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RESEARCH ARTICLE

Soil Properties and Spatial Processes Influence Bacterial Metacommunities within a Grassland Restoration Experiment

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Abstract

Metacommunity theory proposes that a collection of local communities are linked by dispersal and the resulting compositions are a product of both niche-based (species sorting) and spatial processes. Determining which of these factors is most important in different habitats can provide insight into the regulation of community assembly. To date, the metacommunity organization of heterotrophic soil bacteria is largely unknown. Spatial variation of soil bacterial communities could arise from (1) the resource heterogeneity produced by plant communities through root exudation and/or litter inputs; (2) the heterogeneity of soil environmental properties; and (3) pure spatial processes, including dispersal limitation and stochastic assembly. Understanding the relative importance of these factors for soil bacterial community structure and function could increase our ability to restore soil communities. We utilized an ongoing tallgrass prairie restoration experiment in northeastern Kansas to assess if restoring native plant communities produced changes in bacterial communities 6 years after restoration. We further examined the relative importance of the spatial heterogeneity of plant communities, soil properties, and pure spatial effects for bacterial community structure in the old-field restoration site. We found that soil bacterial communities were not influenced by plant restoration, but rather, by the local heterogeneity of soil environmental properties (16.9% of bacterial community variation) and pure spatial effects (11.1%). This work also stresses the idea that restoring bacterial communities can take many years to accomplish due to the inherent changes that occur to the soil after cultivation and the time it takes for the re-establishment of soil quality.

Key words: edaphic soil properties, microbial communities, niche-based processes, plant communities, regional processes, spatial autocorrelation, tallgrass prairie.

Introduction

Agricultural activity since the 1830s has almost eliminated the tallgrass prairie within the Great Plains of North America, leaving less than 1% intact (Samson & Knopf 1994). Thus, native prairie remnants are extremely isolated within a highly fragmented agricultural landscape, increasing the risk for local and regional extinctions of native prairie species (Kindscher & Tieszen 1998). This has led to concern over the ability to effectively restore native tallgrass prairie.

Successful tallgrass prairie restoration not only includes conserving native prairie plant species but also restoring soil microbial communities due to the myriad of ecosystem processes microbes regulate (e.g. decomposition, nutrient availability) (Baer et al. 2002; Allison et al. 2005). Yet, only recently have soil microbes been a focus in restoration experimental studies (Potthoff et al. 2006). These studies have shown that the extent of bacterial community restoration can be highly variable dependent upon factors such as soil pH, texture, time since restoration and landuse history (Bach et al. 2010; Card & Quideau 2010). Thus, 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.

Metacommunity theory proposes that a collection of local communities are linked by dispersal and the resulting compositions are a product of both niche-based and pure spatial processes (Leibold et al. 2004). For example, communities can display spatial patterns because niche partitioning regulates species composition at the local scale and species abundances reflect the environmental gradients of the habitat (species sorting) (Hovatter et al. 2011; Alexander et al. 2012). Community composition may also exhibit spatial structure independent of niche-based processes due to factors such as dispersal limitation and stochastic assembly (pure spatial effects) (Leibold et al. 2004; Martiny et al. 2011). Among different habitats, however, the relative

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importance of species sorting versus pure spatial effects may vary. Determining which of these factors is most important can provide greater understanding of the regulation of community assembly. Currently, the metacommunity dynamics of soil bacteria are largely unknown (Ramette & Tiedje 2007).

Ecologists have predominantly focused upon the role of soil environmental heterogeneity in structuring bacterial composition and found soil pH to be a primary correlate (Fierer & Jackson 2006; Harrison & Bardgett 2010). However, other soil properties, such as texture, moisture, organic matter, nutrient availability, and microbial biomass can be significant depending upon habitat type and land-use history (Fierer et al. 2003; Bach et al. 2010; Dimitriu & Grayston 2010). Continued research into the importance of soil environmental properties will be needed as more habitats are subject to land-use change, potentially leading to alterations in both soil qualities and bacterial composition (Tiessen et al. 1982).

Of potentially equal importance for bacterial composition, but much less studied, is the role of resource heterogeneity generated by the co-occurring plant communities (Hooper et al. 2000). A more diverse plant community could support a more diverse assemblage of soil bacteria by offering a greater variety of resource opportunities via increased root exudate and litter diversities (Hooper et al. 2000; Kowalchuk et al. 2002; De Deyn et al. 2011). Furthermore, restoring native plant communities may potentially lead to changes in the bacterial community as a consequence of these resource differences (Bezemer et al. 2006; Gruter et al. 2006). For example, different plant species can vary in their chemical composition, subsequently altering the types of microbes in the soil based upon their ability to decompose available litter (Hossain et al. 2010). Observing a change in bacterial communities after plant restoration could lend support to the idea that resource heterogeneity created by the plant community could influence soil bacterial communities.

In addition to local niche-based effects, recent work has shown that spatial processes can influence microbial composition (Hovatter et al. 2011; Martiny et al. 2011). Thus, variation in bacterial communities across a landscape could also arise as a result of processes that have acted largely independent from the selective effects of the soil environment or the plant community (Ramette & Tiedje 2007). These three factors (soil environmental properties, plant communities, and spatial effects) could explain a certain amount of variability in bacterial composition. However, each can potentially interact and co-vary. By evaluating their unique contributions, we can further our basic knowledge of the factors structuring soil bacterial communities.

Using an ongoing tallgrass prairie restoration experiment, we assessed the effects of plant restoration after six growing seasons on soil bacterial communities across an abandoned old-field landscape in northeastern Kansas. We had three objectives for this study: (1) Examine if plant restoration produced changes in soil bacterial communities; (2) Examine the extent to which the bacterial communities approached that observed in a nearby native prairie remnant (Reference Prairie) located on similar soil; and (3) Investigate the relative importance of the spatial heterogeneity of soil environmental properties, plant communities, and spatial effects to the variation observed in soil bacterial communities across the Restoration Experiment. By applying a metacommunity viewpoint within a restoration context, not only is our understanding of the structure and dynamics of bacterial community assemblages enhanced, we could also gain insight into the restoration dynamics of soil microbes within degraded land.

Methods

The Restoration Experiment and Reference Prairie (Dogleg Prairie [DLP]) are located at the University of Kansas Field Station (KUFS) in northeastern Kansas. Soils of both sites are Pawnee clay loams (montmorillonitic, mesic Aquic Argiudolls) formed under glacial deposits of till and loess with weathering of interbedded limestones and shales. The region has a mean annual temperature of 12.9°C and mean annual precipitation of 930 mm.

Restoration Experiment

The Restoration Experiment was initiated to explore the effects of seed addition on plant community dynamics, restoration, and ecosystem processes within a degraded former agricultural landscape (Foster et al. 2004). The old-field grassland was historically tallgrass prairie, but in the early 1900s, was plowed for agriculture and later converted into an introduced cool-season hay field (Kettle & Whittemore 1991). The University of Kansas acquired KUFS in 1970 and the experimental study site has been managed as open grassland with periodic mowing to prevent woody plant encroachment.

In 1999, forty 2.5 × 2.5-m blocks were established throughout the grassland (3.5 ha) using a stratified random approach (Foster et al. 2004). In each block, four 1 m^2 quadrats contained 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). For this study, we present results from 18 randomly chosen blocks and from only Control and Restored treatments. In January 2000, seeds from 24 native tallgrass prairie species found within prairie habitats throughout the area were sown into the Restored treatment quadrats by hand. Refer to the study by Foster et al. (2004) for a complete species list.

During the sixth year of the restoration experiment, plants and soils were sampled in June and September 2005. All samples taken during these two time periods were processed separately and later combined to characterize the growing season as a whole (described below).

Soil bacterial communities were sampled in the 1 m^2 quadrats by taking three soil cores (15 cm depth) evenly spaced throughout a 0.1 × 1 m strip and immediately pooled and frozen at −80°C until further processing. The plant community was sampled by clipping a 0.1 × 1 m strip over the same location as the soil samples to optimize the potential association between bacterial and plant communities. Plants were clipped at ground

level and biomass sorted to species. A complete description of the plant communities can be found in the study by Foster et al. (2007).

Several soil properties were used as potential predictors of soil bacterial community structure: total soil carbon (C) and nitrogen (N), organic matter (OM), available N, soil moisture, pH, texture, cation exchange capacity (CEC), elevation, and microbial biomass. A description of all sampling procedures and soil analyses are within Appendix S1, Supporting Information. To evaluate spatial effects within the restoration experiment, the Latitude and Longitude for each quadrat was recorded via GPS.

Reference Prairie

The DLP (2 ha), a tallgrass prairie remnant, was located within 250 m of the Restoration Experimental field site. Management since 1990 has consisted of burning approximately every year including a burn on 31 March 2005, 3 months prior to sampling.

Plant and soil communities were sampled in July and September 2005 and seasonal data were later combined. Eighteen sampling locations were chosen using a stratified random approach to represent the topographical variation at this site. Ocular estimates of percent cover were used to assess plant species abundance using the same sample area as in the restoration experiment. Soil bacterial communities were sampled in the same manner as the Restoration Experiment. Three soil properties were measured in the Reference Prairie: microbial biomass, pH, and elevation. Owing to the disparate soil properties measured between the two sites, metacommunity analyses of the Reference Prairie were not analogous to the Restoration Experiment and not presented here, but can be viewed in the Supporting Information.

Soil Bacterial Community Analysis

After root removal, soils used for bacterial community analyses were sieved (4 mm) and homogenized. Terminal-restriction fragment length polymorphism (T-RFLP) was used to assess the bacterial communities. For a detailed description of the T-RFLP technique and profile analysis, see Appendix S2. Briefly, bacterial DNA was extracted and the 16S ribosomal DNA (rDNA) region polymerase chain reaction (PCR)-amplified using the universal eubacterial primers 6-FAM 8–27 F, a fluorescently labeled forward primer, and 1389R, a non-labeled reverse primer (Fierer et al. 2003). PCR products were cut into terminal restriction fragments (TRFs) using the restriction enzyme *Rsa*I. The fluorescently labeled fragments were analyzed using an Applied Biosystems Instrument 3730 genetic analyzer to generate bacterial community profiles, which were standardized as described by Fierer et al. (2003). To compare bacterial community structure, both the proportional peak area (abundance) and TRF size were used. Because TRFs of similar size could be shared at higher levels of taxonomic organization, TRFs of different lengths were assumed to represent different operational taxonomic units (OTUs) and not necessarily distinct bacterial species.

Bacterial Community Composition Analyses

Because the Reference Prairie was spatially separated from the Restoration Experiment, comparisons between the two sites could not be made using all three "treatments" (Control, Restored, and DLP) simultaneously. Therefore, analyses contrasting the Reference Prairie with the Restoration Experiment were done using each treatment of the Restoration Experiment separately. Because seasonal differences between bacterial community compositions were not observed in either site and to better understand bacterial communities over the entire growing season, for each variable (e.g. plant species biomass/cover, TRF proportional peak area), the maximum value observed for a particular quadrat/location from either season was used. All multivariate analyses used relativized Bray–Curtis abundance matrices. Scores generated from non-metric multidimensional scaling (NMS) analyses (PC-Ord 4.14) were used to graphically display the bacterial communities as ordinations.

To examine differences in bacterial community composition between sowing treatments of the Restoration Experiment and between the Restoration Experiment and Dogleg Prairie, we used permutational multivariate analysis of variance (PERMANOVA) employed by PERMANOVA + for Primer (Clarke & Gorley 2006). A two-way analysis of variance (ANOVA) without Replication was used to analyze bacterial community compositions of the Restoration Experiment, whereas a one-way ANOVA was used for analyses between treatments of the Restoration Experiment and Dogleg Prairie.

Variance Partitioning

To evaluate the unique contributions of plant composition, soil environmental properties and spatial effects to variation in bacterial composition of the Restoration Experiment, we employed a variance partitioning approach that incorporated multiple regression modeling followed by a partial redundancy analysis. For the multiple regressions, distance-based linear models (DISTLM) within PERMANOVA + for Primer (Clarke & Gorley 2006) was used. Analyses of the predictor variables/groups in the restoration experiment were done using all quadrats simultaneously.

To evaluate the dependence of bacterial composition on plant species composition, we reduced the dimensionality of the plant datasets using NMS and used scores from the first two axes as the predictor group, "Plant." This approach reduced the problem of variance inflation that would likely occur if we included all plant species in the regression models. In addition, to determine if the plant community was significantly impacting bacterial compositions via litter inputs, aboveground and root biomass were used as the predictor group, "Bio."

The soil environmental properties used to predict bacterial communities within the Restoration Experiment were grouped and labeled as "Env." Organic matter, N, CEC, and % clay were highly correlated with several other environmental properties and were excluded from all analyses to reduce multicollinearities. The predictor group "Env" included: soil pH, C, % silt, % sand, C/N ratio, soil moisture, available N, microbial biomass, and elevation. In addition, a regression was run on each of the

variables of Env individually to determine which variables significantly predicted bacterial composition. The significant variables from these analyses were then grouped into "'Env2" (soil pH, % sand, elevation, and microbial biomass).

To examine the effect of pure spatial effects on the spatial structure of bacterial community composition, the Latitude and Longitude positions (geographic coordinates) of the quadrats were utilized. To account for complex spatial patterns that correlate beyond just linear gradients (e.g. nonlinear, unimodal patterns of variance) all terms of a cubic trend surface regression were calculated (Borcard et al. 1992). A stepwise regression was performed to select only the significant terms and placed in the predictor group, "Spatial" (x^3, x^2y).

Multiple regression analyses were run in three steps to determine which predictor group(s) were important for variation in bacterial composition. First, bacterial composition was regressed on each predictor group, ignoring all other groups, to determine if a significant relationship existed. Second, a multiple regression, which included the predictor groups, Plant, Bio, Env, and Spatial, was run. Third, a multiple regression using the predictor groups, Plant, Bio, Env2, and Spatial was run to determine if using Env2 improved the final model more than using Env. For all regressions, a stepwise selection procedure, adjusted R^2 selection criterion and 9,999 permutations were used. The model with the highest F-value after all terms were included was deemed the "best" model and reported.

A distance-based Redundancy Analysis (dbRDA) with PERMANOVA + for Primer (Clarke & Gorley 2006) was run to examine the relative contributions of each predictor group to the total explained variation in the "best" model. In this way, partial regression coefficients were calculated and we could partition out pure plant, pure soil environmental properties, and pure spatial effects from any explained variation that was shared among predictor groups.

Results

Plant composition of the Control, Restored, and Dogleg Prairie were all significantly different. A detailed description of the analyses and results of the plant community comparisons can be found within Appendix S3.

PERMANOVA showed that Control and Restored treatments had similar bacterial compositions (Fig. 1; Table 1). However, bacterial compositions of the Reference Prairie were significantly different from the Control and Restored quadrats of the Restoration Experiment.

DISTLM results showed that the bacterial community of the Restoration Experiment varied among quadrats independent of plant community composition and plant biomass (Table 2). However, Env, Env2, and Spatial predictor groups correlated significantly with bacterial OTU community compositions.

Stepwise regression analyses showed that the predictor groups Env2 and Spatial were important correlates for bacterial compositions (Table 3). The predictor groups, Plant and Bio, were not included in the final regression model. Using this final model, the total explained variation (32.2%) was partitioned

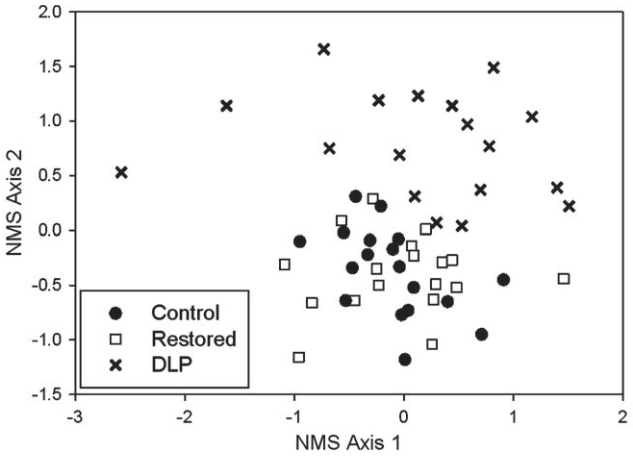

Figure 1. Non-metric multidimensional scaling plots for soil bacterial community composition.

Table 1. Results of two-way ANOVA without replication (Restoration Experiment) and one-way ANOVA (Control versus DLP; Restored versus DLP) for soil bacterial community composition.

		OTU Community Composition[a] [b]	
		Pseudo-F_{df}	*Perm P*
Restoration experiment	Block	$2.54_{17,17}$	*0.001*
	Trt	$1.03_{1,17}$	0.421
Controls versus DLP	Trt	$4.63_{1,34}$	*0.001*
Restored versus DLP	Trt	$5.17_{1,34}$	*0.001*

Significant effects at $\alpha = 0.05$ level are in italics.
[a]Bacterial community composition analyses were performed using a Bray–Curtis abundance matrix in PERMANOVA.
[b]Pseudo-*F*: *F*-value generated from PERMANOVA. Perm *P*: *p*-values generated from 9,999 permutations of the data.

using dbRDA. Results indicated that the predictor group Env2 significantly explained 16.9% of the variation found in bacterial communities, indicating a significant soil environmental property effect ($F_{[7,27]} = 1.69$; $p = 0.001$) (Fig. 2). Spatial effects, independent of the soil 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 soil environmental properties and spatial effects encompassed 4.2% of the total explained variation.

Discussion

Local plant communities did not influence soil bacterial composition within the tallgrass prairie restoration field-site. This suggests that a 6-year period of restoration is insufficient for soil bacterial communities to be influenced by plant-generated resource heterogeneity. Instead, soil bacterial communities within the Restoration Experiment were significantly influenced by both niche-based local effects of soil environmental properties and by pure spatial effects.

Local heterogeneity fostered by elevation, texture, soil pH, and microbial biomass allowed for bacterial OTU sorting across

Table 2. Results from regression analyses between soil bacterial communities and each predictor group/environmental variable individually using DISTLM with PERMANOVA + for Primer.

	Restoration Experiment		
Group/Env. Variable	*Pseudo-F_{df}*[a]	*Perm P*[a]	R^2
Plant[b]	$0.88_{3,31}$	0.667	0.054
Bio[c]	$0.84_{3,31}$	0.658	0.053
Env[d]	$1.39_{12,22}$	*0.003*	0.411
Microbial biomass	$2.04_{2,32}$	*0.008*	0.060
Soil pH	$2.09_{2,32}$	*0.009*	0.061
% Sand	$1.71_{2,32}$	*0.040*	0.051
Elevation	$2.95_{2,32}$	*0.001*	0.084
Env2[e]	$1.95_{5,29}$	*0.001*	0.212
Spatial[f]	$2.81_{3,31}$	*0.001*	0.153

Analyses included all quadrats simultaneously. For each analysis, a Bray–Curtis abundance matrix was used. Significant effects at $\alpha = 0.05$ level are in italics.
[a]Pseudo-*F*: *F*-value generated from DISTLM program. Perm *P*: *p*-values generated from 9,999 permutations of the data.
[b]Plant = predictor group for plant communities portrayed as scores from the first two NMS axes.
[c]Bio = predictor group for plant biomass (aboveground and root biomass).
[d]Env = predictor group for environmental variables. Significant relationships with individual environmental variables are listed below Env.
[e]Env2 = predictor group using only the environmental variables found to be significant correlates with soil bacterial communities individually.
[f]Spatial = predictor group representing the spatial location of the soil bacterial communities. Includes the third polynomial(s) that were significant after stepwise regression: x^3, x^2y.

Table 3. Stepwise regression results between soil bacterial communities and the predictor groups using DISTLM with PERMANOVA + for Primer.

	Predictor Group	*Pseudo-F_{df}*[a]	*Perm P*[a]	*Cumulative Adjusted R^2*	*Cumulative R^2*
Restoration Experiment	Env2	$1.95_{5,29}$	*0.001*	0.103	0.212
	Spatial	$2.21_{7,27}$	*0.001*	0.172	0.322

Analyses included all quadrats simultaneously. For each analysis, a Bray–Curtis abundance matrix was used; adjusted R^2 was the selection criterion. The model with the highest ending Pseudo-*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. Significant effects at $\alpha = 0.05$ level are in italics.
[a]Pseudo-*F*: *F*-value generated from DISTLM program. Perm *P*: *p*-values generated from 9,999 permutations of the data.

the Restoration Experimental field site. Elevation is an integrative variable representing a suite of biotic and abiotic properties to which soil biota can respond (e.g. productivity, nutrients) (Broughton & Gross 2000). In this study, it is unknown to which of these aspects of elevation the bacteria were responding. Moreover, despite sampling from a relatively uniform topography (elevation range = 8 m), the resulting environmental heterogeneity was enough to impact the bacterial communities.

Percent sand in soils sampled throughout the Restoration Experiment ranged from 8.4 to 24.4%, providing variable soil textures. Soil texture can be key in driving microbial communities such that silt and clay particles support more productive and diverse microbial communities than sand, potentially through two routes (Bezemer et al. 2006). First, silt and clay can provide refugia for microorganisms because pore sizes are too small for predators (e.g. protozoa) (Sessitsch et al. 2001). Second,

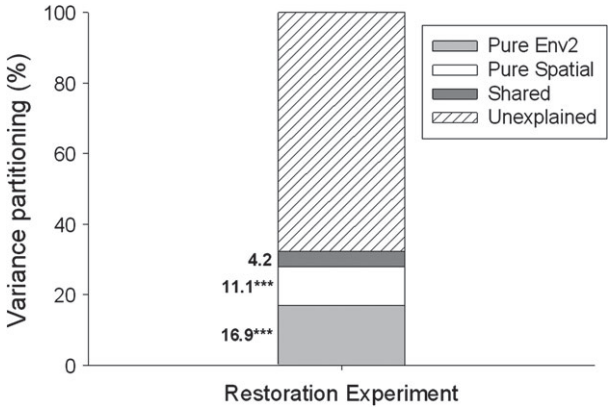

Figure 2. Variance partitioning (%) of the restoration experiment soil bacterial community compositions after DISTLM and dbRDA analyses. Values represent the amount of explained variation for each component. *** $p < 0.001$

sand particles can be depleted in OM and microbial biomass as compared with finer particle sizes, leading to lower nutrient availability in sand. Therefore, only those soil microbes adapted to limited nutrient conditions would be found in sand (Sessitsch et al. 2001).

As in other studies, we found soil pH to be an important determinant of soil bacterial communities (Fierer & Jackson 2006; Dimitriu & Grayston 2010). Soil pH can directly influence bacteria across spatial scales (rhizosphere to continents) and ecosystems by changing biochemical structures and indirectly by altering nutrient solubility (Fierer & Jackson 2006). Furthermore, cultivation can alter soil pH, often by increasing soil acidity (Murphy et al. 2006). Soil pH was significantly lower in the Restoration Experiment than Remnant Prairie and initially an important correlate with bacterial composition for both sites (Appendices S4 and S5). This suggests that prior cultivation could have altered soil pH in the Restoration Experiment, potentially contributing to the distinct bacterial communities between the two sites.

The significant spatial effects found in the Restoration Experiment suggest that bacterial communities were partly influenced by spatial processes acting independent of the environment. For instance, dispersal limitation has been found to explain bacterial community variation in a wide range of systems and could allow for ecological drift of bacterial communities throughout the old-field (Alexander et al. 2012). Over ecological time-scales, restricted movements of bacterial cells could create patches in community composition that are further developed by demographic stochasticity, leading to the spatial variation observed (Martiny et al. 2011). Spatial structure arising from such processes could further be influenced through priority and legacy effects (Fukami et al. 2005). It is important to note that the significant spatial effects found could also be due to unmeasured environmental variables that were spatially structured.

In addition to the significant metacommunity patterns found, the Restoration Experiment had 67.8% unexplained variation in bacterial composition. Potential reasons for this include: error associated with measuring both the soil variables and assessing

the bacterial communities, an unmeasured soil variable that was correlated with the measured soil variables and simultaneously not spatially structured and/or any stochastic effects that were not spatially structured.

As of the sixth year of the Restoration Experiment, variance partitioning analyses showed no correlation between plant and bacterial communities. This result is further demonstrated in two ways. First, no changes in bacterial composition were observed in response to direct experimental manipulation of plant composition and biomass. Second, plant composition among the Control, Restored, and DLP quadrats differed greatly (Appendix S3). If there were direct impacts of plant restoration, bacterial composition should have also differed among all three "treatments." Although this comparison is qualitative, it provides support for our result that plant restoration did not impact bacterial communities.

Bacterial communities may not have responded to plant restoration potentially due to a lack of time. Restoring native plant communities can significantly increase primary production, both above and belowground (Foster et al. 2007). Over the long term, these inputs can improve soil quality, leading to altered bacterial communities (Ettema & Wardle 2002; Allison et al. 2005; Bach et al. 2010). Six years of tallgrass prairie restoration may not have been long enough to "erase" the previous plant communities' legacies in the Restoration Experiment (Card & Quideau 2010). For example, Potthoff et al. (2006) found similar microbial communities when comparing an annual grassland (>60 years) to restored perennial grassland (4 years) in California. Four years of plant restoration may not have allowed time for further build-up of carbon inputs into this system, and did not alter the bacterial communities that had been under permanent grassland for several decades.

Furthermore, there is evidence that restoration age can impact how similar soil microbial communities are between restored and native remnant sites (Allison et al. 2005). Bach et al. (2010) found that microbial communities from older tallgrass prairie restorations (12–18 years) were more similar to native tallgrass prairie than newer restorations (2–12 years). They estimated 30–40 years was needed for microbial communities to be analogous to native tallgrass prairie, potentially due to the time required for further soil-building processes to occur. In this study, the small amount of time since restoration could help explain the similar bacterial communities observed in the Restoration Experiment and why bacterial communities in the Restored quadrats did not approach those of the Reference Prairie.

Several site-specific factors could also be responsible for the different bacterial communities observed between the two sites. For example, recent work has shown that fire can induce changes in microbial community structure as long as 11 years after burning (Xiang et al. 2014). The historical burning of the Dogleg Prairie could have contributed to the disparate bacterial communities. Furthermore, different bacterial communities could arise from the mere fact that they were spatially separated: communities in close proximity tend to be more similar than those located further apart (Horner-Devine et al. 2004).

We found that both niche-based and spatial processes may be important for soil bacterial metacommunity structure. These findings support the recent rethinking of microbial community assembly: not only are microbes influenced by environmental conditions but also by pure spatial effects. Furthermore, our study highlights the importance of considering agricultural legacies for understanding bacterial metacommunities. Continued exploration of bacterial metacommunity dynamics from both native habitats and those with a cultivation history would provide more insight into the generality of our results. Assuming that the build-up of litter inputs is an influence of the plant community upon bacterial communities, time may be required for the previous plant communities' legacies to be replaced by the plant restorations'. Restoring plant communities may not necessarily translate into restored soil bacterial communities over the short term, but will likely take time 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 quality.

Implications for Practice

- To aid the restoration of soil microbial communities, attention should be given to restoring key soil traits such as pH and texture.
- Managing for spatial effects will not be straightforward until more is known about the specific spatial processes acting upon the microbial communities.
- Management should be aware that restoration of soil microbial communities can take several decades to occur despite the restoration of aboveground communities and productivities.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed description of the soil sampling methods and results used within the Restoration Experiment.

Appendix S2. Detailed description of the terminal restriction fragment length polymorphism (T-RFLP) technique used to assess the soil bacterial communities.

Appendix S3. Description of the analyses used to compare plant species communities among the Control treatment, Restored treatment, and Reference Prairie followed by a description of the results.

Appendix S4. Description of the Reference Prairie soil bacteria metacommunity analyses and results.