ILDNR Wildlife Preservation Fund Grant #13-024W
The role of soil microbial communities in regulating the success of prairie restorations

Final Report

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Project objectives:

1) Determine whether soil microbial communities constrain the success of plants in prairie restoration. This will be assessed by determining the growth responses of native species (two grasses and two forbs). As grasses generally succeed at the expense of forbs in prairie restoration, both life forms will be tested to determine whether they are responsive to changes in soil microbial communities. These results will directly come from the soil inoculation experiments.

2) Link the influence of soil microbial communities to the age of restoration. Plant species may respond differentially to local soil communities, but not respond systematically to the gradient from young restoration to prairie remnant. Results from (1) above will be related to the age of the restoration via regression analyses to specifically link microbial responses to plant community development. This will be done for each species separately to understand the range of species' responses.

3) Determine the utility of microbial communities as a management tool for prairie restoration. To be effective as a restoration tool, microbial communities of older restorations must 1) systematically reduce the performance of native prairie grasses and 2) have minimal negative, or beneficial effects on native prairie forbs. In this way microbial communities from older restorations may be used to shift dominance away from aggressive native grasses.

The completion of this project will provide initial evidence of whether soil microbial communities can be used as a prairie restoration tool to generate more diverse prairies. If the experimental evidence is promising, this work may be followed by experimental soil transfer in prairie restorations from remnant or established restorations into new restoration sites to determine their effectiveness in altering species performance and community structure.

Project description:

While grasslands were once a dominant part of Illinois' landscape, the vast majority of prairie lands have been converted to agriculture. Restoration practices aim to recreate functional grasslands to restore both the plant and animal species that reside within these valuable systems and increase their potential habitat. However, restored prairies often do not reflect the diversity and structure seen in remnant prairies - specifically restorations often become dominated by one or more species whereas remnants lack this dominance. Dominance typically decreases with the age of the restoration, more closely approximating the structure of prairie remnants. This project will specifically test whether soil microbial communities regulate the growth and performance of potential dominant grasses in prairie restorations by examining a gradient of restoration ages and remnant prairies. To do this, plants will be experimentally inoculated with microbial communities from restorations of various ages and remnant prairies. If soil microbial communities are important in regulating the dominance of grasses in restoration, we would expect inoculates from older and remnant prairies to be more inhibitory to grasses, whereas microbial communities from young restorations should be more beneficial to grasses, leading to the shift in dominance. Manipulating microbial communities through controlled inoculations or cultural conditions may be an important tool for restoration of diverse and functional prairies

Summary of Project Accomplishments

INTRODUCTION:

Ecology has recently developed an appreciation for the strong impacts that soil microbial communities can exert on plant communities (Bever 2003a, Reynolds et al. 2003). Soil microbial communities may alter competitive hierarchies, determine the abundance of plant species within the community, and their spatial patterning (Klironomos 2002, Bever 2003b, Klironomos 2003, Packer and Clay 2003, Reinhart et al. 2003, Reynolds et al. 2006). Interactions with soil biota may also be important in understanding plant species invasions (Kourtev et al. 2002, Callaway et al. 2003, Lankau 2010, Lankau 2011).

The soil microbial community is a complex assemblage composed of beneficial and antagonistic bacteria, fungi and other organisms (Bever 2003b, Reynolds et al. 2003), the net effect of which may generate positive, neutral or negative effects on a plant species' abundance and shift plant community composition. Microbial communities may also vary spatially and in response to plant species (Kourtev et al. 2002, Zhang et al. 2007). The potential for soil microbes to influence restoration is just now being explored and could be a valuable tool in the restorationist's kit. To date, it is not clear if soil microbial communities are a driver of or a result of restoration activities (Harris 2009). Restoration efforts or other changes in land management may result in changes in in the soil microbial community (Harris 2003, Allison et al. 2005, Smith et al. 2008, Harris 2009). However, in one experimental test, inoculation of soil microbes clearly enhanced the growth of desirable plant species (Middleton and Bever 2012).

Prairie restorations often begin on former agricultural land which has altered soil fertility and soil structure. In addition, former agricultural practices may have shifted the microbial community towards more weedy species that benefit under agricultural conditions (Allison et al. 2005, Smith et al. 2008).

These microbial communities may favor more aggressive species such as C_4 grasses that are similar physiologically to cultured species such as corn. These altered and depauperate soil microbial communities may therefore limit the successful restoration of prairies by favoring dominant matrix species at the expense of forbs, where the majority of prairie diversity lies.

To understand the role of feedbacks from microbial communities in prairie restoration we looked at the performance of two dominant, C_4 grasses and two less abundant prairie forbs (including one legume) in soils from a chronosequnce of prairie restoration. Our goal was to determine whether the dominance of grasses in prairie restorations on the study site was due to species interactions with the soil microbial community. This experiment was conducted to specifically address the following questions: 1) Is there spatial variability in the strength or direction of soil microbial effects on plant performance? 2) Do dominant grass species differ from less abundant forbs in the strength or direction of soil microbial effects? and 3) Is the response of individual species to soil microbial communities related to the restoration chronosequence? The overarching goal of this work was to understand the effect of microbial communities have on prairie restoration and their potential as a restoration tool.

METHODS:

Study site and species – Seed and soil samples for this study were collected from the Richardson Wildlife Foundation (RWF) site in West Brooklyn, IL (X 318252.845105 Y 4620598.215119). This site contains a mosaic of remnant and restored prairies of various ages as well as agricultural areas. The primary prairie remnant is approximately 15ha with several smaller fragments and have been actively managed since the 1970s. Restored prairies of various ages cover an additional 283ha. The history of the remnant prairies includes invasions of trees, mostly willow (*Salix* spp.), and some grazing, prior to protection. Though the remnants were never plowed, the restored areas were largely former agricultural fields. All prairie areas are burned every 3 years in sections.

We selected four species from the site for study. These are the warm-season, C_4 grasses *Andropogon gerardii* (Big bluestem) and *Sorghastrum nutans* (Indian grass), and the forbs *Baptisia leucantha* (White wild indigo, a legume) and *Silphium terebinthinaceum* (prairie dock). These species were selected because they are regionally common components of prairie restorations, and represent the gradient of restoration performance at the site. Neither grass species are planted during prairie restoration, but quickly come to dominate younger restorations. In contrast, the forbs appear slow to establish and flower at the site (J. B. Towey, *personal communication*). All seeds were collected from the RWF property to ensure the appropriateness of the plant-microbe interactions and were stratified as necessary.

Experimental design –We selected 8 different sites at RWF, two of each from fields currently in agriculture (following soybeans and corn), young (3 and 5 y) restorations, old (22 and 28 y) restorations, and remnant prairies. To minimize variation due to soils, we selected locations within each sites that all occurred on the same soil type (Hoopeston fine sandy loam, nearly level and somewhat poorly drained). On 15 February 2013, while the soil microbial community was dormant, 6 soil cores were taken randomly from each site to a depth of 10cm

using a 7cm diameter soil auger. Samples were put in sterile bags and placed on ice during the transport back to the lab and then refrigerated until processed. All sampling equipment was sterilized with a 10% bleach solution between sites. Each sample was processed with a 1.4mm mesh sieve to remove roots and other debris. Samples were then pooled within each site to ensure an even soil inocula. Half of the pooled sample from each site was autoclaved to sterilize the microbial communities. For inoculation, 10 ml of either live or sterilized soil was mixed into the upper 4 cm of a cone-tainer (Stuewe & Sons, Inc., OR) partially filled with sterile potting material. To minimize contamination of across treatments, the inoculum layer was covered with 3 cm of sterile potting mix. This also allowed seedlings to grow through the inoculum layer for colonization (Kardol et al. 2007).

Seedlings were started in the greenhouse on sterile potting mix. After the cone-tainers had been inoculated, similar sized seedlings were transplanted into the experimental treatments. There were 20 replicates of each treatment (8 sites × 4 species × 2 soil sterilization) and therefore 1280 seedlings overall. Each site and treatment was placed in its own rack and location to further minimize the chance of cross contamination. Plants that died within the first week were replaced with similar sized transplants. Plants were watered regularly and monitored for growth and disease. Plant height and flowing buds were recorded throughout the growing period. After 60 days they were harvested, dried and weighed.

ANOVA was used to determine the overall impacts of microbial communities and site age on plant performance. Plants were analyzed between species, inoculate groups, and treatments. Linear and non-linear regression analyses were used to compare responses among species and location.

Measurement of microbial interactions – Plant performance provides an indirect measure of shifts in the soil microbial community during restoration. To link plant performance with the presence of mutualists and provide a direct test of whether microbial communities/activity change during restoration, we also quantified mutualists on plant roots. When the above experiment was harvested, root tissues were collected and preserved to quantify mycorrhizal colonization (all species) and the formation of root nodules (*Baptisia* only). All *Baptisia* roots were cleaned and examined to determine the density of nodules per unit root length and total dry mass of nodules. The analysis of mycorrhizal colonization on a subset of individuals is ongoing. Both measures of microbial interactions are beyond the initial scope of the project.

RESULTS:

All species responded to soil sterilization and the restoration chronosequence (Table 1.) Both grass species responded to soil sterilization with microbial inhibition occurring in the remnant site soils. Between the two grass species *Sorghastrum nutans* experienced stronger inhibitory effects of the soil microbial community than *Andropogon gerardii*. *Sorghastrum nutans* had a strong effect of soil type, soil sterilization and their interaction (Figure 1). This species responded similarly to both dead and live agriculture site soils, with the live soil being

slightly beneficial. There was a slight decrease in biomass from the agricultural sites to the young and to the old restored sites then a slight increase in biomass in the remnant soils. In all three prairie types, the sterilized soil produced more biomass than the live. A similar yet, more complex pattern was seen in the later successional grass species, *A. gerardii.* This species had strong soil type and site by type interaction (Figure 1; Table 1). Again, the most biomass was produced in the agricultural sites with the sterilized soil having slightly more growth. The restoration chronosequence exhibited a decreasing trend in biomass. In both young and old remnant sites, live soil produced more biomass than sterilized soil; this trend reversed in the remnants where the sterilized soil produced twice the biomass of the live soil.

Forbs, in contrast to the grasses, exhibited fewer negative impacts of the soil microbial community, with less supression of growth and no real pattern across the chronosequence. In *Silphium terebinthinaceum,* a similar amounts of biomass were produced across the chronosequence gradient (Table 1) and soil sterilization had no overall effect. There was, hover, a strong interaction between soil sterilization and chronosequence position. Live soil was slightly beneficial to plant growth in the agricultural and remnant sites whereas it was slightly suppressive in the young and old restored sites (Figure 1). There was a dramatically different pattern in the legume *Baptisia leucantha,* where all ANOVA terms were significant (Table 1). Live soils strongly promoted biomass growth in all stages of restoration, with the greatest benefit to growth occurring in soils from young restorations (Figure 1). Live remnant soils produced the least benefit to *B. leucantha* growth.

Looking across sites, we tend to the find the strongest microbial inhibition (or least benefit) to growth in the remnant or old restoration soils. Similarly, we found that agricultural or young remnant soils produced the least inhibitory or greatest beneficial effects on plant growth. However, patterns of plant performance varied among species so that responses to individual sites' soils were not correlated (All $P > 0.05$).

The formation of root nodules also varied across the restoration chronosequence, but not along a single trajectory (Figure 2). Over 50% of plants formed nodules when grown in live agricultural soils, peaked in the young remnants, and then declined dramatically into remnant soils. The impacts of root nodule formation on plant performance also changed with chronosequence position. Plants with root nodules grew larger in agricultural and remnant soils, as would be expected. However, the growth of plants with and without nodules was nearly identical in remnant soils.

DISCUSSION:

Even though sites were selected based on similarity of soil and topographic structure we saw variation due to chemical and physical differences in the soil. The two grass species did the best in the agricultural inoculates. These microbial communities favored more aggressive species such as C_4 grasses that are similar physiologically to cultured species such as corn. The decline in grass performance in soils of later stages of the chronosequence indicates the microbial community shifting from being beneficial to grasses in the early stages to inhibiting grasses in

the later stages. Restored prairies may become dominated by grasses because the altered soil microbial communities that remain following agriculture favor dominant matrix grasses at the expense of forbs. The initial dominance of grasses favored by the soil microbial community then has the potential to persist for long periods, based on the life span of the individuals. As the majority of prairie diversity lies in forbs, this dominance could be combatted by introducing grasses later in the restoration process. Negative biofeedbacks develop very quickly following restoration, which would slow the initial spread of grasses. Alternatively, manipulating microbial communities through controlled inoculations or cultural conditions may be an important tool for restoration of diverse and functional prairies.

The target of a successful prairie restoration is often forb diversity. This project provides evidence that soil microbial communities can be used as a prairie restoration tool to generate forb diversity in addition to reducing grass abundance. However, species responded differently to microbial communities from different sites. *Silphium* growth was largely unresponsive to the restoration chronosquence. *Baptisia*, a legume that forms nitrogen-fixing nodules with bacteria in the soil, performed the worst in agricultural soils were nodule inducing bacteria was low. The reduction of the benefits of root nodules to *Baptisia* growth also provides an argument for increased negative feedback late in restoration. Microbial communities became more antagonistic later in the chronosequence, which would promote diversity and coexistence among forbs. Variation among species and the site-specific nature of feedbacks are likely an important source of heterogeneity in remnant prairies as well as restorations.

SUMMARY:

The overall pattern for this experiment shows strong site variability, representing patchiness in microbe interactions, though older soils were consistently having the strongest inhibitory effect. Our results provide evidence that microbial communities have potential as a potential prairie restoration tool. This work could be followed by experimental soil transfer from prairie remnants or long-established restorations into new restoration sites to determine their effectiveness in altering species performance and community structure. It may also be possible to inoculate species before installation to increase their establishment, survival and performance in restoration settings.

Table 1. Nested ANOVA analysis of species responses to soil microbial communities and position along a restoration chronosequence. Site identity was nested within age to account for variation between the two sites at each age.

Model term	df	MS	*F*	*P*	R^2
Sorghastrum nutans					0.183
Site	4	29179.43	1.33	0.2597	
Age	3	293685.87	13.36	<0.0001	
Soil	1	187786.11	8.54	0.0037	
Soil × Age	3	59331.26	2.70	0.0460	
Error	280	21976.16			
					0.249
Andropogon gerardii.					
Site	4	31021.20	2.16	0.0734	
Age	3	267600.42	18.64	<0.0001	
Soil	1	10797.47	0.75	0.3865	
Soil × Age	3	160246.72	11.16	<0.0001	
Error	300	14358.22			
Silphium terebinthinaceum					0.081
Site	4	5929.49	0.68	0.6031	
Age	3	26065.44	3.01	0.0305	
Soil	1	5389.21	0.62	0.4309	
Soil × Age	3	42072.77	4.86	0.0026	
Error	305	8661.83			
Baptisia leucantha					0.435
Site	4	79549.79	5.22	0.0005	
Age	3	244721.46	16.05	<0.0001	
Soil	1	2048312.94	134.31	<0.0001	
Soil × Age	3	130269.05	8.54	<0.0001	
Error	289	15250.95			

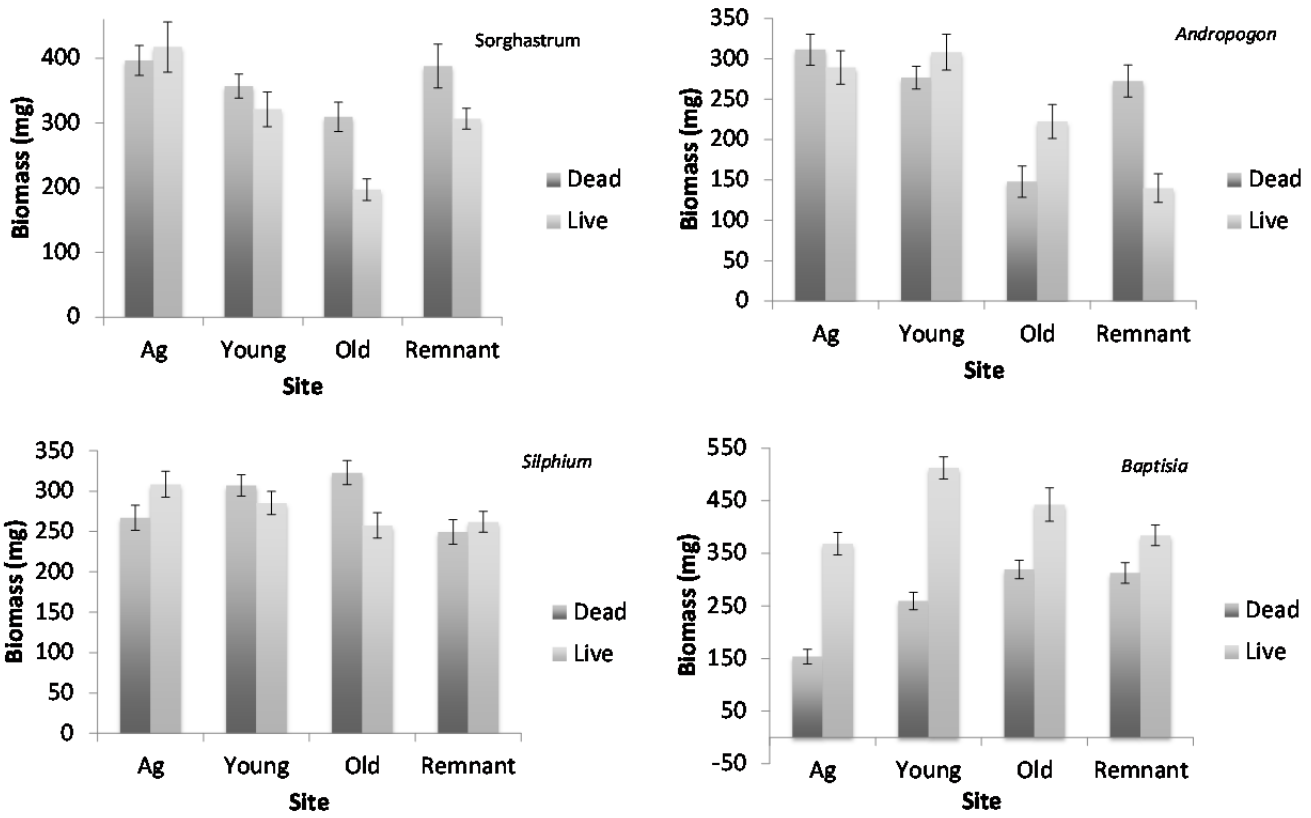

Figure 1. Interactions of four prairie species with soil microbial communities along a chronosequence of prairie restoration from current agriculture field (Ag) to undisturbed remnants.

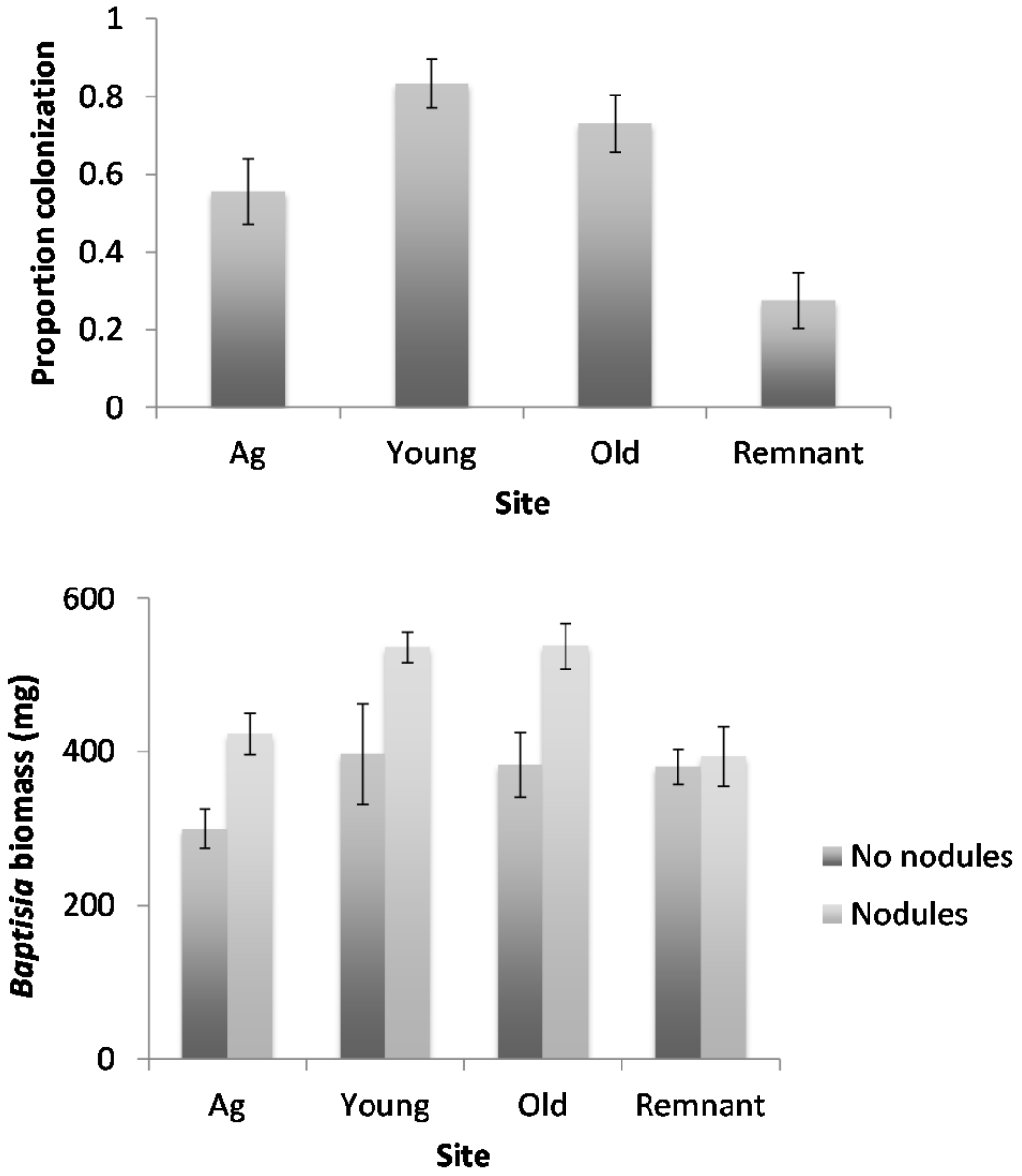

Figure 2. (top) Proportion of *Baptisia* seedlings which had formed root nodules. (bottom) Effect of root nodules on the growth of *Baptisia* seedlings across the restoration chronosequence. Data are from non-sterilized soils only.

PHOTO CAPTIONS:

1 Setup – View of most of the experiment in the greenhouse before the seedlings were transplanted to show the size of the experiment.

2 Baptisia trial – long view of the last species to be harvested. Sterilized (dead) soils are in the foreground and live soil inoculates are in the back. Plants which have formed root nodules are visibly greener.

3 Baptisia root with nodules – close up of a cluster of nodules formed from inoculation by live soil from one of the remnants.

4 Baptisia live young restoration 6. View of *Baptisia* plants that had been inoculated with live soil from one of the young restoration sites (site 6). Photo taken at harvest.

5 Baptisia dead young restoration 6 View of *Baptisia* plants that had been inoculated with sterilized (dead) soil from one of the young restoration sites (site 6). Compare with photo 4 (live soil from the same site) to see the influence of live soil on plant health. These plants are much more chlorotic and grew less overall than plants in live soil. Photo taken at harvest.

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