani* for this study may have contributed further to the lack of relationship between plant richness and rhizosphere bacterial diversities. It is postulated that an increase in plant richness could increase soil diversity, and therefore ecosystem performance, through either niche complementarity or chance events (Loreau et al. 2001). For example, niche

complementarity promotes higher diversity, or ecosystem functioning, because plant communities with higher species richness increase diversity and resource utilization of the soil biota to levels greater than expected from individual plant species grown in monocultures (Tilman et al. 2001). In contrast, higher diversity in the soil could result from the greater chance of a highly productive or keystone plant species influencing the soil community more than plant richness (i.e. the sampling effect) (Carney and Matson 2006; Ladygina and Hedlund 2010). Mixture treatments that included *H. maximiliani* in the plant community tended to have lower rhizosphere bacterial diversities than the other treatments. This could have drawn down the average diversities from the two and three plant species communities (Figure 1), negating any plant richness effects. Thus, the absence of a plant richness-rhizosphere bacterial diversity relationship may be the consequence of plant communities that contain a plant species (e.g. *H. maximiliani*) exerting a strong influence upon the rhizosphere community (Bremer et al. 2009).

Not only did rhizosphere bacterial communities exhibit lower diversities when *H. maximiliani* was part of the plant community, the presence of *H. maximiliani* also impacted rhizosphere bacterial community compositions. Monocultures of *H. maximiliani* had distinct compositions from the monocultures of the two grass species, which were similar (*A. gigantea* and *A. gerardii*) (Figure 3 and 4). Furthermore, when *H. maximiliani* was part of mixture plant communities, the presence of this species shifted compositions towards that of the *He* monocultures. Thus, this study showed a strong plant identity effect with *H. maximiliani* while the two grass species exhibited similar responses from the rhizosphere bacterial community.

Despite the prevalent idea that differing plant species can have unique rhizosphere communities, the extent of the plant identity effect is often variable (Smalla et al. 2001; Kowalchuk et al. 2002; de Ridder-Duine et al. 2005). For example, Westover et al. (1997) found

distinct microbial communities when examining the rhizospheres of *Anthoxanthum odoratum* and *Plantago lanceolata*. In contrast, Kielak et al. (2008) observed very little effect upon rhizosphere bacterial communities when using 12 different late-successional plant species. The authors suggested that the field's agricultural history and resulting soil edaphic properties superseded any plant species effects. Different rhizosphere sampling strategies could contribute to these varying results by inadvertently collecting differing amounts of bulk soil and potentially diluting any rhizosphere effects (Broughton and Gross 2000; Kowalchuk et al. 2002). Further, different groups of microbes could have varied levels of specificity. Within a grassland in southern Scotland, a weak relationship between rhizosphere bacterial communities and plant species was found, whereas, a plant identity effect was observed with fungal community structure (Ridgeway et al. 2003; Nunan et al. 2005; Singh et al. 2007).

It is possible that grass species may not display as strong of an effect on microbial communities as forbs or legumes, as observed in this study, because of differing root exudation patterns (Ladygina and Hedlund 2010). For example, Warembourg et al. (2003) found that different plant families partition C belowground differently. Non-legume forbs (Asteraceae family in particular) invested more C into root biomass whereas grasses allocated more C into root exudation. Using the plant species *Holcus lanatus* (grass), *Lotus corniculatus* (legume), and *Plantago lanceolata* (forb), Ladygina and Hedlund (2010) observed these same C allocation patterns (higher root exudation in the grass species, *H. lanatus*). They also found *H. lanatus* exhibited a plant identity effect upon both bacterial and fungal compositions because of those exudations. Despite this finding, it is widely unknown whether particular plant families and/or functional groups have distinct impacts upon rhizosphere microbial communities.

To my knowledge, differences in the quantity and quality of root exudates among the three plant species used in this study are unknown. If plant family root exudation patterns are similar, this could potentially explain the similarities between *A. gigantea* and *A. gerardii* rhizosphere communities and the distinct rhizosphere bacterial communities of *H. maximiliani*. In addition, annual sunflower (*Helianthus annuus*) has been shown to have high allelopathic potential (Spring and Benz 1989). More than 200 natural allelopathic compounds have been isolated with corresponding reductions of microorganisms in the rhizosphere of *H. annuus* such as Azospirillum and Rhizobium (Kamal and Bano 2008). Although beyond the scope of this study, it would be interesting to investigate if other plant species within this genus, such as *H. maximiliani*, exuded allelopathic compounds. If so, it also could potentially explain the lower rhizosphere bacterial diversities in plots with *H. maximiliani* and their distinct rhizosphere bacterial community composition.

Not only did *H. maximiliani* display a plant identity effect upon rhizosphere bacterial communities, the presence of this plant species impacted several environmental variables as well. Plots with *H. maximiliani* had significantly higher aboveground productivity, higher rhizosphere microbial biomass and lower soil moisture than the two grass species monocultures and their mixtures. However, none of these environmental variables helped explain differences among the rhizosphere bacterial communities when used as covariates (Table 5). There was some indication that *A. gigantea* and *H. maximiliani* root biomass influenced rhizosphere bacterial OTU Shannon diversity (Table 6). Overall, rhizodeposition could be presumed to be the primary factor, with root biomass playing a minor role, in shaping the bacterial communities found in the rhizospheres of these three plant species (Eisenhauer et al. 2010).

In addition to plant identity, I found an effect of plant presence (also known as the rhizosphere effect): bacterial compositions of the bulk soil were significantly different from those of the rhizosphere (Figure 4). Bacterial alpha diversity also showed signs of a plant presence response, albeit not as striking as the bacterial compositions. Bacterial richness and Shannon diversity of the NP control was either similar to or tended to be slightly higher than within the rhizosphere. These results are consistent with several other studies that found lower rhizosphere bacterial diversities and distinct compositions when compared to bulk soil (Marilley et al. 1998; Kowalchuk et al. 2002; de Ridder-Duine et al. 2005; Zul et al. 2007; Kielak et al. 2008). Differences in microbial diversities and compositions between bulk and rhizosphere soil has often been attributed to the effects of root exudation (Zul et al. 2007). For example, within a mesocosm study, Bremer et al. (2009) showed that the *nir*K-type denitrifier community in bulk soil was most strongly impacted by the presence of plants and secondarily by plant composition, through differences in the amounts and compositions of root exudates.

In conclusion, the influence of plant identity, particularly *H. maximiliani*, and plant presence was more important in structuring rhizosphere bacterial communities than plant richness. A lack of response by the rhizosphere bacterial community to increased plant richness was potentially because of the small range in richness levels used, because of the dominant nature of *H. maximiliani*, and/or because additional rhizosphere environmental heterogeneity was not provided by the two grass species (*A. gigantea, A. gerardii*). The plant identity effect of *H. maximiliani* was most likely due to different root exudation patterns from those of the two grass species. By increasing the number of plant species used, further investigations could determine i) if there is a minimum number of plant species that are required to show a relationship with soil biota, ii) how important the plant identity effect is to the outcome of the plant richness and soil

biota relationship, iii) if plant families or functional groups impact rhizosphere bacterial communities to a greater level than plant species and iv) what types and amounts of root exudates are common for these species and if allelopathy contributes to the plant identity effect of *H. maximiliani*.

Table 1. Effects of plant richness upon microbial biomass, inorganic N and soil moisture. Results of the LSD post-hoc tests are superscripted next to the means for each variable and plant richness level. Differing letters signify significant differences at $\alpha = 0.05$ level. Significant *p*-values are in bold. NA: Microbial biomass was analyzed using 1-Way ANOVA; block factor was not included.

	Microbial Biomass (cells/g soil)		**Inorganic N (mg/L)**		**Soil Moisture (%)**	
NP	$3.44 \times 10^{6\,b}$		18.49^{a}		37.29^{ab}	
SB	$9.96 \times 10^{6\,a}$		1.96^{b}		36.35^{abc}	
1	$9.93 \times 10^{6\,a}$		1.64^{b}		37.55^{a}	
2	$10.12 \times 10^{6\,a}$		1.71^{b}		36.38^{bc}	
3	$10.05 \times 10^{6\,a}$		1.39^{b}		34.80^{c}	
	F_{df}	**P**	**F_{df}**	**P**	**F_{df}**	**P**
Block	NA	NA	$0.97_{9,76}$	0.469	$2.02_{9,76}$	0.049
Plant Richness Factor	$4.41_{4,13}$	**0.018**	$21.02_{4,76}$	**0.001**	$2.43_{4,76}$	**0.012**

Table 2. Results of the plant richness factor from ANCOVA analyses. Separate analyses were done for each covariate listed below. Results of the plant richness factor from RCB ANOVA are for comparison. Significant values are in bold; $\alpha = 0.05$. df = 4, 75.

	OTU Richness		**OTU H′**	
	F	**P**	**F**	**P**
Plant Richness Factor: RCB ANOVA	3.30	**0.015**	2.84	**0.030**
Root Biomass	3.03	**0.023**	2.19	0.079
Aboveground Biomass	2.58	**0.044**	1.45	0.225
Microbial Biomass	3.38	**0.014**	2.83	**0.030**
Inorganic N	3.27	**0.016**	2.48	0.051
Soil Moisture	3.12	**0.020**	2.69	**0.037**

Table 3. *P*-values for pair-wise comparisons between rhizosphere bacterial communities performed with PERMANOVA; RCB ANOVA. Significant values are in bold; $\alpha = 0.05$.

	NP	SB	*Ag*	*An*	*He*	*AgAn*	*AgHe*	*AnHe*	*AgAnHe*
NP									
SB	**0.002**								
Ag	**0.001**	**0.005**							
An	**0.019**	0.163	0.153						
He	**0.004**	**0.003**	**0.004**	**0.003**					
AgAn	**0.009**	0.080	0.209	0.080	**0.002**				
AgHe	**0.003**	**0.008**	0.241	**0.011**	**0.025**	0.080			
AnHe	**0.001**	**0.013**	0.107	**0.012**	**0.018**	**0.020**	0.349		
AgAnHe	**0.001**	**0.008**	0.142	0.202	**0.037**	0.285	0.683	0.481	

Table 4. Effects of plant community compositions upon microbial biomass, inorganic N and soil moisture. Results of the LSD post-hoc tests are superscripted next to the means for each variable and treatment. Differing letters signify significant differences at $\alpha = 0.05$ level. Significant *p*-values are in bold; $\alpha = 0.05$. NA: Microbial biomass was analyzed using 1-Way ANOVA; block factor was not included.

	Microbial Biomass (cells/g soil)		**Inorganic N (mg/L)**		**Soil Moisture (%)**	
NP	$3.44 \times 10^{6\,d}$		$18.49^{\,a}$		$37.29^{\,ab}$	
SB	$9.96 \times 10^{6\,b}$		$1.96^{\,b}$		$36.35^{\,bc}$	
Ag	$7.82 \times 10^{6\,c}$		$1.37^{\,b}$		$38.32^{\,a}$	
An	$8.65 \times 10^{6\,c}$		$1.30^{\,b}$		$38.5^{\,a}$	
He	$13.33 \times 10^{6\,a}$		$2.24^{\,b}$		$35.82^{\,bc}$	
AgAn	$7.89 \times 10^{6\,c}$		$0.90^{\,b}$		$37.45^{\,ab}$	
AgHe	$10.17 \times 10^{6\,b}$		$1.29^{\,b}$		$36.25^{\,bc}$	
AnHe	$12.30 \times 10^{6\,a}$		$2.93^{\,b}$		$35.44^{\,c}$	
AgAnHe	$10.05 \times 10^{6\,b}$		$1.39^{\,b}$		$34.80^{\,c}$	
	F_{df}	**P**	**F_{df}**	**P**	**F_{df}**	**P**
Block	NA	NA	$0.93_{9,72}$	0.502	$2.33_{9,72}$	**0.023**
Treatment	$76.48_{8,9}$	**0.001**	$10.20_{8,72}$	**0.001**	$3.96_{8,72}$	**0.001**

Table 5. Results of the treatment factor from ANCOVA analyses. Separate analyses were done for each covariate listed. Results of the treatment factor RCB ANOVA are for comparison. Pseudo-F: F-value generated from PERMANOVA. Perm P: *p*-values generated from 9999 permutations of the data in PERMANOVA. Significant values are in bold; $\alpha = 0.05$. df = 8, 71.

	OTU Richness		**OTU H′**		**OTU Community Compositions**	
	F	**P**	**F**	**P**	**Pseudo-F**	**Perm P**
Treatment Factor: RCB ANOVA	2.66	**0.013**	3.71	**0.001**	3.16	**0.001**
Root Biomass	2.49	**0.019**	3.30	**0.003**	2.68	**0.001**
Aboveground Biomass	2.11	**0.045**	2.09	**0.048**	2.60	**0.001**
Microbial Biomass	3.03	**0.008**	3.38	**0.004**	2.12	**0.001**
Inorganic N	2.64	**0.014**	3.47	**0.002**	2.58	**0.003**
Soil Moisture	2.45	**0.021**	3.28	**0.003**	3.02	**0.003**

Table 6. Results of the treatment factor from ANCOVA analyses. Target species root biomass was used as a covariate. Controls were excluded from the analyses. Results of the treatment factor from RCB ANOVA are for comparison. Pseudo-F: F-value generated from PERMANOVA. Perm P: *p*-values generated from 9999 permutations of the data in PERMANOVA. Significant values are in bold; $\alpha = 0.05$. df = 6, 53.

	OTU Richness		**OTU H′**		**OTU Community Compositions**	
	F	**P**	**F**	**P**	**Pseudo-F**	**Perm P**
Treatment Factor: RCB ANOVA Controls Excluded	1.97	0.087	2.84	**0.018**	2.29	**0.001**
Agrostis	1.96	0.087	2.08	0.071	1.94	**0.001**
Andropogon	1.93	0.093	2.68	**0.024**	2.16	**0.001**
Helianthus	2.04	0.077	2.25	0.052	1.89	**0.001**

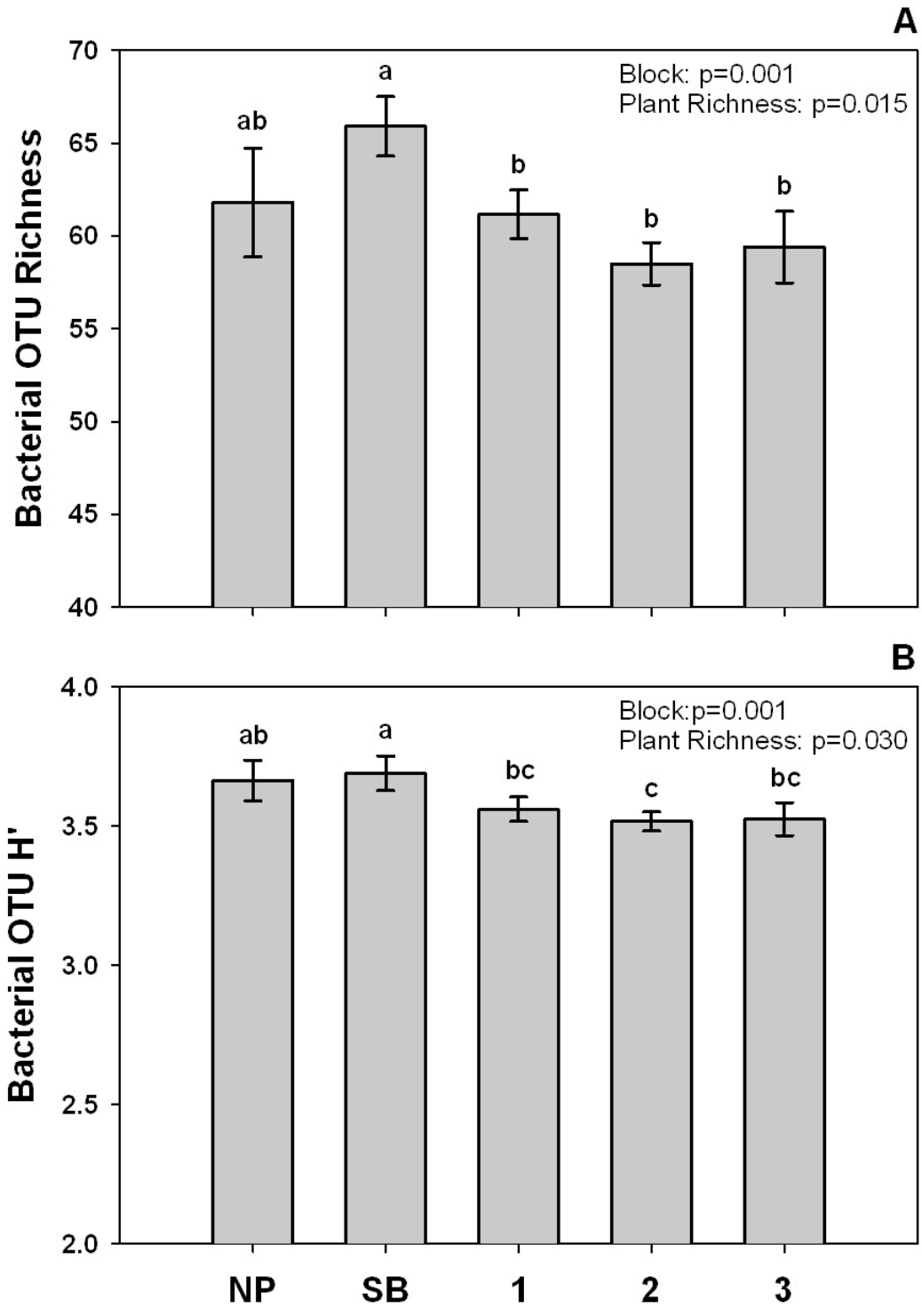

Figure 1. Effects of plant richness upon rhizosphere bacterial alpha diversity; A) OTU richness; B) OTU Shannon diversity. Differing letters signify significant differences at $\alpha = 0.05$ level using least significance difference tests. Error bars represent SE.

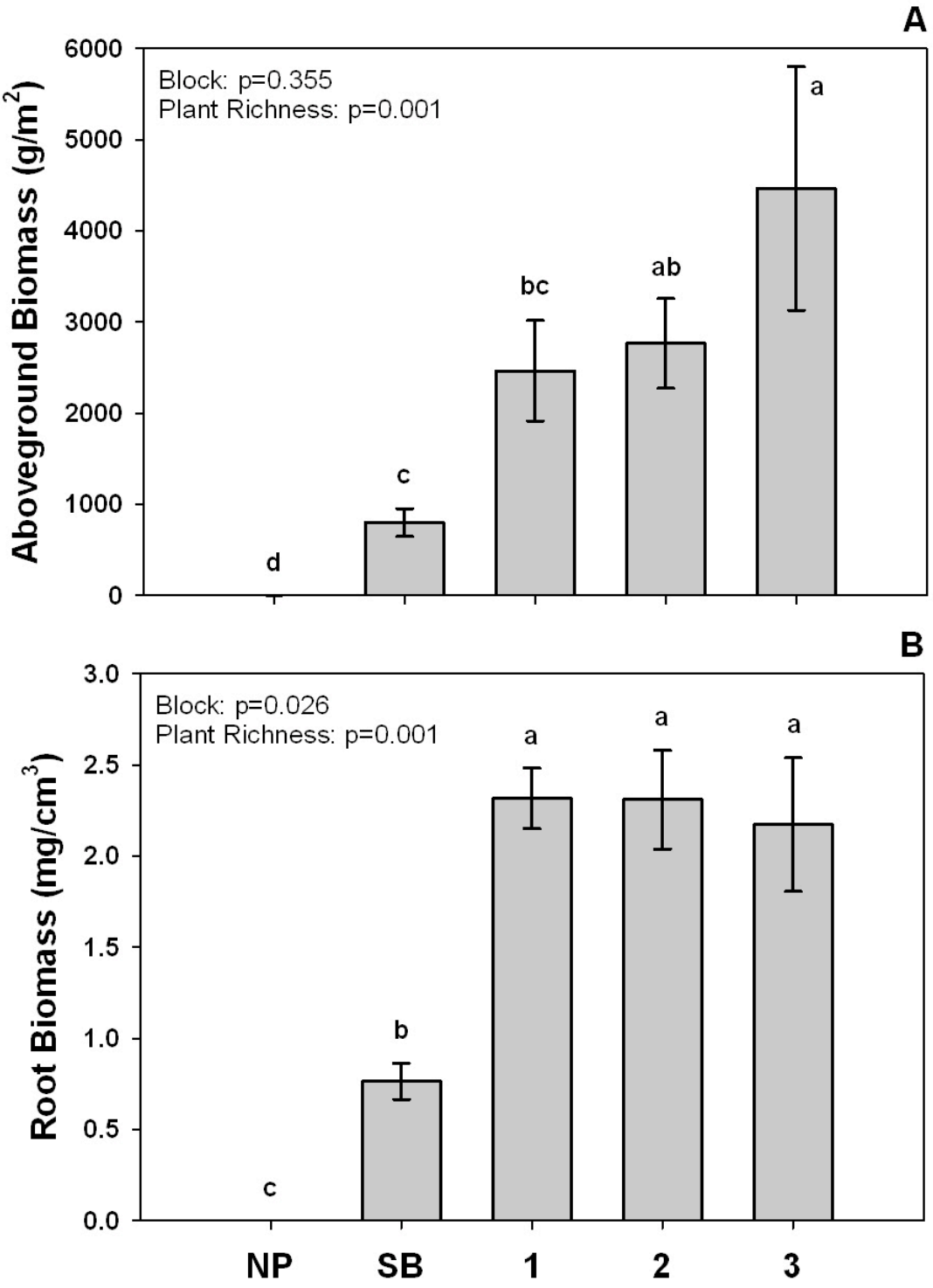

Figure 2. Effects of plant richness upon A) aboveground biomass and B) root biomass. Differing letters signify differences at $\alpha = 0.05$ level using least significance difference tests. Error bars represent SE.

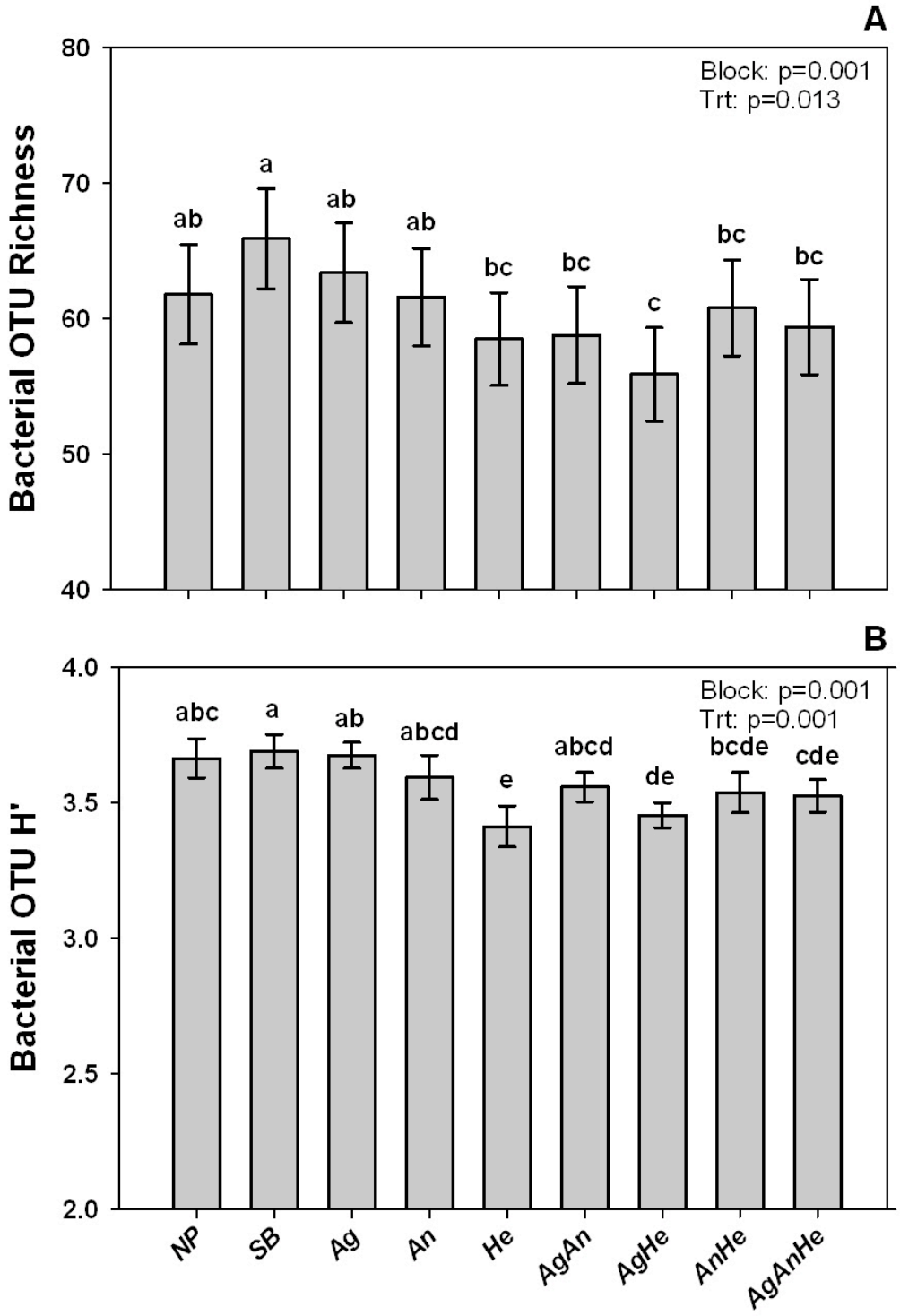

Figure 3. Rhizosphere bacterial alpha diversity by treatment; A) OTU richness; B) OTU Shannon diversity. Differing letters signify significant differences at $\alpha = 0.05$ level using least significance difference tests. Error bars represent SE.

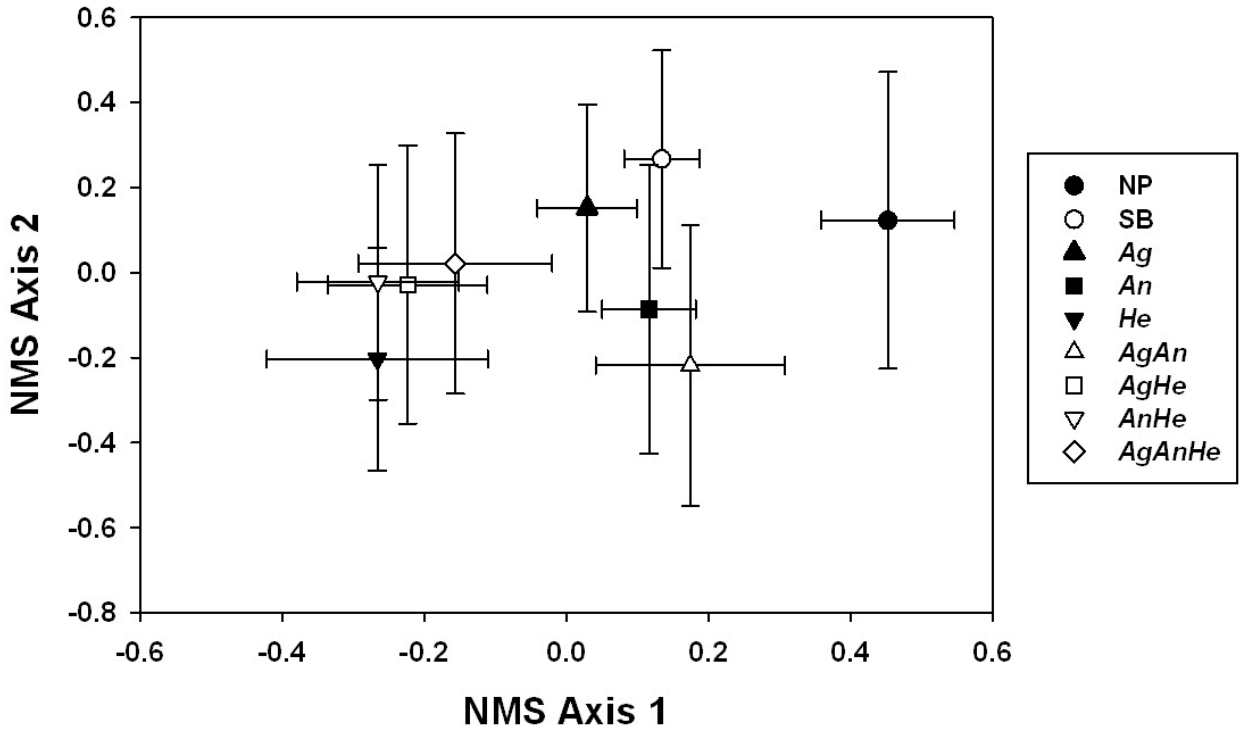

Figure 4. NMS plots of rhizosphere bacterial communities by treatment. Symbols represent the average NMS score. Error bars represent SE of the NMS scores. Block factor Pseudo-$F_{9,72}$ = 14.33 p = 0.001; Trt factor Pseudo-$F_{8,72}$ = 3.16 p = 0.001.

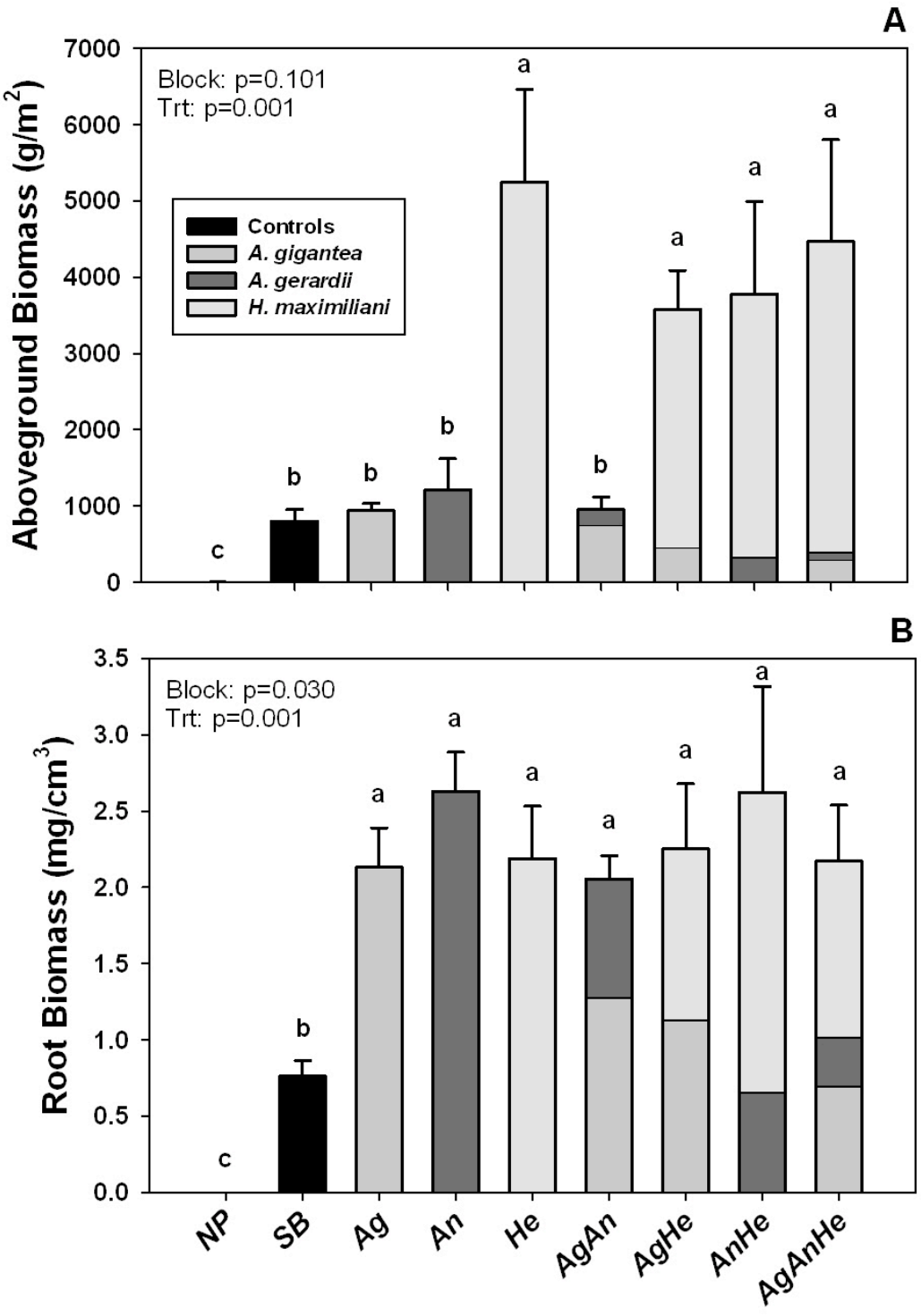

Figure 5. Effects of treatment upon A) aboveground biomass and B) root biomass, distinguished by target species. Differing letters signify differences at $\alpha = 0.05$ level using least significance difference tests. Analyses and error bars (SE) are based off of total biomass values.

CHAPTER 3: **Temporal dynamics of soil bacterial communities in the rhizospheres of three perennial grassland species**

Abstract:

Plant-specific root exudates can provide an environment within the rhizosphere that harbors unique microbial communities: the plant identity effect. However, detection of distinct rhizosphere communities with different plant species has been variable. Potentially, this is because the plant identity effect is not permanent as root exudation patterns can vary with plant phenology. Additionally, if rhizosphere communities change in response to root exudation patterns associated with plant phenology, rhizosphere microbial communities could exhibit seasonal patterns. In a Kansas field experiment, over two growing seasons, I sampled rhizosphere bacterial communities during the active growth and flowering stages of *Agrostis gigantea*, *Andropogon gerardii* and *Helianthus maximiliani* to 1) determine the extent of the plant identity effect among three perennial grassland species and if the effect was maintained over time and 2) assess if rhizosphere bacterial communities showed seasonal patterns, potentially corresponding with plant phenology. I found that *Helianthus* rhizosphere bacterial communities were distinct from those of *Agrostis* and *Andropogon* (which were similar) only when *Helianthus* was flowering and this plant identity effect grew stronger during the second year. Further, seasonal shifts in *Helianthus* rhizosphere bacteria indicated that community changes could potentially be associated with plant phenology. In contrast, *Agrostis* and *Andropogon* rhizosphere bacterial communities were similar over time and both showed gradual non-seasonal changes in compositions over the two growing seasons. Overall, the observance of a plant identity effect depended upon the plant species and when the rhizosphere bacterial community was sampled.

Moreover, temporal patterns of rhizosphere bacteria from perennial plant species likely reflect the complex interactions of factors such as plant phenology, plant functional group and soil properties.

INTRODUCTION

The rhizosphere is generally defined as the soil fraction influenced by the root system (Berg and Smalla 2009). Although the actual physical extent of the rhizosphere can vary both spatially and temporally, at any given time, the rhizosphere is presumed to extend 2-10mm from the root surface (Gregory et al. 2006; Sauer et al. 2006). Within this region, abiotic and biotic factors such as root exudation, water uptake, soil pH and competition for nutrients create a unique environment for soil microbes compared to bulk soil (Curl and Truelove 1986; Hinsinger et al. 2005). The rhizosphere habitat, therefore, commonly displays increased microbial biomass and activity, decreased diversity and microbial compositions that are distinct from those of the bulk soil (Steer and Harris 2000; Baudoin et al. 2002; Kowalchuk et al. 2002; Bardgett et al. 2005). This phenomenon is commonly referred to as the 'rhizosphere effect' (Gregory 2006; Berg and Smalla 2009; Raaijmakers et al. 2009).

In addition to the effect of plant presence, individual plant species can also influence microbial diversities and compositions within the rhizosphere. The amounts and types of root exudates, most abundantly in the forms of organic acids, amino acids and sugars, can vary among plant species, selecting for or against particular soil microbes within the region of the root (Berg and Smalla 2009; Raaijmakers et al. 2009). This can potentially lead to rhizosphere microbial communities that are unique for each plant species (Kowalchuk et al. 2002; Berg and Smalla

2009; Bardgett and Wardle 2010). Over the long-term, this 'plant identity effect' could have important implications for plant growth, vegetation succession and nutrient cycling through the beneficial (e.g. nitrogen fixation, produce plant growth hormones) and adverse (e.g. pathogens) effects the microorganisms may have upon their associated plant species (Westover et al. 1997; Nunan et al. 2005; Bever et al. 2010; Milcu et al. 2010). Even with this potential feedback upon native plant communities, most of our knowledge about plant identity effects on rhizosphere microbial communities comes from studies of economically important annual plant species or from bioremediation studies (Hawkes et al. 2007). There is some evidence that native vegetation can have very high plant specificity, but our knowledge of the impacts that native perennial plant species have upon their associated rhizosphere communities is limited (Berg and Smalla 2009).

Despite a number of studies showing plant-specific rhizosphere microbial communities, unique associations are not consistently found within the literature (Nunan et al. 2005; Kielak et al. 2008). For instance, denaturing gradient gel electrophoresis showed that rhizosphere bacterial communities of strawberry were different from those of both potato and oilseed rape, which were similar to each other (Smalla et al. 2001). Differing degrees of the plant identity effect could arise because soil type and edaphic soil properties, not plant species, are most important in determining rhizosphere microbial communities (Innes et al. 2004; de Ridder-Duine et al. 2005; Singh et al. 2007). Further, the extent of the plant-dependent rhizosphere community could be a result of the methods used to sample the rhizosphere or the management practices employed (e.g. tillage, crop rotation) (Lupwayi et al. 1998; Smalla et al. 2001; Nunan et al. 2005; Kielak et al. 2008).

Detecting a plant identity effect within the rhizosphere could also depend upon sampling time (Berg and Smalla 2009). Patterns of root exudation can vary due to plant phenology (Steer and Harris 2000; Singh et al. 2007). In general, when compared to the flowering stage, higher

concentrations of root exudates have been found during the initial growth phase of younger plants (Hamlen et al. 1972). Most likely this is because plants allocate less C belowground when flowering (Rovira 1959; Marschner et al. 2002). If rhizosphere microbial communities change in response to differences in root exudation patterns driven by plant phenology, as some evidence indicates, it is possible that the extent of the plant identity effect could also differ temporally (Smalla et al. 2001; Baudoin et al. 2002). However, very little is known about the permanence of the plant identity effect over time.

Furthermore, if rhizosphere microbial community structure responds to altered root exudation associated with plant phenology, the rhizosphere communities could exhibit seasonal patterns (Schmidt et al. 2007). For instance, when examining the rhizosphere of *Spartina alterniflora*, Gamble et al. (2010) found that Gammaproteobacteria dominated rhizosphere communities and observed an increase in diazotrophic bacterial activity during periods of plant growth compared to flowering. This was most likely due to increased root exudation and OM availability during plant growth (Gregory 2006). However, this study, and most examining temporal dynamics of rhizosphere communities have sampled throughout only one year (Chiarini et al. 1998; Kowalchuk et al. 2002). Determining if any observed changes in rhizosphere microbial communities are seasonal in nature is, therefore, confounded by the possibility that the rhizosphere biota are responding to climatic seasonal differences in temperature and precipitation instead of plant phenology. In addition, by sampling over a single year, one cannot dismiss the probability that the study was carried out during an unusually hot, cold, wet or dry growing season or that stochastic chance events are the reason for any observed patterns (Hubbell 2001; Bell et al. 2009).

In contrast to these studies sampling over one year, a study by Smalla et al. (2001) provides an instance when rhizosphere bacterial communities were sampled multiple times over two growing seasons. Using strawberry, oilseed rape and potato, rhizosphere bacterial communities displayed seasonal patterns for each plant species in which the flowering stage had the strongest enrichment of some bacterial populations. Sampling rhizosphere microbial communities several times over multiple years could provide insight towards whether changes in rhizosphere communities are seasonal in nature, thereby, furthering our understanding of how rhizosphere communities may change due to plant phenology.

The following field experiment is unique in that after one year of establishment, rhizosphere bacterial communities were sampled twice in each of the following two growing seasons, corresponding with the active growth and flowering stages of three contrasting perennial grassland plant species. The main goal of this study was to explore the temporal changes in rhizosphere bacterial diversities and compositions of these three plant species. Encompassed within this goal were two objectives. First, I investigated the potential differences in the diversities and compositions of rhizosphere bacterial communities among the plant species over the two growing seasons. In this way, I tested for a plant identity effect among the plant species and whether the plant identity effect was maintained or changed over time. Second, I assessed if rhizosphere bacterial diversities and compositions exhibited seasonal patterns corresponding to plant phenology. If a plant species' rhizosphere community exhibits primarily seasonal patterns, I would expect a consistent change in rhizosphere bacterial communities for each year sampled.

METHODS

Study Site

The study site is located within a former pasture/hay field at the University of Kansas Field Station (KUFS) (lat 39°03′ N, long 95°12′ W). Located along the eastern deciduous forest-tallgrass prairie ecotone of Northeastern Kansas, the area consists of undulating, ridge-to-swale topography. The study site is situated on an upland ridgetop, gently sloping downwards from north to south. Soils are Pawnee clay loams (montmorillonitic, mesic Aquic Argiudolls) formed under glacial deposits of till and loess with weathering of interbedded limestones and shales (U.S. Department of Agriculture Soil Conservation Service 1977). The mean annual temperature is 12.9°C and mean annual precipitation is 930 mm (Atmospheric Science Library 1990).

The experiment was located within a 25m x 55m enclosure (3m high fence) and used to exclude deer and other large vertebrate herbivores. Between 1997 and 2000, the enclosed area was used to study changes in the frequency of crop-specific genetic markers of wild sunflower (*Helianthus annuus*) (Cummings et al. 2002). After the wild sunflower experiment, the field site had been periodically mown. Before setting up the current experiment, a survey showed that the plant community was dominated by *Bromus inermis* (cool-season introduced grass). Also prevalent were saplings of *Ulmus rubra*, perennial native warm-season grasses (*Andropogon virginicus* and *Sporobolus* sp.) and forbs (*Solidago canadensis*) and the shrub, *Rubus occidentalis*.

Experiment Design and Establishment

In May 2006, soil within the enclosure was plowed, tilled and roto-tilled to diminish potential impacts of prior vegetation upon the soil bacterial community. The experiment was set

up as a randomized complete block design with ten blocks of nine treatments. Plots were 1.5m x 1.5m with alleyways between each. Two treatments were considered controls. One control did not contain any plants, was continually weeded, and used to measure bulk soil and the effect of no plant inputs (NP). The other control treatment consisted of allowing plants to germinate from the seed bank and grow unchecked (SB). This treatment allowed me to measure rhizosphere soil from plant species growing naturally within the area and served as a comparison for the three target plant species. Overall, weedy annual forbs (*Lactuca serriola*, *Ambrosia artemisiifolia*, *Ambrosia trifada*), annual grasses (*Setaria faberi*), the perennial forb, *Solidago canadensis,* and the shrub *Rubus occidentalis* dominated the SB control plots. Plant richness within these plots had an average of 23.5 species over the two growing seasons. The other seven treatments were monocultures and mixtures (2-species; 3-species) of the three target plant species. For the purpose of this study, only the monoculture treatments and the NP and SB controls were analyzed.

The target species were *Agrostis gigantea* (C_3 grass), *Andropogon gerardii* (C_4 grass) and *Helianthus maximiliani* (forb). The original intent of this experiment was to use native tallgrass prairie species as the target plant species. Initially, *Koeleria macrantha* was chosen as the cool-season grass. However, after treatment application (seed sowing, described below), I observed that *K. macrantha* seed were contaminated with seeds of *A. gigantea*, a non-native perennial C_3 grass. All monocultures targeting *K. macrantha* were ultimately monocultures of *A. gigantea* during the first growing season (2006) due to competitive exclusion. Therefore, *A. gigantea* was used as a target species.

Seeds of the target species were sown on 6 June 2006. For each plot, the amount sown was based upon percent germination of each species and a goal of 1000 seedlings per plot. Seeds

were broadcast and the soil was raked to scrape the surface and cover the seeds. Alleyways and plots were weeded throughout the duration of the experiment.

Rhizosphere Bacterial Community Structure

To assess rhizosphere bacterial communities, soils from each target species were sampled in June and August of the 2007 and 2008 growing seasons (four sampling periods total). These sampling times coincided with the active growth and peak flowering stages of the target species: *A. gigantea* flowers in June with active growth periods in August, *A. gerardii* and *H. maximiliani* have active growth stages in June and flower in August. For each sampling period, three locations were randomly chosen and the soil beneath the plant closest to that location was sampled (2.5cm x 15cm). Soils were frozen at -80°C until further processing could occur. To collect rhizosphere soil, the soil cores were carefully broken apart to expose the roots. Any soil that was still attached to the root was considered rhizosphere soil, brushed into a collection tray and stored at -80°C until DNA analyses.

T-RFLP Procedure: Terminal-Restriction Fragment Length Polymorphism (T-RFLP) was used to assess rhizosphere bacterial communities. Even though T-RFLP does not allow for estimating diversity and structure at fine taxonomic levels, this technique is a reproducible method having sufficient resolution to detect differences in soil bacterial community structure (Fierer et al. 2003). Rhizosphere soil DNA was extracted in duplicate for each plot using the MoBio PowerSoil DNA extraction kit as recommended by the manufacturer. The duplicate extractions were pooled and DNA was quantified by gel electrophoresis. Extractions were either concentrated or diluted to obtain a final concentration of 10ng DNA/uL extract.

16S rDNA from the extracted DNA samples was PCR-amplified using the universal eubacterial primers 6-FAM 8-27F, a fluorescently labeled forward primer (5′-AGRGTTTGATCMTGGCTCAG-3′), and 1389R, a non-labeled reverse primer (5′-ACGGGCGGTGTGTACAAG-3′) (Fierer et al. 2003). For each plot, PCR reactions were performed in triplicate. Each 50μL PCR reaction contained 50ng DNA, 0.5uM of each primer, and 1 x HotStar Taq Master Mix Kit (Qiagen), which included a final concentration of 2.5 units HotStarTaq DNA Polymerase, 1.5nM $MgCl_2$ and 200uM of each dNTP. Reaction mixtures were held for 15 minutes at 95°C for activation. Reactions were cycled 32 times through three steps: 45s of denaturation at 94°C, 45s of annealing at 58°C and 90s for primer extension at 72°C. The final extension lasted 10 minutes at 72°C. The triplicate PCR reactions were pooled, purified using the MoBio UltraClean PCR Clean-Up Kit, and yields quantified using gel electrophoresis.

Products of PCR reactions were initially digested using both *Rsa*I and *Msp*I restriction enzymes (New England Biolabs). However, only the *Rsa*I enzyme completely digested PCR product. Therefore, analyses of rhizosphere bacterial communities only included digestions using *Rsa*I. Each 20μL reaction contained 100ng of purified PCR product, 10 units of restriction enzyme and two μL of buffer. Each reaction was digested for three hours at 37°C with an inactivation period of 20 minutes at 65°C. The fluorescently labeled fragments were analyzed using an Applied Biosystems Instrument 3730 genetic analyzer. Terminal restriction fragment (TRFs) sizing between 25 and 1000 bp, with peak heights > 25 fluorescent units were measured using Peak Scanner 1.0 analytical software (Applied Biosystems).

T-RFLP Profile Alignment: All rhizosphere bacterial community profiles, from the four sampling periods, were analyzed simultaneously using the program T-REX (Culman et al. 2009). T-REX is

a free, online-based tool that was developed to aid in the alignment and analysis of T-RFLP datasets. Within T-REX, TRFs were first filtered to find 'true peaks' within the profiles, using peak area. TRFs were aligned such that fragment sizes differing by ≤ 0.5 bp were considered identical and clustered. To avoid clustering 'distinct close peaks', the maximum number of fragments assigned to a cluster was limited to the number of profiles being aligned. Once the maximum number was reached, a new cluster was created. For each cluster of TRFs, the average size was calculated and used to distinguish TRFs. The data matrix was exported and the peak areas for each community profile were relativized for each TRF within that community profile. Peak areas with a proportion <0.5% were removed (Fierer et al. 2003).

In order to compare rhizosphere bacterial community structures, both the proportional peak area (abundance) and TRF size were used. Because different organisms can produce TRFs of similar size, TRFs of different lengths were assumed to represent different operational taxonomic units (OTUs) and not necessarily distinct bacterial species.

Soil Moisture

Because there is some evidence that soil characteristics, rather than plant species, can be the primary influence acting upon rhizosphere bacterial community diversity and composition, soil properties were measured two times during the course of the experiment. First, prior to incorporating treatments (June 2006), baseline soil measurements were taken that included total soil C, total soil N, available P, soil pH, mineralizable C and N and soil moisture. Second, during the 2008 growing season, available N and soil moisture were measured concurrently with the sampling of rhizosphere bacterial soils. Because all baseline measurements in 2006 and available N values in 2008 were similar among all treatments containing plants (i.e. plots in which

rhizosphere soils were sampled), they are not reported here. However, in 2008, soil moisture values exhibited differences among these treatments and are reported. The day before soil sampling in both June and August 2008, percent soil moisture was measured from the center of each plot using a Trime-FM TDR instrument.

Data Analyses

Soil moisture and bacterial alpha diversity (richness and Shannon diversity, H′) (calculated in PC-Ord 4.14) (McCune and Mefford 1999) were analyzed using repeated measures ANOVA in SPSS 19. Tukey HSD was used as post-hoc tests. Rhizosphere bacterial community compositions were first analyzed using Non-Metric Multidimensional Scaling (NMS) within PC-Ord 4.14 using a Bray-Curtis similarity matrix. To graphically display the rhizosphere bacterial communities, averages and standard errors were calculated by treatment and sampling date from the NMS scores. Repeated measures ANOVA was used to analyze rhizosphere bacterial community compositions using the program Permutational Multivariate Analysis of Variance (PERMANOVA) within PERMANOVA+ for Primer, Primer v6 software (Anderson 2001; Clarke and Gorley 2006). PERMANOVA tests for differences among community matrices using ANOVA designs and calculating *p*-values through permutations of observations. The software will also perform a posteriori pair-wise comparisons among factor levels. All PERMANOVA analyses used relativised abundance matrices, 9999 permutations and the Bray-Curtis similarity distance measure.

A significant treatment x sampling date interaction was found for both bacterial alpha diversity and community compositions (Table 1). Therefore, pair-wise comparisons (alpha diversity = Tukey HSD; bacterial compositions = PERMANOVA) among treatments for each

sampling date were calculated to assess the extent of the plant identity effect over time. Pair-wise comparisons were also calculated among sampling dates for each treatment separately to evaluate if rhizosphere bacterial community diversities and compositions exhibited seasonal patterns. Temporal shifts were considered seasonal if the changes in rhizosphere bacterial communities over the growing season were the same for both years.

RESULTS

Soil Moisture

Significant treatment, season and treatment x season effects were found when analyzing percent soil moisture from the 2008 growing season (Block: $F_{9,36} = 3.75$ $p = 0.002$; Treatment: $F_{4,36} = 8.81$ $p = 0.001$; Season: $F_{1,36} = 80.91$ $p = 0.001$; Treatment x Season interaction: $F_{4,36} = 8.06$ $p = 0.001$) (Figure 1). Overall, soil moisture was significantly higher in June 2008 than in August 2008. In June 2008, soil moisture was not significantly different among treatments with the exception of *Andropogon*. *Andropogon* was significantly higher than both the SB control and *Helianthus* treatment. In August 2008, soil moisture in *Agrostis* plots had significantly higher soil moisture than the NP control, the SB control and *Helianthus* treatment. Further, *Agrostis* and *Andropogon* soil moisture values in August 2008 were not significantly different.

Plant Identity Effects

In June 2007, rhizosphere OTU richness was similar among all treatments (Figure 2A). However, in August 2007, *Helianthus* rhizosphere bacteria had significantly lower OTU richness than all other treatments. In June 2008, bacterial OTU richness associated with *Agrostis*, was

significantly lower than the NP control and *Andropogon* treatments. Once again, in August 2008, the rhizosphere of *Helianthus* had significantly lower bacterial OTU richness than all other treatments. Bacterial OTU Shannon diversity exhibited a comparable pattern to that of OTU richness (Figure 2B). In June 2007 and 2008, there were no significant differences among treatments, while in August of both years, *Helianthus* rhizosphere bacteria had significantly lower Shannon diversities compared to all other treatments.

Significant treatment, season and treatment x season effects were found when analyzing rhizosphere bacterial community compositions (Table 1), indicating that the relationship among target plant species changed over time. All three target plant species had similar rhizosphere bacterial compositions on the first sampling date (Table 2a; Figure 3A). In August 2007, *Agrostis* and *Helianthus* rhizosphere bacterial compositions were significantly different, but both were not significantly different from *Andropogon* rhizosphere bacterial compositions (Table 2b; Figure 3B). Thus, bacterial community compositions associated with *Andropogon* were intermediate between those of *Agrostis* and *Helianthus*. The following June (2008), rhizosphere bacterial compositions of *Helianthus* were intermediate between compositions of *Agrostis* and *Andropogon*, which were significantly different (Table 2c; Figure 3C). Finally, in August 2008, the rhizosphere bacterial communities of both grass species had similar compositions and were both significantly different from *Helianthus* bacterial compositions (Table 2d; Figure 3D). In fact, *Helianthus* rhizosphere bacterial community compositions were significantly different from all other treatments in August 2008. The NP control had soil bacterial compositions that were either significantly different, or nearly so (e.g. June 2007), from all other treatments for each season that was sampled. In contrast, the SB control rhizosphere bacterial communities were similar to those of each target plant species for each season, with two exceptions. The SB control had

significantly different rhizosphere bacterial compositions from *Agrostis* and *Helianthus* treatments in June 2008 and August 2008, respectively.

Seasonal Patterns

Examining how alpha diversity changed over time for each treatment separately showed that rhizosphere bacterial OTU richness and Shannon diversities did not change significantly over the two growing seasons for all treatments, except *Helianthus* (Figure 2). Rhizosphere bacterial communities form the forb species exhibited a seasonal pattern: OTU richness and Shannon diversities were similar from June of both years, as were communities from August of both years. However, rhizosphere bacterial OTU alpha diversities significantly decreased in August.

When assessing rhizosphere bacterial community compositions over time for each treatment, I found only the *Helianthus* treatment displayed a seasonal pattern (Table 3e; Figure 4E). This plant species had bacterial compositions that were similar in June of both years. Likewise, in August of both years, rhizosphere compositions were also similar. However, rhizosphere compositions between June and August were significantly different. In contrast, the SB control and *Andropogon* treatments exhibited gradual changes in rhizosphere bacterial community compositions, but these changes were not significant until June 2008 (June 2008 ≠ August 2008) (Table 3b, 3d; Figure 4B, 4D). *Agrostis* rhizosphere bacterial community compositions were similar between June 2007 and August 2007, but changed significantly thereafter with each season (August 2007 ≠ June 2008 ≠ August 2008) (Table 3c; Figure 4C). The bacterial communities of the NP control, representing bulk soil, were not significantly different over the two growing seasons, except for one sampling date. Soil bacterial community compositions from August 2007 were significantly different from all other sampling times.

DISCUSSION

By sampling rhizosphere bacterial communities multiple times over two growing seasons, I was able to examine the persistence of the plant identity effect among three perennial plant species. In addition, I was able to evaluate if these species had rhizosphere bacterial communities exhibiting seasonal patterns potentially corresponding with plant phenology. My results indicated that the observance of a plant identity effect depended upon the plant species examined and when rhizosphere bacterial communities are sampled: *Helianthus* had unique rhizosphere bacterial diversities and compositions in August, but the effect grew stronger during the second year. Further, *Helianthus* rhizosphere bacterial communities displayed seasonal patterns, whereas both *Agrostis* and *Andropogon* had similar rhizosphere bacterial communities and both showed gradual changes in compositions over the two growing seasons.

Seasonal patterns were observed for *Helianthus* rhizosphere bacterial diversities (lower in August) and compositions (June ≠ August) indicating that root exudation patterns, potentially associated with phenology, could be important in determining the rhizosphere communities of this forb species. *Helianthus* rhizosphere bacterial communities also exhibited a plant identity effect. However, this effect was observed only in August of both years (lower diversities and distinct compositions from the other rhizosphere soils), potentially reflecting the seasonal shifts in rhizosphere bacterial community diversities and compositions. Moreover, the plant identity effect grew stronger during the second year as *Helianthus* bacterial communities were different from the SB control, *Agrostis*, and *Andropogon* treatments. These results suggest two possibilities: observing a plant identity effect could depend upon the time of year (or year) soils are sampled and the effect could potentially require time to develop. Smalla et al. (2001) also observed a delay

in plant identity effects when examining bacterial communities in the rhizospheres of strawberry, oilseed rape and potato. One month after planting, all rhizosphere communities were similar. Thereafter, strawberry and oilseed rape rhizosphere communities differed over two growing seasons. Furthermore, during year two, strawberry and potato rhizosphere communities became distinct. These findings were partially attributed to the fact that strawberry is a perennial while oilseed rape and potato are annual species. However, because all target species within this study were perennials, this life history trait is most likely not a reason why differences were observed. The stronger plant identity effect in August 2008 potentially could be attributed to the simultaneous temporal changes in bacterial compositions that were occurring within the SB control, *Agrostis*, and *Andropogon* treatments.

In contrast to what was found with *Helianthus* rhizospheres, both *Agrostis* and *Andropogon* had similar rhizosphere bacterial diversities and compositions throughout all sampling dates, except June 2008, and did not display seasonal patterns over time. Instead, the rhizosphere bacterial community composition of these two grasses progressively changed over time in a similar manner, albeit with a higher level of significance for *Agrostis*. These results, along with the fact that the active growth and flowering stages differed between these two grasses, suggest that any root exudation patterns potentially associated with plant phenology was not influencing the rhizosphere bacterial communities. It is conceivable that *Agrostis* and *Andropogon* rhizosphere bacterial compositions were responding to root exudation patterns that correspond with a plant trait common to both grass species. For instance, as plants age, root exudation quantities can decrease and change in quality (Hamlen et al. 1972). Further as plants age, the rhizosphere microbial communities can change from ones that are dominated by populations with rapid growth and turnover (e.g. r-strategists such as Proteobacteria) to

communities that are dominated by K-strategists such as mutualistic bacteria and fungi, suggesting a succession of the rhizosphere communities (de Leij et al. 1993; Chiarini et al. 1998; Steer and Harris 2000; Smalla et al. 2001; Baudoin et al. 2002; Marschner et al. 2002; Thirup et al. 2003). Assessing root exudation patterns and the specific bacterial populations within the rhizosphere were beyond the scope of this study, so it is unknown if *Agrostis* and *Andropogon* rhizospheres were responding to plant age-root exudation and/or undergoing succession, but remain as potential explanations for the observed patterns.

It is also possible that the rhizosphere bacterial diversities and compositions could respond in accordance to plant functional groups (e.g. grasses vs. forbs) (Singh et al. 2007). Functional group differences could arise through contrasting root exudations: non-legume forbs can invest more long-term C storage into roots, reducing the amount of root exudation, and potentially exerting a stronger selective force upon rhizosphere communities compared to grass species (Kowalchuk et al. 2002; Marschner et al. 2002; Warembourg et al. 2003). Further grasses and forbs have distinct root architectures (fibrous vs. taproot, respectively), which have also been found to potentially impact rhizosphere microbial communities (Gregory 2006). Functional group traits could have given rise to the differences found between *Helianthus* and the two grass species in August, but does not explain the similarities found in June 2007 and the different rhizosphere bacterial communities between *Agrostis* and *Andropogon* in June 2008. Therefore, functional group traits may not fully explain the relationships among the rhizosphere bacterial communities of the three target plant species. In addition, the two grass species exhibited rhizosphere bacterial diversities, compositions and temporal patterns similar to the SB control, suggesting that the rhizospheres of the grass species could be selecting for a more general bacterial community,

reflecting that of an entire plant community developed from the existing seed bank and not necessarily functional group traits.

Soil edaphic properties can potentially determine rhizosphere microbial communities to a greater extent than plant species (Innes et al. 2004; Singh et al. 2007). Within this study, there were no treatment differences observed for soil pH, available N and OM prior to plant establishment and throughout the 2008 growing season (unpublished results). Thus, the observed differences in rhizosphere bacterial diversities and compositions of the three target species were most likely not because of these soil characteristics. Although soil moisture was not measured in 2007, based upon the 2008 data, the significantly lower soil moisture within *Helianthus* plots compared to *Agrostis* and *Andropogon* in August could partially explain the unique rhizosphere bacterial communities of *Helianthus* during this season. However, patterns of rhizosphere bacterial diversities and compositions in June 2008 did not correlate with soil moisture. It is likely that the factors influencing rhizosphere bacterial communities of the three target plant species could be a complex array of many variables including, but not limited to, root exudation patterns, plant functional traits and soil properties.

In addition to the results observed among the various rhizosphere soils, I also found evidence of a rhizosphere effect: bacterial community compositions within the bulk soil (NP control) were significantly different from all rhizosphere soils for most sampling dates. Although the types of bacterial populations inhabiting the bulk and rhizosphere soils are not known in the current study, others have found that soils without plant inputs can favor spore-forming bacteria such as Firmicutes (Singh et al. 2007). In contrast, the presence of roots can prompt an immediate shift in the bacterial community towards Proteobacteria, which typically show fast growth rates

and responses to the easily degradable C that are released by roots (Grayston et al. 1999; Gros et al. 2006; Kielak et al. 2008).

In June 2007, the NP control had bacterial community compositions similar to the SB control and *Andropogon* treatment, suggesting the rhizosphere effect might not yet have emerged for these rhizosphere soils. Despite its common reference throughout the literature, there is some evidence that the rhizosphere effect could require time to develop and strengthen (Smalla et al. 2001; Baudoin et al. 2002). For instance, distinct microbial communities between bulk and rhizosphere soils only became apparent 90 days after planting *Agrostis stolinifera* (Steer and Harris 2000). It has been suggested that the general pool of microbial populations already inhabiting the bulk soil are the primary source of rhizosphere microbial communities (de Ridder-Duine et al. 2005; de Boer et al. 2006; Singh et al. 2007). Therefore, a time lag for the rhizo-competent populations to become dominant within the rhizosphere seems plausible. Because I did not sample rhizosphere soils during the first year of plant establishment, I do not know when the rhizosphere effect first occurred for *Agrostis* and *Helianthus* treatments. However, the results point to the possibility that the rhizosphere effect was still developing into the second year of plant establishment for the SB control and *Andropogon* treatments.

Part of the general concept of the rhizosphere effect is that bulk soil harbors higher bacterial diversity than the rhizosphere due to a greater diversity of microhabitats (Gros et al. 2006). Within this study, this pattern was observed for the *Helianthus* rhizospheres in August of both years. Nevertheless, in general, bacterial diversities were similar between bulk (NP control) and most rhizosphere soils. It is not clear why these results are in contrast to this general trend, but could be due to the T-RFLP technique used. By excluding the rare OTUs within a community profile, T-RFLP can limit the number of OTUs detected (Bent and Forney 2008). Further, T-

RFLP could mask any subtle differences occurring at lower taxonomic resolutions between the bulk and rhizosphere soils (Danovaro et al. 2006). Despite these inherent biases, T-RFLP can be a powerful tool to distinguish amongst fundamentally different bacterial communities (Fierer and Jackson 2006).

Soil bacterial community compositions of the NP control generally did not change significantly over time. This is in contrast to several studies reporting temporal dynamics of biota within the bulk soil (Bardgett et al. 2005). For example, within alpine environments, fungi can dominate the microbial community during winter, corresponding to high levels of biomass and litter decomposition (Schmidt et al. 2007). Then, fueled by rhizodeposition, bacteria can dominate the microbial community in the summer months. It is not fully known why the bulk soil within this experiment did not display temporal changes in bacterial compositions, but could be because only a few months out of each year were sampled and the full range of shifts in bacterial communities were not detected. A lack of temporal change in soil bacterial communities could also be due to an absence of plant litter inputs to support such changes (Bardgett and Wardle 2010).

In conclusion, the observance of a plant identity effect can depend upon both the plant species and when during the growing season rhizosphere bacterial communities are sampled. These qualifications are most likely the result of the disparate temporal dynamics of the rhizosphere bacterial communities that can occur with different plant species. In all likelihood, temporal patterns of rhizosphere communities from different perennial plant species reflect complex interactions of numerous factors such as plant phenology, plant age, functional group, root morphology and soil properties. This study provides support towards the necessity of taking

multiple samples over the course of the year (and over several years) to capture the essence of the entire rhizosphere microbial community (Schmidt et al. 2007). Further understanding of the temporal dynamics of rhizosphere microbial communities is important if we are to gain greater insight into the nature and significance of above- and belowground relationships (Bardgett et al. 2005).

Table 1. Results of Repeated Measures ANOVA for rhizosphere bacterial OTU richness, Shannon diversity and community compositions. Pseudo-F: F-value generated from PERMANOVA. Perm P: *p*-values generated from 9999 permutations of the data. Significance differences at $\alpha = 0.05$ level.

ANOVA Factor$_{df}$	OTU Richness		OTU H′		OTU Community Composition	
	F	P	F	P	Pseudo-F	Perm P
Block$_{9,22}$	2.26	0.058	1.56	0.188	7.00	**0.001**
Treatment$_{4,22}$	5.22	**0.004**	8.26	**0.001**	5.62	**0.001**
Season$_{3,66}$	4.32	**0.008**	4.22	**0.009**	11.11	**0.001**
Treatment x Season$_{12,66}$	1.92	**0.048**	2.93	**0.003**	1.58	**0.001**

Table 2. *P*-values for pair-wise comparisons of the rhizosphere bacterial OTU community compositions among treatments for each season separately. Comparisons were analyzed within PERMANOVA. Significance differences at $\alpha = 0.05$ level.

a. June 2007

	NP	SB	*Agrostis*	*Andropogon*
NP				
SB	0.058			
Agrostis	**0.007**	0.071		
Andropogon	0.083	0.262	0.466	
Helianthus	**0.018**	0.076	0.246	0.217

b. August 2007

	NP	SB	*Agrostis*	*Andropogon*
NP				
SB	**0.033**			
Agrostis	**0.004**	0.526		
Andropogon	**0.005**	0.267	0.406	
Helianthus	**0.019**	0.108	**0.018**	0.081

c. June 2008

	NP	SB	*Agrostis*	*Andropogon*
NP				
SB	**0.002**			
Agrostis	**0.001**	**0.010**		
Andropogon	0.062	0.267	**0.011**	
Helianthus	**0.037**	0.072	0.119	0.190

d. August 2008

	NP	SB	*Agrostis*	*Andropogon*
NP				
SB	**0.003**			
Agrostis	**0.012**	0.301		
Andropogon	**0.030**	0.287	0.366	
Helianthus	**0.001**	**0.001**	**0.003**	**0.003**

Table 3. *P*-values for pair-wise comparisons of the rhizosphere bacterial OTU community compositions among seasons for each treatment separately. Comparisons were analyzed within PERMANOVA. Significance differences at $\alpha = 0.05$ level.

a. NP

	June 2007	August 2007	June 2008
June 2007			
August 2007	**0.031**		
June 2008	0.085	**0.029**	
August 2008	0.078	**0.009**	0.075

b. SB

	June 2007	August 2007	June 2008
June 2007			
August 2007	0.179		
June 2008	**0.044**	0.427	
August 2008	0.068	0.105	**0.025**

c. *Agrostis*

	June 2007	August 2007	June 2008
June 2007			
August 2007	0.082		
June 2008	**0.033**	**0.024**	
August 2008	**0.009**	**0.023**	**0.003**

d. *Andropogon*

	June 2007	August 2007	June 2008
June 2007			
August 2007	0.181		
June 2008	0.294	0.063	
August 2008	0.147	**0.027**	**0.035**

e. *Helianthus*

	June 2007	August 2007	June 2008
June 2007			
August 2007	**0.028**		
June 2008	0.170	**0.030**	
August 2008	**0.002**	0.160	**0.001**

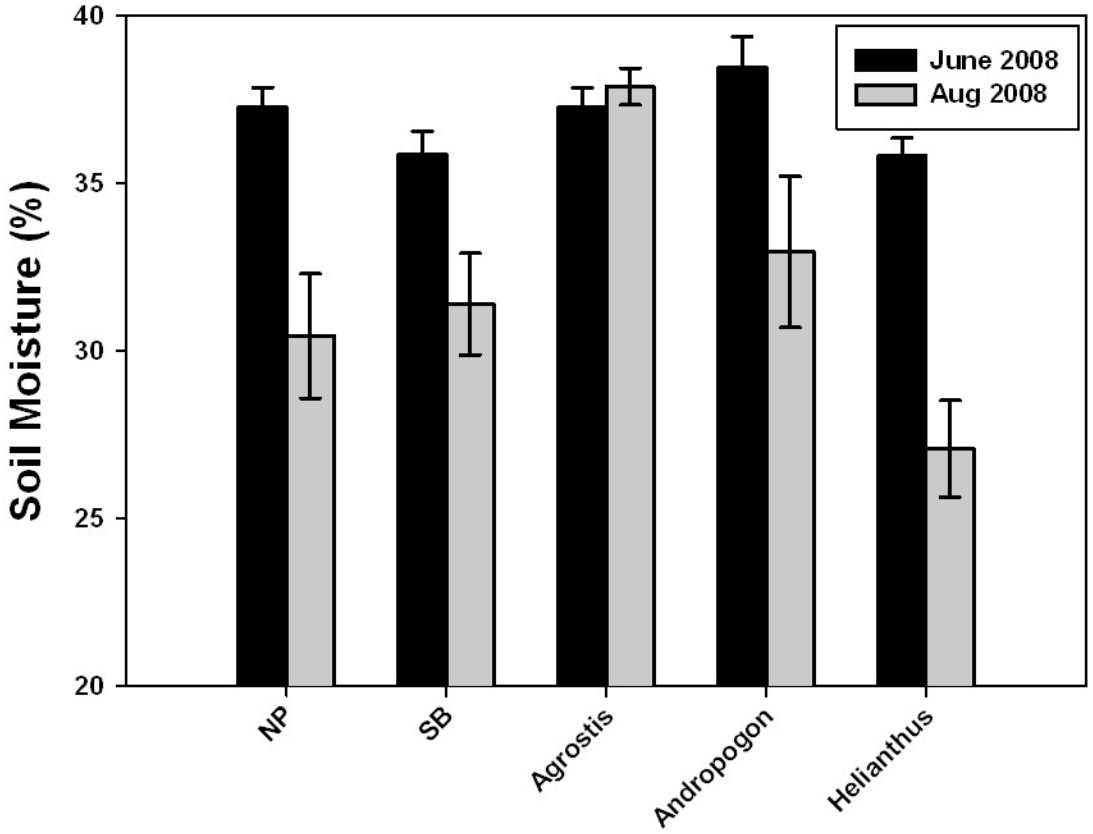

Figure 1. Percent soil moisture from June 2008 and August 2008. Means and SE are displayed for each treatment. Block: $F_{9,36} = 3.75$ $p = 0.002$; Treatment: $F_{4,36} = 8.81$ $p = 0.001$; Season: $F_{1,36} = 80.91$ $p = 0.001$; Treatment x Season interaction: $F_{4,36} = 8.06$ $p = 0.001$

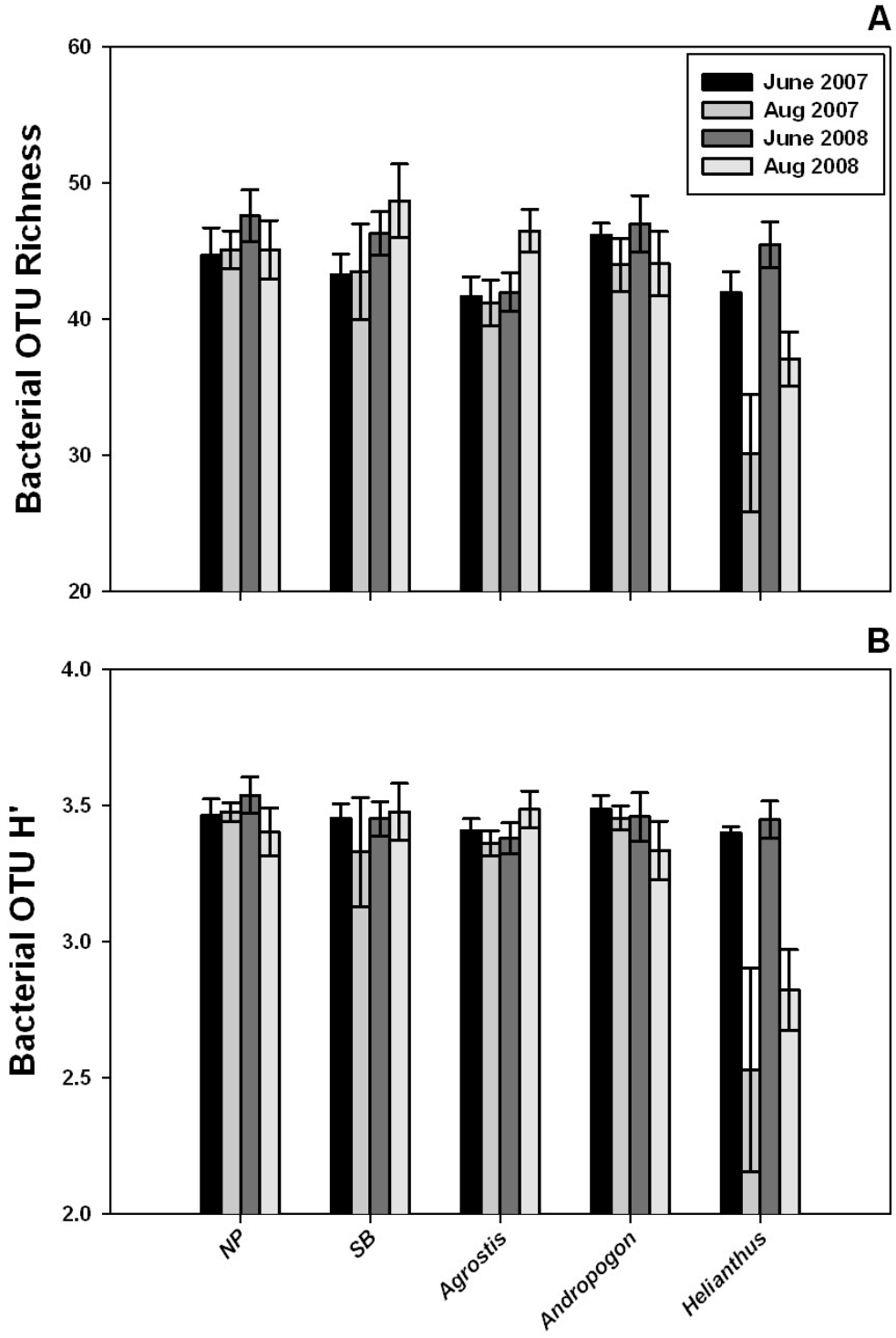

Figure 2. Rhizosphere bacterial OTU A) richness and B) Shannon diversity across all sampling dates. Means and SE are displayed for each treatment. OTU Richness: Block factor $F_{9,22} = 2.26$ $p = 0.058$; Trt factor $F_{4,22} = 5.22$ $p = 0.004$; Season factor $F_{3,66} = 4.32$ $p = 0.008$; Trt x Season factor $F_{12,66} = 1.92$ $p = 0.048$. OTU Shannon Diversity: Block factor $F_{9,22} = 1.56$ $p = 0.188$; Trt factor $F_{4,22} = 8.26$ $p = 0.001$; Season factor $F_{3,66} = 4.22$ $p = 0.009$; Trt x Season factor $F_{12,66} = 2.93$ $p = 0.003$.

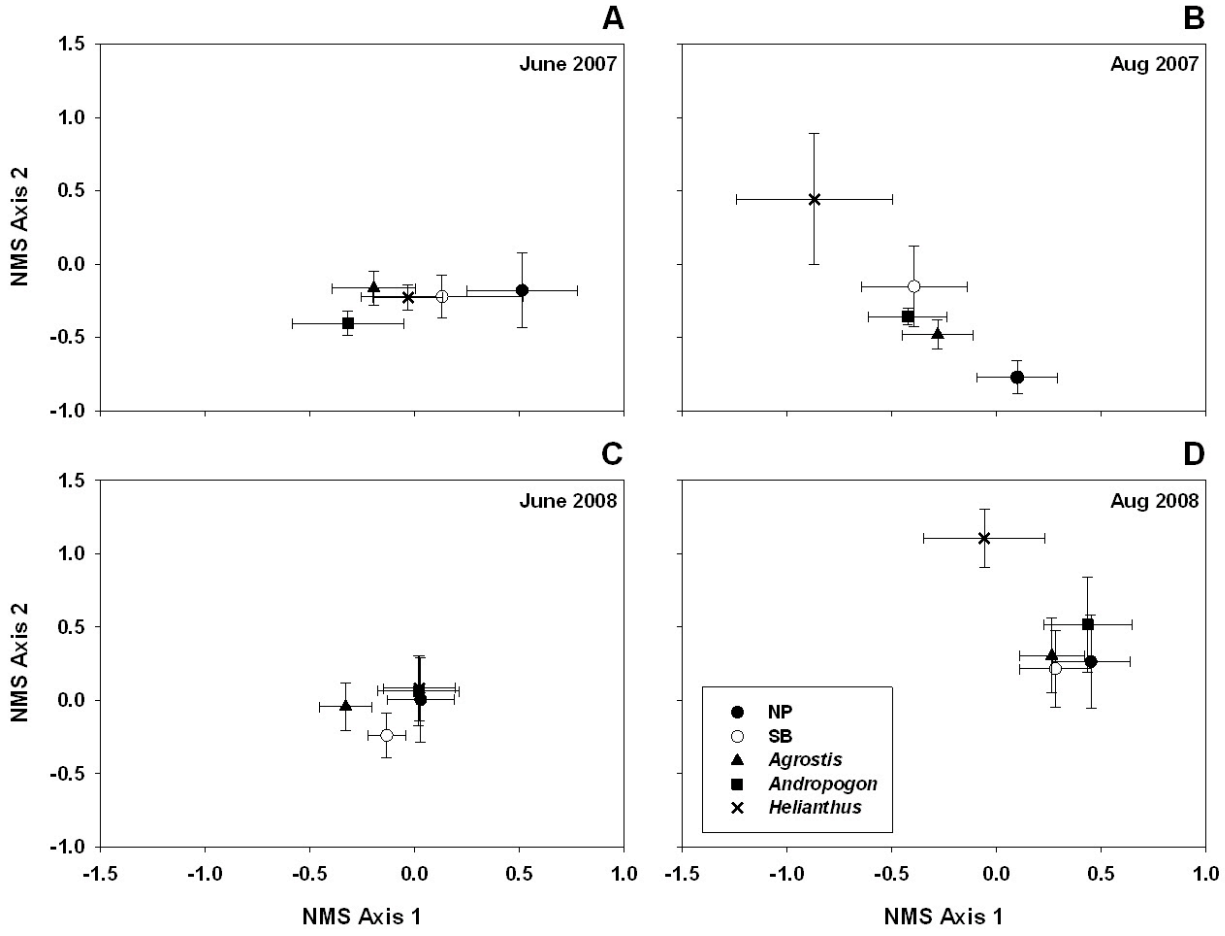

Figure 3. NMS plots of rhizosphere bacterial OTU community compositions comparing treatments for each season separately. A) June 2007, B) August 2007, C) June 2008 and D) August 2008. Symbols represent the average NMS score. Error bars represent SE of the NMS scores. Block factor Pseudo-$F_{9,22} = 7.00$ $p = 0.001$; Trt factor Pseudo-$F_{4,22} = 5.62$ $p = 0.001$; Season factor Pseudo-$F_{3,66} = 11.11$ $p = 0.001$; Trt x Season factor Pseudo-$F_{12,66} = 1.58$ $p = 0.001$.

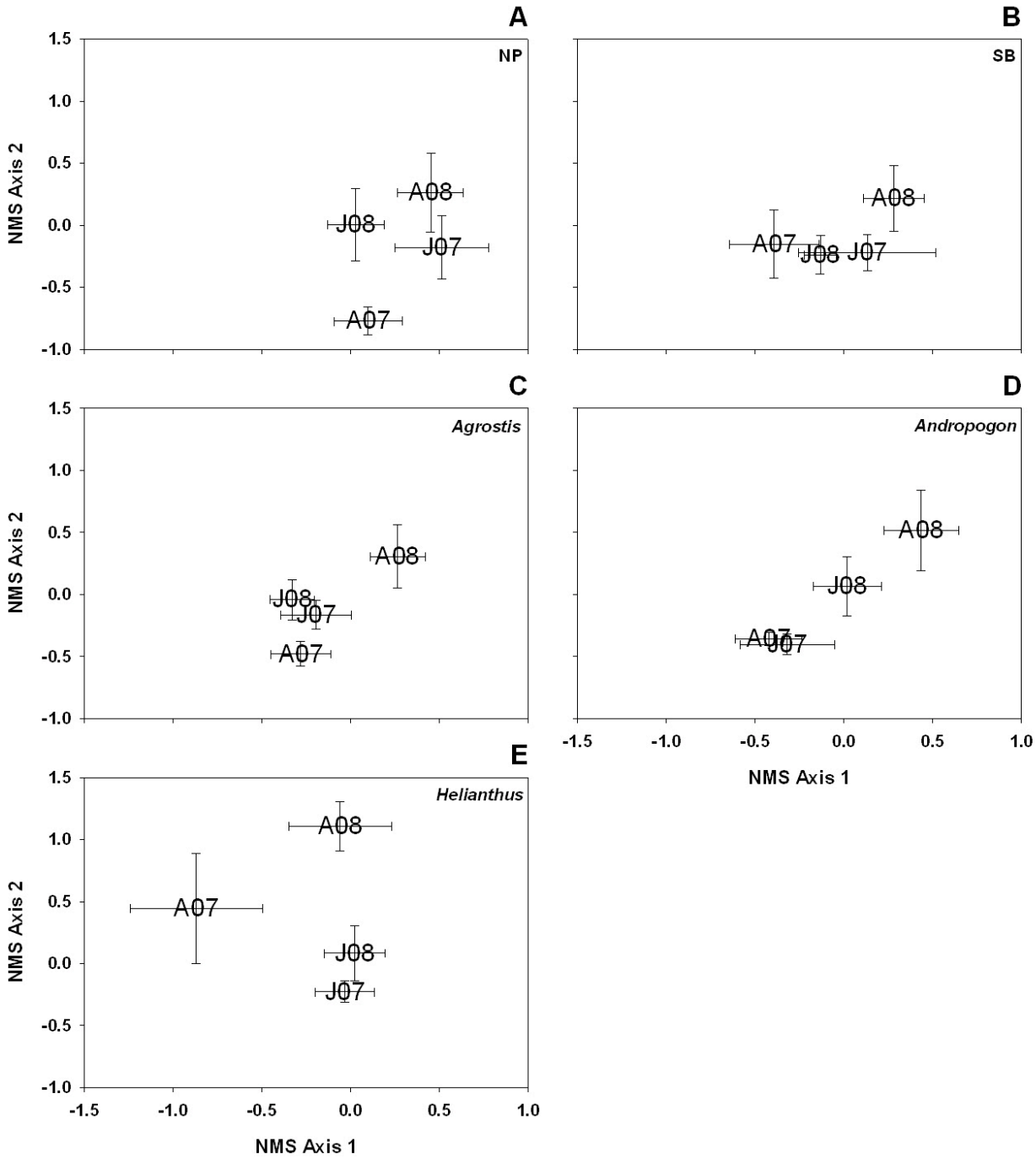

Figure 4. NMS plots of rhizosphere bacterial OTU community compositions comparing seasons for each treatment separately. A) NP, B) SB, C) *Agrostis*, D) *Andropogon* and E) *Helianthus*. Symbols represent the average NMS score: J07 = June 2007; A07 = August 2007; J08 = June 2008; A08 = August 2008. Error bars represent SE of the NMS scores. Block factor Pseudo-$F_{9,22}$ = 7.00 p = 0.001; Trt factor Pseudo-$F_{4,22}$ = 5.62 p = 0.001; Season factor Pseudo-$F_{3,66}$ = 11.11 p = 0.001; Trt x Season factor Pseudo-$F_{12,66}$ = 1.58 p = 0.001.

GENERAL DISCUSSION

In this dissertation, I used ecological theory, initially developed for macroorganisms, and applied it towards soil microbes to better understand the factors that structure these communities. More specifically, I investigated the role that environmental heterogeneity and plant communities play in structuring soil bacterial communities. In Chapter 1, within a restoration experiment, I compared the importance of local environmental heterogeneity created by the plant community to that of the heterogeneity of chemical and physical soil characteristics and to regional processes, all of which could be potentially acting upon the bulk soil bacteria. In Chapter 2, I reduced the spatial scale of inquiry and examined if an increase in rhizosphere bacterial diversity, and differing bacterial compositions, were associated with an increase in plant richness, theoretically through the increased diversity of resources provided by plant communities with higher richness. Further, resources provided by plants can vary over time, thus creating environmental heterogeneity over temporal scales. In Chapter 3, I applied this idea to rhizosphere bacterial communities by assessing how compositions changed over time in association with plant phenology. Overall, my results show that resource heterogeneity promoted by plant communities can be an important, but not an exclusive component, in shaping soil bacterial communities.

The results from my first chapter show that the capacity of plant communities to influence soil bacterial communities can vary depending upon restoration age and land-use history. Three avenues of inquiry (i.e. the restoration experiment, multivariate regression analyses, and comparison with a remnant prairie) led me to conclude that there was no association between the restored plant community and the soil bacterial community of the

restoration experiment. Within the bulk soil, the main influence of the plant community can be through litter inputs (long-term, indirect effects) and time may be required for the build-up of root systems and carbon in the soil. The time since restoration (six years) may have been too short to elicit a response by the bacterial community to plant restoration. Instead, bacterial communities throughout the experimental field-site were primarily shaped by both niche-based local effects of soil environmental properties (e.g. soil pH, texture) and by pure spatial processes (e.g. dispersal limitations, historical legacies). Variation in soil bacterial community compositions could be due to ecological drift manifested by dispersal limitations of bacterial cells, creating patches in community compositions. Further, the legacy effects of cultivation history can be very long-lasting, influencing which bacterial populations are present at the beginning of restoration. In contrast to the restoration experiment, I found that the spatial variation observed in bacterial communities within a remnant prairie was due to the spatial variation of the plant community (primarily) and soil properties (secondarily) and not regional processes. The differences observed between the restoration experiment and remnant prairie were potentially due to the contrasting land-use histories (e.g. cultivation) of these two field sites. My results further suggest that several years or decades are necessary in order to restore soil bacterial communities and the key factors shaping these communities could change depending upon land-use history.

In Chapter 2, I found that rhizosphere bacterial diversity did not respond to an increase in plant species richness when using three perennial plant species. It is possible that the plant communities used did not generate enough environmental heterogeneity within the overall rhizospheres to bring about a response by the bacteria. This is demonstrated in several ways. First, two out of the three plant species used (*Agrostis gigantea*, *Andropogon gerardii*) had

similar rhizosphere bacterial diversities and compositions, suggesting that plant-derived resources were similar between these grass species. Therefore, an increase in resource diversity might not have occurred in the mixture plots that included these two species. Second, the forb, *Helianthus maximiliani*, provided a strong selective force within the rhizosphere, such that bacterial diversities were significantly lower when compared to the other rhizosphere communities and lowered the overall average in the mixture plots. These aspects, together with an indication from the SB control that higher plant species richness (approximately 30 species) could display higher rhizosphere bacterial diversity, demonstrates that more than three species may have been necessary to generate a response by the bacterial community. Despite an absence of a plant richness effect, I found a strong plant presence and plant identity effect. The presence of plants altered soil bacterial compositions when compared to the bulk soil. The plant identity effect was very striking with the forb, *H. maximiliani*. Rhizosphere bacterial communities associated with this species had distinct compositions from all other treatments. Furthermore, when this species was included in a mixture, the bacterial community shifted towards that of *H. maximiliani*. It is possible that the disparities in soil bacterial communities found between the two grass species and the forb species could be due to general differences in root exudation patterns associated with the plant families, but more work is needed to determine if this was the case in this study.

In addition to the environmental heterogeneity that plants can produce spatially, variation in plant root exudation over time can promote temporal environmental heterogeneity within the rhizosphere. In Chapter 3, *H. maximiliani* rhizosphere bacterial communities exhibited seasonal patterns suggesting that resources within the rhizosphere were potentially changing in association with plant phenology. In contrast, the rhizosphere communities of the two grass

species (*A. gigantea*, *A. gerardii*) were similar over time and both showed gradual non-seasonal changes in compositions over the two growing seasons. Therefore, these different plant species could be generating temporal heterogeneity through different routes resulting in a plant identity effect with *H. maximiliani* that was not permanent and took time to strengthen. Overall, the observance of a plant identity effect depended upon the plant species and when the rhizosphere bacterial community was sampled. Moreover, temporal patterns of rhizosphere bacteria from perennial plant species likely reflected the complex interactions of factors such as plant phenology, plant functional group and soil properties.

The results of this dissertation show that environmental heterogeneity provided by the plant community, both spatially and temporally, can be important for structuring soil bacterial communities. It is also clear that other factors (soil environmental properties, regional processes) simultaneously help govern soil biota. Within the bulk soil, the degree of influence the plant community exerts upon bacterial communities can depend upon land-use history and the time allowed for an association between the above- and belowground components to form. A higher degree of association between plants and soil bacteria occurs within the rhizosphere. Even with this greater interaction, the extent of influence upon soil bacteria can vary depending upon plant species, plant phenology, and plant community composition. Overall, the factors governing soil bacterial community structure can be numerous and very complex. Deciphering which are most important within environments that are continually changing will be an ongoing, important task for soil ecologists.

Throughout this dissertation there were many compelling discoveries concerning the factors that govern soil bacterial community structure. These findings also generated many

potential avenues of exploration for future studies, some of which I discuss below. First and foremost, because there were distinct differences between bacterial communities between the restoration experiment and Dogleg Prairie and among the rhizospheres of the three grassland plant species, one of the next steps for each of the chapters could be to determine the types of bacteria within the bulk and rhizosphere soils. This could provide insight into how the compositions of the bacterial communities were different. Various DNA-based techniques have become available to characterize the bacteria within the soil, but one of the most recent, pyrosequencing, could provide a thorough overview of the bacteria that are present. In addition, one could investigate the bacterial genes that are being expressed within the soil communities such as the genes that code for ammonia-oxidation and de-nitrification. This would paint a picture of not only the kinds of bacteria within the soil, but also give a sense of the ecosystem processes being carried out by the bacterial communities, providing a link between bacterial compositions and ecosystem function.

My research focused upon the bacteria, essentially ignoring any other soil organisms. But bacteria not only interact with plant communities, but with a suite of other organisms such as archaea, fungi, protozoa, and nematodes. Examining how interactions amongst these organisms affect bacterial populations could give a broader picture of the bulk and rhizosphere environments. It would also be interesting to apply the hypotheses and objectives within this dissertation towards these other soil organisms as this would greatly expand our knowledge about soil ecology. For example, the only metacommunity analyses performed on soil organisms have been for the bacteria, whereas the metacommunity dynamics of other soil microorganisms are unknown.

Based upon the results of Chapter 1, future studies could examine the metacommunity dynamics from more than one restoration and from multiple remnant prairies. This would not only advance our basic knowledge about metacommunity dynamics of soil bacteria, but 1) provide evidence as to if the plant communities within the remnants have a strong influence upon soil bacterial communities, or if what was found within Chapter 1 was unique for the Dogleg Prairie, and 2) give a broader perspective upon what factors are important within a restoration context. Revisiting the same plots after 20 or 30 years and comparing the bacterial communities within the restoration experiment could give insight into how long it would take soil bacterial communities to respond to plant restoration within this system.

Several patterns concerning rhizosphere bacterial communities emerged from the results found in both Chapters 2 and 3. A challenge for future studies will be to determine the underlying mechanisms for the observed patterns. It is hypothesized that the primary reason for the plant identity effect is root exudation for each plant species, but the root exudation compounds and quantities are unknown for the grassland plant species used in my research. Examining 1) what compounds are exuded by these plant species, 2) how much of those compounds are exuded, 3) how the root exudates may change over time and 4) if the exudates correlate with the compositions of the rhizosphere bacteria could provide several routes for determining if root exudation is an underlying mechanism for the observed rhizosphere bacterial communities.

Additionally, my results suggest that the rhizosphere bacteria may be responding to root exudation patterns associated with plant functional group rather than plant species. However, it is not known how widespread a plant functional group response is for the rhizosphere bacteria. Future research could include using additional plant species classified by plant functional group

and/or plant family, determining how root exudation patterns correlate to these groups and if plant functional group root exudation traits contribute to any differences observed within the rhizosphere communities. More specifically, it would be very interesting to determine if *Helianthus maximiliani* exhibited allelopathic tendencies within the roots, its exudates and leaf litter, giving rise to potential mechanisms explaining the lower bacterial diversities and distinct compositions found within its rhizospheres. How these allelopathic compounds change over time could also elucidate the seasonal patterns observed within the rhizospheres of *Helianthus maximiliani* and the varied plant identity effect.

From Chapter 2, I found that the range of plant richness levels used might not have been high enough to observe a response by the rhizosphere bacterial community. By increasing the number of plant species used, further investigations could determine 1) if there is a minimum number of plant species that are required to show a relationship with soil biota and 2) how important the plant identity effect is to the outcome of the plant richness and soil biota relationship. And finally, from the results in Chapter 3, the rhizosphere bacterial communities of the two grass species changed over time, but not in a seasonal pattern. It would be interesting to monitor these rhizospheres for a longer period of time to examine if the rhizosphere bacterial communities continue to change and potentially result in a 'climax' community.

LITERATURE CITED

Allison, V. J., R. M. Miller, J. D. Jastrow, R. Matamala and D. R. Zak. 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Sci Soc Am J 69: 1412-1421.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol 26: 32-46.

Anderson, M. J. 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62: 245-253.

Atmospheric Science Library. 1990. United States Centennial Cooperative Weather Station, Lawrence, KS. 1957-1990. University of Kansas, Lawrence, KS. 4 p.

Bach, E. M., S. G. Baer, C. K. Meyer and J. Six. 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. Soil Biol Biochem 42: 2182-2191.

Baer, S. G., D. J. Kitchen, J. M. Blair and C. W. Rice. 2002. Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecol Appl 12: 1688-1701.

Baker, K. L., S. Langenheder, G. W. Nicol, D. Ricketts, K. Killham, C. D. Campbell and J. I. Prosser. 2009. Environmental and spatial characterization of bacterial community composition in soil to inform sampling strategies. Soil Biol Biochem 41: 2292-2298.

Bardgett, R. D., W. D. Bowman, R. Kaufmann and S. K. Schmidt. 2005. A temporal approach to linking aboveground and belowground ecology. Trends Ecol Evol 20: 634-641.

Bardgett, R. D. and D. A. Wardle. 2010. Aboveground-Belowground Linkages: Biotic Interactions, Ecosystem Processes and Global Change. Oxford University Press.

Baudoin, E., E. Benizri and A. Guckert. 2002. Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Appl Soil Ecol 19: 135-145.

Bell, C., V. Acosta-Martinez, N. McIntyre, S. Cox, D. Tissue and J. Zak. 2009. Linking microbial community structure and function to seasonal differences in soil moisture and temperature in a Chihuahuan Desert grassland. Microbial Ecol 58: 827-842.

Bent, S. J. and L. J. Forney. 2008. The tragedy of the uncommon: understanding limitations in the analysis of microbial diversity. The ISME J 2: 689-695.

Berg. G. and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68: 1-13.

Bever, J. D., I. A. Dickie, E. Facelli, J. M. Facelli, J. Klironomos, M. Moora, M. C. Rillig, W. D. Stock, M. Tibbett and M. Zobel. 2010. Rooting theories of plant community ecology in microbial interactions. Trends Ecol Evol 25: 468-478.

Bezemer, T. M., C. S. Lawson, K. Hedlund, A. R. Edwards, A. J. Brook, J. M. Igual, S. R. Mortimer and W. H. Van Der Putten. 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. J Ecol 94: 893-904.

Borcard, D., P. Legendre and P. Drapeau. 1992. Partialing out the spatial component of ecological variation. Ecology 73: 1045-1055.

Bossio, D. A., K. M. Scow, N. Gunapala and K. J. Graham. 1998. Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecol 36: 1-12.

Boyle, S. I., S. C. Hart, J. P. Kaye and M. P. Waldrop. 2005. Restoration and canopy type influence soil microflora in a Ponderosa Pine forest. Soil Sci Soc Am J 69: 1627-1638.
Brodie, E., S. Edwards and N. Clipson. 2002. Bacterial community dynamics across a floristic gradient in a temperate upland grassland ecosystem. Microbial Ecol 44: 260-270.

Bremer, C., G. Braker, D. Matthies, C. Beierkuhnlein and R. Conrad. 2009. Plant presence and species combination, but not diversity, influence denitrifier activity and the composition of *nir*K-type denitrifier communities in grassland soil. FEMS Microbiol Ecol 70: 377-387.

Brodie, E., S. Edwards and N. Clipson. 2002. Bacterial community dynamics across a floristic gradient in a temperate upland grassland ecosystem. Microbial Ecol 44: 260-270.

Broughton, L. C. and K. L. Gross. 2000. Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. Oecologia 125: 420-427.

Buckley, D. and T. Schmidt. 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microbial Ecol 42: 11-21.

Card, S. M. and S. A. Quideau. 2010. Microbial community structure in restored riparian soils of the Canadian prairie pothole region. Soil Biol Biochem 42: 1463-1471.

Carney, K. M. and P. A. Matson. 2006. The influence of tropical plant diversity and composition on soil microbial communities. Microbial Ecol 52: 226-238.

Cheng, W. and A. Gershenson. 2007. Carbon fluxes in the rhizosphere. *In:* The Rhizosphere: An Ecological Perspective. Eds. Z. G. Cardon and J. L. Whitbeck. Pp. 31-56. Elsevier Academic Press. Burlington, MA.

Chesson, P. and N. Huntly. 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. The Am Nat 150: 519-553.

Chiarini, L., A. Bevivino, C. Dalmastri, C. Nacamulli and S. Tabachioni. 1998. Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Appl Soil Ecol 8: 11-18.

Clarke, K. R. and R. N. Gorley. 2006. *PRIMER v6: User Manual/Tutorial*. PRIMER-E Ltd, Plymouth.

Collins, C. D. and B. L. Foster. 2008. The role of topography and soil characteristics in the relationship between species richness and primary productivity in a Kansas grassland. Trans Kansas Acad Sci 111:105-117.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science 199: 1302-1310.

Cottenie, K., E. Michels, N. Nuytten and L. De Meester. 2003. Zooplankton metacommunity structure: Regional vs. local processes in highly interconnected ponds. Ecology 84: 991-1000.

Cottenie, K. 2005. Integrating environmental and spatial processes in ecological community dynamics. Ecol Lett 8: 1175-1182.

Culman, S. R. Bukowski, H. Gauch, H. Cadillo-Quiroz and D. Buckley. 2009. T-REX: software for the processing and analysis of T-RFLP data. BMC Bioinformatics 10: 171.

Cummings, C. L., H. M. Alexander, A. A. Snow, L. H. Rieseberg, M. J. Kim and T. M. Culley. 2002. Fecundity selection in a sunflower crop-wild study: can ecological data predict crop allele changes? Ecol Appl 12: 1661-1671.

Curl, E. A. and B. Truelove (Eds). 1986. The Rhizosphere. Springer, New York, 288 pp.

Danovaro, R., G. M. Luna, A. Dell'Anno and B. Pietrangeli. 2006. Comparison of two fingerprinting techniques, terminal restriction fragment length polymorphism and automated ribosomal intergenic spacer analysis, for determination of bacterial diversity in aquatic environments. Appl Environ Microbiol 72: 5982-5989.

de Boer, W., G. A. Kowalchuk and J. A. van Veen. 2006. 'Root-food' and the rhizosphere microbial community composition. New Phytol 170: 3-6.

De Deyn, G. B., H. Quirk and R. D. Bardgett. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biol Lett 7: 75-78.

DeGrood, S. H., V. P. Claassen and K. M. Scow. 2005. Microbial community composition on native and drastically disturbed serpentine soils. Soil Biol Biochem 37: 1427-1435.

de Leij, F. A. A. M., J. M. Whipps and J. M. Lynch. 1993. The use of colony development for the charactization of bacterial communities in soil and on roots. Microb Ecol 27: 81-97.

de Ridder-Duine, A. S., G. A. Kowalchuk, P. J. A. Klein Gunnewiek, W. Smant, J. A. van Veen and W. de Boer. 2005. Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition. Soil Biol Biochem 37: 349-357.

Dimitriu, P. and S. Grayston. 2010. Relationship between soil properties and patterns of bacterial *β*–diversity across reclaimed and natural boreal forest soils. Microbial Ecol 59: 563-573.

Dunbar, J., L. O. Ticknor and C. R. Kuske. 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Appl Environ Microbiol 67: 190-197.

Eisenhauer, N., H. Bevuler, C. Engels, G. Gleixner, M. Habekost, A. Milcu, S. Partsch, A. C. W. Sabais, C. Scherber, S. Steinbeiss, A. Weigelt, W. W. Weisser and S. Scheu. 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91: 485-496.

Ettema, C. H. and D. A. Wardle. 2002. Spatial soil ecology. Trends Ecol Evol 17: 177-183.

Farrar, J. F., M. Hawes, D. L. Jones and S. Lindow. 2003. How roots control the flux of carbon to the rhizosphere. Ecology 84: 827-837.

Fierer, N., J. P. Schimel and P. A. Holden. 2003. Influence of drying-rewetting frequency on soil bacterial community structure. Microbial Ecol 45: 63-71.

Fierer, N. and R. B. Jackson 2006. The diversity and biogeography of soil bacterial communities. Proc Nat Acad Sci 103: 626-631.

Fierer, N., M. A. Bradford and R. B. Jackson. 2007. Toward an ecological classification of soil bacteria. Ecology 88: 1354-1364.

Findlay, R. H., G. M. King and L. Watling. 1989. Efficacy of phospholipid analysis in determining microbial biomass in sediments. Appl Environ Microbiol 55: 2888-2893.

Foster, B. L., T. L. Dickson, C. A. Murphy, I. S. Karel, and V. H. Smith. 2004. Propagule pools mediate community assembly and diversity-ecosystem regulation along a grassland productivity gradient. J Ecol 92: 435-449.

Foster, B. L., C. A. Murphy, K. R. Keller, T. A. Aschenbach, E. J. Questad and K. Kindscher. 2007. Restoration of prairie community structure and ecosystem function in an abandoned hayfield: A sowing experiment. Rest Ecol 15: 652-661.

Freeman, C. C., W. D. Kettle, K. Kindscher, R. E. Brooks, V. C. Varner and C. M. Pitcher. 1991. Vascular plants of the Kansas ecological reserves. *In:* Ecology and hydrogeology of the Kansas Ecological Reserves and the Baker University wetlands. Eds. W. D. Kettle and D. O Whittemore. Open file report 91-35. University of Kansas. Lawrence, KS.

Gamble, M. D., C. E. Bagwell, J. LaRocque, P. W. Bergholz and C. R. Lovell. 2010. Season variability of diazotroph assemblages associated with the rhizosphere of the salt marsh cordgrass, *Spartina alterniflora*. Microbial Ecol 59: 253-265.

Grayston, S. J. S. Wang, C. D. Campbell and A. C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30: 369-378.

Great Plains Flora Association. 1986. Flora of the Great Plains. University Press of Kansas. Lawrence, KS.

Gregory, P. J. 2006. Roots, rhizosphere and soil: the route to a better understanding of soil science? European J Soil Sci. 57:2-12.

Gros, R. L. Jocteur Monrozier and P. Faivre. 2006. Does disturbance and restoration of alpine grassland soils affect the genetic structure and diversity of bacterial and N_2-fixing populations? Environment Microbiol 8: 1889-1901.

Gruter, D., B. Schmid and H. Brandl. 2006. Influence of plant diversity and elevated atmospheric carbon dioxide levels on belowground bacterial diversity. BMC Microbiol 6: 1-8.

Haland, A. 1992. The effects of fertilizer type on grassland soil pH and yield. Norsk Landbruksforsking 6: 297-304.

Hamlen, R. A., F. L. Lukezic, and J. R. Bloom. 1972. Influence of age and stage of development on the neutral carbohydrate components in root exudates from alfalfa plants grown in a gnotobiotic environment. Can J Plant Sci 52: 633-642.

Harner, R. F. and K. T. Harper. 1976. The role of area, heterogeneity and favorability in plant species diversity of Pinyon-Juniper ecosystems. Ecology 57: 1254-1263.

Harris, J. 2009. Soil microbial communities and restoration ecology: Facilitators or followers? Science. 325: 573-574.

Harrison, K. A. and R. D. Bardgett. 2010. Influence of plant species and soil conditions on plant-soil feedback in mixed grassland communities. J Ecol 98: 384-395.

Hawkes, C. V., K. M. DeAngelis and M. K. Firestone. 2007. Root interactions with soil microbial communities and processes. *In:* The Rhizosphere: An Ecological Perspective. Eds. Z. G. Cardon and J. L. Whitbeck. Pp. 1-29. Elsevier Academic Press. Boston, MA.

Hazra, C. R. and S. G. Tripathi. 1986. Influence of nitrogen on some soil properties and forage production of Safflower and Chinese cabbage with and without tree association. J Indian Soc Soil Sci 34: 275-280.

Hedlund, K., I. Santa Regina, W. H. Van der Putten, J. Leps, T. Diaz, G. W. Korthals, S. Lavorel, V. K. Brown, D. Gormsen, S. R. Mortimer, C. R. Barrueco, J. Roy, P. Smilauer, M. Smilauerova and C. van Dijk. 2003. Plant species diversity, plant biomass and responses of the soil community on abandoned land across Europe: idiosyncrasy or above-belowground time lags. Oikos 103: 45-58.

Hinsinger, P., G. R. Gobran, P. J. Gregory and W. W. Wenzel. 2005. Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytol 168: 293-303.

Hooper, D. U., D. E. Bignell, V. K. Brown, L. Brussard, J. M. Dangerfield, D. H. Wall, D. A. Wardle, D. C. Coleman, K. E. Giller, P. Lavelle, W. H. Van der Putten, P. C. De Ruiter, J. Rusek, W. L. Silver, J. M. Tiedje and V. Wolters. 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms and feedbacks. BioScience 50: 1049-1061.

Horner-Devine, M., K. Carney and B. J. M. Bohannan. 2004a. An ecological perspective on bacterial biodiversity. Proc R Soc Lond 271: 113-122.

Horner-Devine, M., M. Lage, J. B. Hughes and B. J. M. Bohannan. 2004b. A taxa-area relationship for bacteria. Nature 432: 750-753.

Hossain, M., A. Okubo and S. Sugiyama. 2010. Effects of grassland species on decomposition of litter and soil microbial communities. Ecol Res 25: 255-261.

Hovatter, S. R., C. Dejelo, A. L. Case and C. B. Blackwood. 2011. Metacommunity organization of soil microorganisms depends on habitat defined by presence of *Lobelia siphilitica* plants. Ecology 92: 57-65.

Hubbell, S. P. 2001. The Unified Neutral Theory of Biodiversity and Biogeography, Monographs in Population Biology. Princeton University Press, Princeton.

Innes, L., P. J. Hobbs and R. D. Bardgett. 2004. The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. Biol Fert Soils 40: 7-13.

Jangid, K., M. A. Williams, A. J. Franzluebbers, T. M. Schmidt, D. C. Coleman and W. B. Whitman. 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biol Biochem 43: 2184-2193.

Jog, S., K. Kindscher, E. Questad, B. Foster and H. Loring. 2006. Floristic quality as an indicator of native species diversity in managed grasslands. Nat Areas J 26: 149-167.

Kamal, J. and A. Bano. 2008. Potential allelopathic effects of sunflower (*Helianthus annuus* L.) on microorganisms. African J Biotech 7: 4208-4211.

Karlen, D. L., M. J. Rosek, J. C. Gardner, D. L. Allan, M. J. Alms, D. F. Bezdicek, M. Flock, D. R. Huggins, B. S. Miller and M. L. Staben. 1999. Conservation Reserve Program effects on soil quality indicators. J Soil Water Conserv 54: 439-444.

Kennedy, N., E. Brodie, J. Connolly and N. Clipson. 2004. Impact of lime, nitrogen and plant species on bacterial community structure in grassland microcosms. Environ Microbiol 6: 1070-1080.

Kent, A. D. and E. W. Triplett. 2002. Microbial communities and their interactions in soil and rhizosphere ecosystems. An Rev Microbiol 56: 211-236.

Kettle, W. D. and D. O. Whittemore. 1991. Ecology and hydrology of the Kansas Ecological Reserves and the Baker Wetlands. Open file report 91-35. University of Kansas, Lawrence, KS.

Kettle, W. D., P. M. Rich, K. Kindscher, G. L. Pittman and P. Fu. 2000. Land-use history in ecosystem restoration: a 40-yr study in the prairie-forest ecotone. Rest Ecol 8: 307-317.

Kielak, A., A. G. Pijl, J. A. van Veen and G. A. Kowalchuk. 2008. Differences in vegetation composition and plant species identity lead to only minor changes in soil-borne microbial communities in a former arable field. FEMS Microbiol Ecol 63: 372-382.

Kindscher, K. and L. L. Tieszen. 1998. Floristic and soil organic matter changes after five and thirty-five years of native tallgrass prairie restoration. Rest Ecol 6: 181-196.

King, A. J., K. R. Freeman, K. F. McCormick, R. C. Lynch, C. Lozupone, R. Knight and S. K. Schmidt. 2010. Biogeography and habitat modeling of high-alpine bacteria. Nature Comm 1: 1-6.

Knops, J. M. H. and D. Tilman. 2000. Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. Ecology 81: 88-98.

Kowalchuk, G. A., A. W. Stienstra, G. H. J. Heilig, J. R .Stephen and J. W. Woldendorp. 2000. Composition of communities of ammonium-oxidising bacteria in wet, slightly acid grassland soils using 16S rDNA-analysis. FEMS Microbiol Ecol 31: 207-215.

Kowalchuk, G. A., D. W. Buma, W. de Boer, P. G. L. Klinkhamer and J. A. van Veen. 2002. Effects of aboveground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie van Leeuwenhoek 81: 509-520.

Kulmatiski, A. and K. H. Beard. 2011. Long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community structure. Soil Biol Biochem 43: 823-830.

Ladygina, N. and K. Hedlund. 2010. Plant species influence microbial diversity and carbon allocation in the rhizosphere. Soil Biol Biochem 42: 162-168.

Lamb, E. G., N. Kennedy and S. D. Siciliano. 2011. Effects of plant species richness and evenness on soil microbial community diversity and function. Plant Soil 338: 483-495.

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

Loranger-Merciris, G., L. Barthes, A. Gastine and P. Leadley. 2006. Rapid effects of plant species diversity and identity on soil microbial communities in experimental grassland ecosystems. Soil Biol Biochem 38: 2336-2343.

Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, J. P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D. Raffaelli, B. Schmid, D. Tilman and D. A. Wardle. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294: 804-808.

Lupwayi, N. A., W. A. Rice and G. W. Clayton. 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. Soil Biol Biochem 30: 1733-1741.

MacArthur, R. H. 1958. Population ecology of some warblers of Northeastern coniferous forests. Ecology 39: 599-619.

Marilley, L. and M. Aragno. 1999. Phylogenetic diversity of bacterial communities differing in degree of proximity of *Lolium perenne* and *Trifolium repens* roots. Appl Soil Ecol 13: 127-136.

Marshall, C. B., J. R. McLaren and R. Turkington. 2011. Soil microbial communities resistant to changes in plant functional group composition. Soil Biol Biochem 43: 78-85.

Marschner, P., G. Neumann, A. Kania, L. Weiskopf and R. Lieberei. 2002. Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (*Lupinus albus* L.). Plant Soil 246: 167-174.

Martiny, J. B. H., J. A. Eisen, K. Penn, S. D. Allison and M. Horner-Devine. 2011. Drivers of bacterial beta diversity depend on spatial scale. Proc Nat Acad Sci 108: 7850-7854.

McArdle, B. H. and M. J. Anderson. 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82: 290-297.

McCune, B. and M. J. Mefford. 1999. PC-ORD for Windows. Multivariate Analysis of Ecological Data Version 4.14. MjM Software, Gleneden Beach, Oregon, U.S.A.

McKinley, V. L., A. D. Peacock and D. C. White. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biol Biochem 37: 1946-1958.

Metting, F. B., J. L. Smith, J. S. Amthor and R. C. Izaurralde. 2001. Science needs and new technology for increasing soil carbon sequestration. Climate Change 51: 11-34.

Milcu, A., E. Thebault, S. Scheu and N. Eisenhauer. 2010. Plant diversity enhances the reliability of belowground processes. Soil Biol Biochem 42: 2101-2110.

Millard, P. and B. K. Singh. 2010. Does grassland vegetation drive soil microbial diversity? Nutr Cycl Agroecosyst 88: 147-158.

Mummey, D. L., J. T. Clarke, C. A. Cole, B. G. O'Connor, J. E. Gannon and P. W. Ramsey. 2010. Spatial analysis reveals differences in soil microbial community interactions between adjacent coniferous forest and clearcut ecosystems. Soil Biol Biochem 42: 1138-1147.

Murphy, C. A., B. L. Foster, M. E. Ramspott and K. P. Price. 2004. Grassland management effects on soil bulk density. Trans Kansas Acad Sci 107: 45-54.

Murphy, C. A., B. L. Foster, M. E. Ramspott and K. P. Price. 2006. Effects of cultivation history and current grassland management on soil quality in northeastern Kansas. J Soil Water Conserv 61: 75-84.

Nannipieri, P., J. Ascher, M. T. Ceccherini, L. Landi, G. Pietramellara and G. Renella. 2003. Microbial diversity and soil functions. Euro J Soil Sci 54: 655-670.

Nguyen, C. 2003. Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23: 375-396.

Norris, M. D., J. M. Blair, L. C. Johnson and R. B. McKane. 2001. Assessing changes in biomass, productivity, and C and N stores following *Juniperus virginiana* forest expansion into tallgrass prairie. Can J Forest Res 31: 1940-1948.

Nsabimana, D., R. J. Haynes and F. M. Wallis. 2004. Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Appl Soil Ecol 26: 81-92.

Nunan, N., T. J. Daniell, B. K. Singh, A. Papert, J. W. McNicol, and J. I. Prosser. 2005. Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. Appl Environ Microbiol 71: 6784-6792.

Olsson, S. and P. Persson. 1999. The composition of bacterial populations in soil fractions differing in their degree of adherence to barley roots. Appl Soil Ecol 12: 205-215.

Paine, R. T. 1966. Food web complexity and species diversity. The Am Nat 100: 65-75.

Parker, I. M., S. K. Mertens, and D. W. Schemske. 1993. Distribution of seven native and two exotic plants in a tallgrass prairie in southeastern Wisconsin: The importance of human disturbance. Am Mid Nat 130: 43-55.

Plassart, P., M. A. Vinceslas, C. Gangneux, A. Mercier, S. Barray and K. Laval. 2008. Molecular and functional responses of soil microbial communities under grassland restoration. Agr Ecosys Environ 127: 286-293.

Polley, H. W., J. D. Derner and B. J. Wilsey. 2005. Patterns of plant species diversity in remnant and restored tallgrass prairies. Rest Ecol 13: 480-487.

Porazinska, D. L., R. D. Bardgett, M. B. Blaauw, H. W. Hunt, A. N. Parsons, T. R. Seastedt and D. H. Wall. 2003. Relationships at the aboveground-belowground interface: plants, soil biota and soil processes. Ecol Monographs 73: 377-395.

Potthoff, M., K. L. Steenwerth, L. E. Jackson, R. E. Drenovsky, K. M. Scow and R. G. Joergensen. 2006. Soil microbial community composition as affected by restoration practices in California grassland. Soil Biol Biochem 38: 1851-1860.

Prosser, J. I., B. J. M. Bohannan, T. P. Curtis, R. J. Ellis, M. K. Firestone, R. P. Freckleton, J. L. Green, L. E. Green, K. Killham, J. J. Lennon, A. M. Osborn, M. Solan, C. J. van der Gast and P. W. Young. 2007. The role of ecological theory in microbial ecology. Nat Rev Microbiol 5: 384-392.

Qian, P. and J. J. Schoenau. 1996. Ion exchange resin membrane (IERM): A new approach for in situ measurement of nutrient availability in soil. Plant Nutr Fert Sci 2: 322-330.

Raaijmakers, J. M., C. T. Paulitz, C. Steinberg, C. Alabouvette and Y. Moenne-Loccoz. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil DOI: 10.1007/s11104-008-9568-6.

Ramette, A. and J. M. Tiedje. 2007. Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. Proc Nat Acad Sci 104: 2761-2766.

Reynolds, H. L., A. Packer, J. D. Bever and K. Clay. 2003. Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84: 2281-2291.

Ridgeway, K. P., M. J. Duck and P. W. Young. 2003. Identification of roots from grass swards using PCR-RFLP and FFLP of the plastid trnL (UAA) intron. BMC Ecol 3:8.

Risser, P. G. 1996. Summary: A new framework for prairie conservation. *In:* Prairie Conservation. Eds: F. B. Samson and F. L. Knopf. Pp 261-274. Island Press, Washington, D. C.

Robertson, G. P., P. Sollins, B. G. Ellis and K. Lajtha. 1999. Exchangeable ions, pH, and cation exchange capacity. *In:* Standard Soil Methods for Long-Term Ecological Research. Eds. G. P. Robertson, D. C. Coleman, C. S. Bledsoe and P. Sollins. Oxford University Press. New York, NY.

Rosenzweig, M. L. 1995. Species Diversity in Space and Time. Cambridge University Press, Cambridge, UK.

Rovira, A. D. 1959. Root excretions in relation to the rhizosphere effect. IV. Influence of plant species, age of plants, light, temperature and calcium nutrition on exudation. Plant Soil 11: 53-64.

Rovira, A. D. 1969. Plant root exudates. Bot Rev 35: 35-59.

Samson, F. and F. Knopf. 1994. Prairie conservation in North America. BioScience 44: 418-421.

Sauer, D., Y. Kuzyakov and K. Stahr. 2006. Spatial distribution of root exudates of five plant species as assessed by ^{14}C labeling. J Plant Nutr Soil Sci 169: 360-362.

Schmidt, S. K., E. K. Costello, D. R. Nemergut, C. Cleveland, S. C. Reed, M. N. Weintraub, A. F. Meyer and A. M. Martin. 2007. Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. Ecology 88: 1379-1385.

Sessitsch. A., A. Weilharter, M. H. Gerzabek, H. Kirchmann and E. Kandeler. 2001. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Appl Environ Microbiol 67: 4215-4224.

Singh, B. K., S. Munro, J. M. Potts and P. Millard. 2007. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl Soil Ecol 36: 147-155.

Singh, B. K., N. Nunan, K. P. Ridgeway, J. McNicol, P. W. Young, T. J. Daniell, J. I. Prosser and P. Millard. 2008. The relationships between assemblages of mycorrhizal fungi and bacteria on grass roots. Environ Microbiol 10: 534-541.

Smalla, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer and G. Berg. 2001. Bulk and rhizosphere soil bacterial communities studies by denaturing gradient gel electrophoresis: Plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67: 4742-4751.

Smit, E., P. Leeflang, S. Gommans, J. van den Broek, S. van Mil and K. Wernars. 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl Environ Microbiol 67: 2284-2291.

Smith, M. D. and A. K. Knapp. 2003. Dominant species maintain ecosystem function with non-random species loss. Ecol Lett 6: 509-517.

Smith, V. H., B. L. Foster, J. P. Grover, R. D. Holt, M. A. Leibold and F. deNoyelles, Jr. 2005. Phytoplankton species richness scales consistently from laboratory microcosms to the world's oceans. Proc Nat Acad Sci 102: 4393-4396.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd Edition. W. H. Freeman and Company, New York, NY, U.S.A.

Spehn, E. M., J. Joshi, B. Schmid, J. Alphei, C. Korner. 2000. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant Soil 224: 217-230.

Sperry, T. M. 1994. The Curtis Prairie restoration: using the single species planting method. Nat Areas J 14: 124-127.

Spring, O. and T. Benz. 1989. Sesquiterpene lactones of the capitates glandular trichomes of *Helianthus annuus*. Phytochemistry 28: 745-749.

Steer, J. and J. A. Harris. 2000. Shifts in the microbial community in rhizosphere and non-rhizosphere soils during the growth of *Agrostis stolonifera*. Soil Biol Biochem 32: 869-878

Stephan, A., A. H. Meyer and B. Schmid. 2000. Plant diversity affects culturable soil bacteria in experimental grassland communities. J Ecol 88: 988-998.

Strickland, M. S., C. Lauber, N. Fierer and M. A. Bradford. 2009. Testing the functional significance of microbial community composition. Ecol 90: 441-451.

Thirup, L., A. Johansen and A. Winding. 2003. Microbial succession in the rhizosphere of live and decomposing barley roots as affected by the antagonistic strain *Pseudomonas fluorescens* DR54-BN14 or the fungicide imazalil. FEMS Microbiol Ecol 43: 383-392.

Thompson, J. 1992. Prairies, forests, and wetlands: the restoration of natural landscape communities in Iowa. University of Iowa Press, Iowa City, Iowa.

Tiessen, H., J. W. B. Stewart and J. R. Bettany. 1982. Cultivation effects on the amounts and concentration of carbon, nitrogen, and phosphorus in grassland soils. Agron J 74: 831-835.

Tiessen, H., E. Cuevas and P. Chacon. 1994. The role of soil organic matter in sustaining soil fertility. Nature 371: 783-785.

Tilman, D. and S. Pacala. 1993. The maintenance of species richness in plant communities. *In:* Species diversity in ecological communities: Historical and geographical perspectives. Eds. R. Ricklefs and D. Schluter. University of Chicago Press, Chicago, Il.

Tilman, D., D. Wedin and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379: 718-720.

Tilman, D. 1997. Community invasibility, recruitment limitation, and grassland biodiversity. Ecology 78: 81-92.

Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science 294: 843-845.

Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion and community assembly. Proc Nat Acad Sci 101: 10854-10861.

Torsvik, V., J. Goksoyr and F. L. Daae. 1990. High diversity in DNA of soil bacteria. Appl Environ Microbiol 56: 782-787.

U.S. Department of Agriculture Soil Conservation Service. 1977. Soil survey of Douglas County, Kansas. U.S. Department of Agriculture and the Kansas Agriculture Experiment Station, Washington, D. C.

U.S. Department of Agriculture. Natural Resources Conservation Service. Plants Database. http://plants.usda.gov.

van der Gast, C. J., D. Ager and A. K. Lilley. 2008. Temporal scaling of bacterial taxa is influenced by both stochastic and deterministic ecological factors. Environ Microbiol 10: 1411-1418.

van Elsas, J. D., M. Chiurazzi, C. A. Mallon, D. Elhottova, V. Kristufek and J. F. Salles. 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. Proc Natl Acad Sci 109: 1159-1164.

Vitousek, P. M, H. A. Mooney, J. Lubchenco and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science 277: 494-499.

Walkley, A. and I. A. Black. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37: 29-37.

Wang, J., Y. Wu, H. Jiang, C. Li, H. Dong, Q. Wu, J. Soininen and J. Shen. 2008. High beta diversity of bacteria in the shallow terrestrial subsurface. Environ Microbiol 10: 2537-2549.

Wardle, D. A., G. W. Yeates, W. Williamson and K. I. Bonner. 2003. The response of three trophic level soil food web to the identity and diversity of plant species and functional groups. Oikos 102: 45-56.

Warembourg, F. R., C. Roumet and F. Lafont. 2003. Differences in rhizosphere carbon-partitioning among plant species of different families. Plant Soil 256: 347-357.

Westover, K. M., A. C. Kennedy and S. E. Kelley. 1997. Patterns of rhizosphere microbial community structure associated with co-occurring plant species. J Ecol 85: 863-873.

Zak, D. R., W. E. Holmes, D. C. White, A. D. Peacock and D. Tilman. 2003. Plant diversity, soil microbial communities and ecosystem function: Are they any links? Ecology 84: 2042-2050.

Zul, D., S. Denzel, A. Kotz and J. Overmann. 2007. Effects of plant biomass, plant diversity and water content on bacterial communities in soil lysimeters: Implications for the determinants of bacterial diversity. Appl Environ Microbiol 73: 6916-6929.