

RESEARCH ARTICLE

Plant–microbe interactions change along a tallgrass prairie restoration chronosequence

Anna J. Herzberger¹, Scott J. Meiners^{1,2}, J. Brian Towey³, Paula A. Butts¹, Daniel L. Armstrong¹

Soil microbial communities are critical in determining the performance and density of species in plant communities. However, their role in regulating the success of restorations is much less clear. This study assessed the ability of soil microbial communities to regulate the growth and performance of two potentially dominant grasses and two common forbs in tallgrass prairie restorations. Specifically, we examined the effects of soil microbial communities along a restoration chronosequence from agricultural fields to remnant prairies using experimentally inoculated soils. The two grass species, *Andropogon gerardii* and *Sorghastrum nutans*, grew best with the agricultural inoculates and experienced a decline in performance in later stages of the chronosequence, indicating that the microbial community shifted from being beneficial to grasses in the early stages to inhibiting grasses in the later stages of restoration. Growth of the forb, *Silphium terebinthinaceum*, varied little with inoculation or position along the restoration chronosequence. Growth of *Baptisia leucantha*, a legume, appeared limited by nodule formation in agricultural soils, peaked in young restoration soils along with nodule formation, but decreased in older soils as the microbial community became more antagonistic. Overall, negative feedbacks tended to be less important early in restoration, but appeared important in remnant and older restored prairies. Our results provide evidence that it may be advantageous for management practices to take negative soil feedbacks into consideration when trying to recreate the diversity of tallgrass prairies.

Key words: C₄ grasses, grassland, legumes, negative feedbacks, root nodules, soil microbial communities

Implications for Practice

- Soil microbial communities could be a potential prairie restoration tool: when restoring a prairie, inoculates containing microbial communities from remnants or long established restorations could set up negative feedbacks that would prevent grass dominance and promote forb diversity.
- The development of negative feedbacks in grasses over time suggests that delaying the introduction of grasses until later in the restoration process may limit grass dominance and their competitive suppression of forbs.

Introduction

Ecology has historically given little attention to the interactions of soil microbial communities with plant communities, particularly within the context of restoration. The high diversity of soil microbes provides a significant research challenge as these communities contain both beneficial and antagonistic organisms in the form of an interacting suite of bacteria, mutualistic and pathogenic fungi, nematodes, and other organisms (Bever 2003; Reynolds et al. 2003; Middleton & Bever 2012; Padilla et al. 2012; Sikes et al. 2012). However, microbial community composition is critical to the development, abundance, and diversity of the aboveground plant community. Plant species differ widely in response to individual microbial species, with positive and

negative effects often being host specific, and the net microbial community effect impacting plant performance (van der Heijden et al. 2008; Bever et al. 2010). Methodologically, microbes are often considered an extension of the plant or are experimentally eliminated by using sterile soil mixes that contain nutrients sufficient to reduce the influence of communities already present (Reynolds et al. 2003). Recent studies have documented that the effects of microbial communities can dramatically control plant performance, generating patterns of abundance, diversity, and coexistence in plant communities (Reinhart 2012; Sikes et al. 2012; Hodge & Fitter 2013).

Plant interactions with the soil microbial community can be either direct or indirect and lead to net negative or positive feedbacks (Bever et al. 2010). These net interactions can facilitate or inhibit further growth of both the plant community and the soil microbial community (Kardol et al. 2007; Sikes et al. 2012). Plant–soil feedbacks are generated by plants inducing changes in the composition of their soil microbial community, which then affects plant performance (Bever 2003; Bever

Author contributions: AH, SM, JT conceived and designed the research; JT provided soil and seed materials; AH, PB, DA conducted the experiment; AH, SM wrote and edited the manuscript.

¹Department of Biological Sciences, Eastern Illinois University, Charleston, IL 61920, U.S.A.

²Address correspondence to S. J. Meiners, email sjmeiners@eiu.edu

³Richardson Wildlife Foundation, West Brooklynn, IL, U.S.A.

et al. 2010). If changes in soil biota increase performance relative to other plants, the positive feedback may increase abundance, maintain dominance and slow successional replacement (Reynolds et al. 2003; Kardol et al. 2007; Faber & Markham 2012). Beneficial microbes such as nitrogen fixing bacteria and mycorrhizal fungi can directly enhance plant fitness by allowing greater access to mineral resources (Allen & Allen 1984; Smith et al. 1998; Kardol et al. 2007; Bever et al. 2010; Fitzsimons & Miller 2010; Hodge & Fitter 2013). Microbial communities that decrease plant performance generate negative feedbacks that reduce species abundance and favor plant coexistence and diversity or may lead to successional replacement. (Janzen 1970; Connell 1971; Mills & Bever 1998; Kardol et al. 2007; Petermann et al. 2008; Fitzsimons & Miller 2010). Although individual plant–microbial community interactions will be positive or negative, the structure and dynamics of entire plant communities can be influenced by negative and positive feedbacks across species (Bever et al. 2010).

Microbial community composition is context dependent (Shannon et al. 2012) and can be altered by a number of local environmental factors (Hodge & Fitter 2013). A major anthropogenic activity that severely alters microbial communities is agricultural disturbance. The mechanical disruption of soil structure through plowing, alteration of nutrient dynamics via chemical inputs, and the maintenance of plant monocultures leads to a disturbed microbial community (Middleton & Bever 2012; Hansen & Gibson 2013). In the Midwestern United States, attempts to restore croplands to tallgrass prairie often lead to mediocre results that may be the result of a depauperate microbial community that lacks the negative feedbacks characteristic of natural systems (Anderson 2008; Fitzsimons & Miller 2010). Restored prairies typically fall short of prairie remnants in both plant species diversity and structure (Beyhaut et al. 2014). They often become heavily dominated by C_4 grasses, limiting the establishment of other species (Anderson 2008). Several mechanisms have been proposed to explain the dominance of grasses in many prairie restorations, including initial planting density, degraded native seed banks, the timing of management fires that enhance C_4 plant growth, the absence of grazing animals, residual fertility from agricultural amendments, and the lack of established microbial feedbacks (Collins et al. 1998; Anderson 2008; Fitzsimons & Miller 2010; McCain et al. 2010; Goldblum et al. 2013). Species that are fast to establish in restoration because of their associations with microbial communities may compete strongly with other native species, slowing their establishment and reducing the diversity and therefore success of the restoration (Anderson 2008). As diversity within a restored prairie is critical to the wide breadth of ecological services they provide (Fitzsimons & Miller 2010; Goldblum et al. 2013), proper restoration and management practices are critical to generating fully functional communities.

To understand the role of feedbacks from soil 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 prairie restoration chronosequence. This experiment was conducted to specifically

address the following questions: (1) Does the net impact of the microbial feedbacks on plant performance change along a restoration gradient? (2) Do differences in microbial response between grasses and forbs explain early dominance by grasses in restoration? (3) How does the presence of nitrogen fixing bacteria alter legume response to the microbial community along the restoration chronosequence?

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, U.S.A. (41.709°N, 89.187°W). This site contains a mosaic of remnant and restored tallgrass prairies of various ages as well as agricultural areas. The primary prairie remnant is approximately 15 ha with several smaller fragments that have been actively managed since the 1970s. Restored prairies of various ages cover an additional 283 ha. The history of the remnant prairies includes invasions of trees, mostly willow (*Salix* spp.) and some grazing, prior to protection. Although the remnants were never plowed, the restored areas were largely former agricultural fields. All prairie areas are burned every 3 years in sections. Although older restorations at the site included grasses as part of the seed mix, C_4 species such as *Andropogon gerardii* (big bluestem) and *Sorghastrum nutans* (indian grass) are no longer planted during 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 observation).

We selected four species from the site for study. These were the warm-season C_4 grasses, *A. gerardii* and *S. nutans*, 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 tallgrass prairie restorations and perform differently in restorations at the site. The grasses tend to be very aggressive in restorations, whereas both forbs are much slower to establish. Seeds and soil communities were collected from the RWF property to encompass any local variation in plant–microbe interactions. All seeds were collected at the end of the previous growing season and stored dry at 4°C before usage. *Silphium terebinthinaceum* was cold-moist stratified at 4°C for 60 days and *B. leucantha* for 10 days following scarification with sandpaper to break dormancy.

Experimental Design

We employed a soil sterilization approach to determining the net impacts of soil microbial communities of different restoration ages on plant performance. In this method, plants are inoculated with either live or sterilized samples from a study site and the difference in plant growth between the two treatments reflects the net impact of the soil microbes. Although this methodology can assess net impacts, the actual composition of the soil

microbial community remains unknown. Genetic or lipid profiling (e.g. Kardol et al. 2007; Pendergast et al. 2013) can be used to document variation in the composition of soil microbes, but given the species-specific nature of plant–microbe interactions (Klironomos 2003), composition does not necessarily translate to function. We chose to focus on the entire soil microbial community as it reflects the complete suite of interactions that a plant in a restoration could experience. Patterns found with this broad approach could then justify more complex and detailed analysis of microbial communities. To minimize the effect of sterilization on nutrient availability, studies typically use small amounts of soil inocula relative to the total soil volume (e.g. Pendergast et al. 2013). Following this, we used 10 mL inocula, representing approximately 6% of the experimental soil volume.

We selected eight different sites at RWF, two of each from four age classes along a tallgrass restoration chronosequence: fields currently in agriculture (following soybeans and corn), young (3 and 5 years) restorations, old (22 and 28 years) restorations, and remnant prairies. On 15 February 2013, while the soil was frozen, six soil cores were taken randomly from each site to a depth of 10 cm using a 7-cm diameter soil auger. To minimize variation due to soils, we selected locations within each site that occurred on the same soil type (Hoopston fine sandy loam, nearly level and somewhat poorly drained). Samples were put in sterile bags and placed on ice during transport back to the lab and refrigerated until processed. All sampling equipment was sterilized with a 10% bleach solution between sites. Each sample was processed with a 1.4-mm mesh sieve to remove roots and other debris. Samples were then pooled within each site to ensure an even soil inoculant. 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, Tangent, OR, U.S.A.) partially filled with sterile potting material (Fafard 2, Sun Gro Horticulture, Agawam, MA, U.S.A.). The inoculum layer was covered with 3 cm of the sterile potting mix, which minimized contamination across treatments and allowed seedlings to grow through the inoculum layer for colonization (Kardol et al. 2007).

In late February, large numbers of seeds were started in the greenhouse on the sterile potting mix 1–2 weeks prior to transplantation to ensure similar age and size of plants used in the experiment. After the cone-tainers had been inoculated, similar-sized seedlings were transplanted into the experimental treatments. There were three treatment types (chronosequence position [4, with 2 sites in each] × species [4] × soil sterilization [2]), each with 20 replicates of for a total of 1,280 plants. 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. After 60 days, aboveground tissues were harvested, dried, and weighed. Analyses of variance (ANOVA) were used to determine the impact of microbial communities and chronosequence position on aboveground biomass (PROC GLM; SAS Institute, Inc., Cary, NC, U.S.A.). In all analyses, site identity nested within chronosequence position was included to account for variation between chronosequence

replicates. ANOVAs were conducted for each target species including soil sterilization, chronosequence position (age), and their interaction as explanatory variables to determine individual species' responses. In addition, a pooled model that included species identity and its interactions was run to determine overall effects of soil sterilization along the restoration chronosequence. Biomass data did not require transformation in any of these analyses.

Formation of Root Nodules

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 nodules on *Baptisia* roots. During harvest, *Baptisia* root tissues were collected, cleaned, and examined with the aid of a dissecting microscope to determine whether the plant was colonized (binary) and the total number of nodules present. The dry mass of all nodules was also measured, but preliminary analyses found this to be redundant with nodule number. Plant colonization and nodule number data were analyzed with a chi-square test and ANOVA, respectively. To assess how the benefits of nodule formation changed along the chronosequence, *Baptisia* biomass was compared between colonized and uncolonized plants (live soils only) in a nested ANOVA design as described above.

Results

The model that included all four species found all factors and all interactions to be significant predictors of aboveground plant biomass in this experiment (Table 1). Overall, the chronosequence separated into young sites (agricultural fields and young restorations) and older sites (old restorations and remnants) with statistical significance between the two (Fig. 1). The net effects of the soil community on plant biomass shifted from overall positive early in the chronosequence, to neutral (old restorations) or slightly negative (remnants). When examined individually, all species responded to both soil sterilization and the restoration chronosequence (Table 2) varying from the overall pattern across species.

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 chronosequence position, soil sterilization, and their interaction (Fig. 2A). This species responded similarly to both dead and live agriculture site soils, with the live soil being slightly beneficial. 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 (Fig. 2B; Table 2). Again, the most biomass was produced in

Table 1. Aboveground biomass response to chronosequence position (age) and soil microbial communities (sterilization) pooling across all species. ANOVA model with site nested within chronosequence position

| Model Term | df | Mean square | F | p | R ² |
|-------------------------------|----|-------------|-------|---------|----------------|
| Site (age) | 4 | 41,496 | 2.75 | 0.0272 | 0.309 |
| Age | 3 | 163,748 | 10.83 | <0.0001 | |
| Sterilization | 1 | 166,974 | 11.05 | 0.0009 | |
| Species | 3 | 658,616 | 43.57 | <0.0001 | |
| Age × Sterilization | 3 | 156,186 | 10.33 | <0.0001 | |
| Age × Species | 9 | 222,576 | 14.73 | <0.0001 | |
| Sterilization × Species | 3 | 704,303 | 46.59 | <0.0001 | |
| Age × Species × Sterilization | 9 | 78,151 | 5.17 | <0.0001 | |

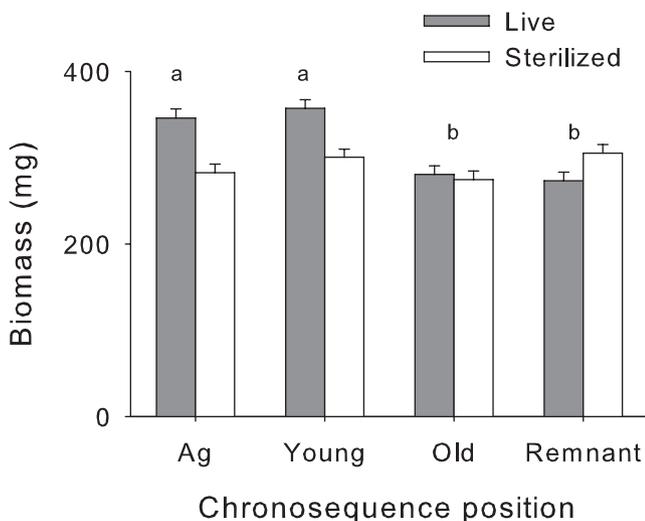


Figure 1. Aboveground biomass (mg) responses to live and sterilized soil along the restoration chronosequence for all species pooled together. Data plotted are least square means (\pm SE) from the analysis presented in Table 1. Different letters represent statistical significance between chronosequence positions from a Tukey post hoc test.

the agricultural sites with the sterilized soil having slightly more growth. 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 suppression of growth and no real pattern across the chronosequence. In *Silphium terebinthinaceum*, similar amounts of biomass were produced across the chronosequence gradient (Table 2) and soil sterilization had no overall effect. There was, however, an 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 (Fig. 2C). There was a dramatically different pattern in the legume *Baptisia leucantha*, where all ANOVA terms were significant (Table 2). Live soils promoted growth in all stages of restoration, with the greatest benefit to growth occurring in soils from young restorations

Table 2. Aboveground biomass response of plant species to chronosequence position (age) and soil microbial communities (sterilization). ANOVA model with site nested within chronosequence position

| Model Term | df | Mean square | F | p | R ² |
|----------------------------------|-----|--------------|--------|---------|----------------|
| <i>Sorghastrum nutans</i> | | | | | 0.183 |
| Site (age) | 4 | 29,179.43 | 1.33 | 0.2597 | |
| Age | 3 | 293,685.87 | 13.36 | <0.0001 | |
| Sterilization | 1 | 187,786.11 | 8.54 | 0.0037 | |
| Age × Sterilization | 3 | 59,331.26 | 2.70 | 0.0460 | |
| Error | 280 | 21,976.16 | | | |
| <i>Andropogon gerardii</i> | | | | | 0.249 |
| Site (age) | 4 | 31,021.20 | 2.16 | 0.0734 | |
| Age | 3 | 267,600.42 | 18.64 | <0.0001 | |
| Sterilization | 1 | 10,797.47 | 0.75 | 0.3865 | |
| Age × Sterilization | 3 | 160,246.72 | 11.16 | <0.0001 | |
| Error | 300 | 14,358.22 | | | |
| <i>Silphium terebinthinaceum</i> | | | | | 0.081 |
| Site (age) | 4 | 5929.49 | 0.68 | 0.6031 | |
| Age | 3 | 26,065.44 | 3.01 | 0.0305 | |
| Sterilization | 1 | 5389.21 | 0.62 | 0.4309 | |
| Age × Sterilization | 3 | 42,072.77 | 4.86 | 0.0026 | |
| Error | 305 | 8661.83 | | | |
| <i>Baptisia leucantha</i> | | | | | 0.435 |
| Site (age) | 4 | 79,549.79 | 5.22 | 0.0005 | |
| Age | 3 | 244,721.46 | 16.05 | <0.0001 | |
| Sterilization | 1 | 2,048,312.94 | 134.31 | <0.0001 | |
| Age × Sterilization | 3 | 130,269.05 | 8.54 | <0.0001 | |
| Error | 289 | 15,250.95 | | | |

(Fig. 2D). Live remnant soils produced the least benefit to *B. leucantha* growth.

The proportion of *B. leucantha* plants colonized and the number of nodules produced varied across the chronosequence. Colonization was highest in the restored prairies, intermediate in agricultural soils, and lowest in remnant prairie soils ($\chi^2 = 28.4$, $df = 3$, $p < 0.001$; Fig. 3). The number of nodules formed followed the same pattern ($F_{[3,145]} = 11.42$, $p < 0.0001$). Site identity was not significant in this analysis and was dropped from the model. Growth of *B. leucantha* was always higher in colonized plants, and there was variation with chronosequence position (Fig. 4; Table 3). Although the biomass difference between colonized and uncolonized plants disappeared in remnant soils, there was no interaction between colonization and chronosequence position.

Discussion

We used a restoration chronosequence to represent the temporal dynamics of plant–soil community interactions. Although there are limitations (Pickett 1989; Johnson & Miyanishi 2008), the chronosequence approach has been quite useful in assessing temporal changes in plant communities and soil (Vankat & Snyder 1991; Lawson et al. 1999; Walker et al. 2010). This experimental design allowed us to examine the development of soil microbial communities during restoration to determine if they have the potential to regulate restoration success.

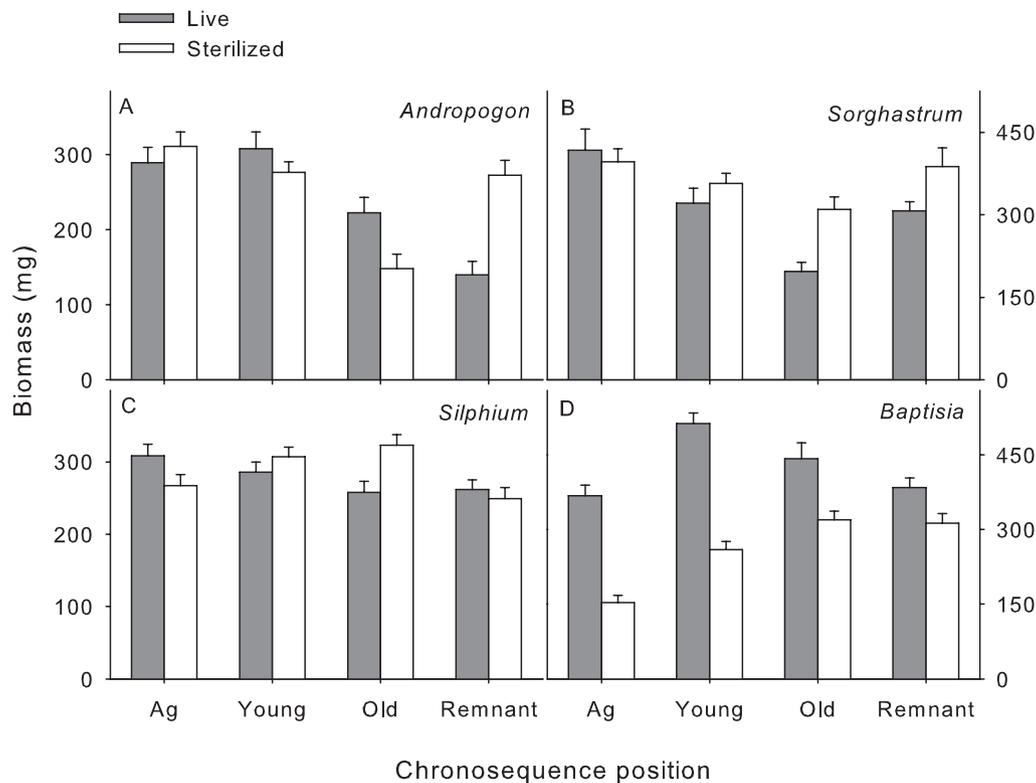


Figure 2. Aboveground biomass (mg) responses to live and sterilized soil along the restoration chronosequence: (A) *Andropogon gerardii*; (B) *Sorghastrum nutans*; (C) *Silphium terebinthinaceum*; (D) *Baptisia leucantha*. Bars are mean \pm SE.

Collection locations were selected based on similarity of soil and topographic structure, and small amounts of inocula were used to minimize variation in the abiotic soil conditions. Despite this, we saw significant site variation in sterilized soils that maybe contributed to chemical and physical differences (Kardol et al. 2007; Anderson 2008). In sterilized controls, we saw similar performance patterns for *Andropogon gerardii* and *Sorghastrum nutans*. Both species did relatively well in the sterile agricultural soils and performance decreased with restoration age. However, biomass in sterile remnant soils rebounded equivalent to the sterile agricultural soils. This pattern suggests that fertility carryover from agricultural application may have influenced growth initially, but that these sources are depleted in time. Remnant sites appeared to have greater organic matter (personal observation) that may have served as a source of additional fertility during the experiment. The two forbs differed slightly in their response to abiotic soil conditions. *Baptisia leucantha* showed a steady increase in growth along the restoration chronosequence, whereas the growth of *S. terebinthinaceum* slightly peaked in sterilized soil from old restored sites. This variation among sites and species could be due to changes in soil characteristics or species-specific interactions (Middleton & Bever 2012), despite the usage of small amounts of soil inocula relative to the volume of fertile potting mix. Shifts in plant performance with abiotic soil properties are not uncommon in such studies (Anderson 2008; Faber & Markham 2012).

Grass Responses to the Microbial Chronosequence

Performance of both grass species was greatest in the agricultural and young restored soils, with little difference between live and sterilized soils. Microbial communities resulted in marked depression of performance in older soils, except that *A. gerardii* growth increased in the live soil communities from old restorations. These differences may partly reflect the successional status of these species. The earlier dominance of restorations by *S. nutans* reflects its fast establishment (Smith et al. 1998; Anderson 2008), which may make it vulnerable to negative feedbacks (Reynolds et al. 2003). *Andropogon gerardii* is typically somewhat slower to establish, and benefited from the microbial community of old restored soils where it would be expected to dominate (Smith et al. 1998; Anderson 2008).

The agricultural and young restored soil microbial communities were less antagonistic to the aggressive C₄ grasses, likely because they are similar physiologically to cultured species such as corn (Anderson 2008; Middleton & Bever 2012). A lack of negative feedback early in prairie restoration would lead to grasses rapidly becoming dominant before stronger negative feedbacks develop. This dominance would likely suppress forb growth and other restoration grasses (Kardol et al. 2007; Anderson 2008). Such temporally restricted opportunities for establishment can be critical as plant–soil feedbacks that develop early in succession can have long-term effects on community assembly and affect future patterns of dominance (Kardol et al. 2007).

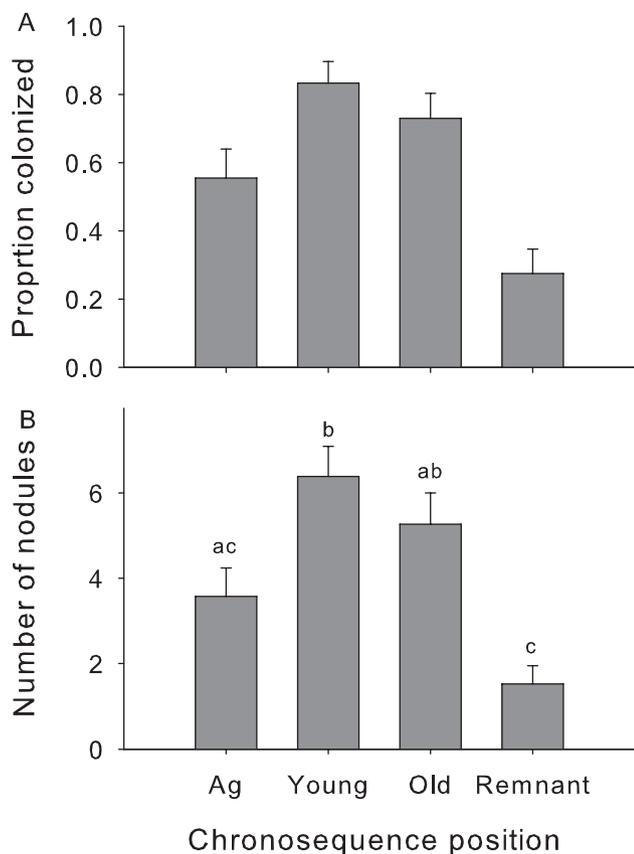


Figure 3. Effects of chronosequence position on the formation of root nodules. (A) Proportion of *Baptisia leucantha* colonized and (B) number of nodules formed. Bars are mean \pm SE. Different letters represent statistical significance between chronosequence positions from a Tukey post hoc test.

The microbially induced decline in grass performance in soils from later stages of the chronosequence indicates the microbial community shifts from being largely benign to grasses in the early stages, to inhibiting grasses in the remnants. Successional development in restoration leads to changes in the microbial community that are responsible for reduced growth of early dominating species (Kardol et al. 2007). The data presented here suggest that restored prairies may become dominated by grasses because the soil microbial communities of post-agricultural restorations initially favor dominant matrix grasses at the expense of forbs. Similar to our findings, Faber and Markham (2012) found differences in the feedbacks associated with remnant and restored prairies. However, the microbial community of remnant sites in that study produced positive feedbacks on *A. gerardii* growth, whereas our live remnant soil inoculates produced negative feedbacks. Carbajo et al. (2011) also found that late successional plants benefit from late successional soil inoculates.

Forb Responses to the Microbial Chronosequence

Tallgrass prairie restoration efforts largely focus on forb diversity, which provides benefits such as increased nutrient retention

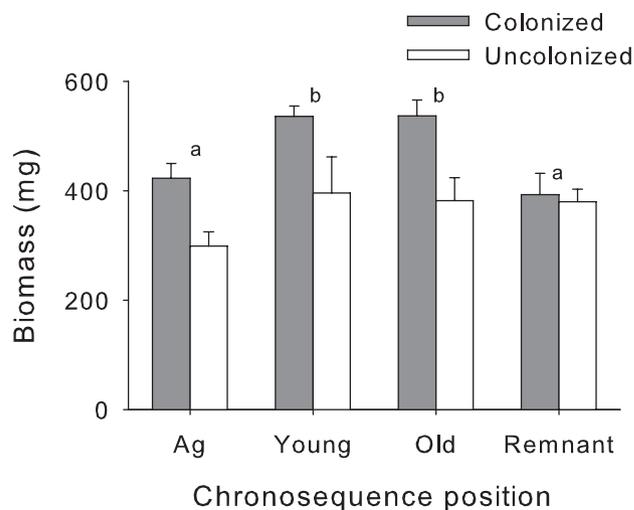


Figure 4. Changes in the benefits of nodule formation to *Baptisia* along the restoration chronosequence. Only data from unsterilized inoculations are included in this analysis. Bars are mean \pm SE of aboveground biomass. Different letters represent statistical significance between chronosequence positions from a Tukey post hoc test.

Table 3. Aboveground biomass response of *Baptisia leucantha* to colonization by root nodules along the restoration chronosequence (age). ANOVA model with site nested within chronosequence position

| Model Term | df | MS | F | p | R ² |
|---------------------------|-----|---------|-------|---------|----------------|
| Site (age) | 4 | 27,856 | 1.78 | 0.1367 | |
| Colonization | 1 | 272,550 | 17.40 | <0.0001 | 0.346 |
| Age | 3 | 81,820 | 5.22 | 0.0019 | |
| Colonization \times Age | 3 | 24,258 | 1.55 | 0.2048 | |
| Error | 141 | 16,010 | | | |

and productivity (McCain et al. 2010) and reduced susceptibility to invasive species (Goldblum et al. 2013). In contrast to the grass species, the two forbs varied dramatically in their response to microbial communities along the restoration chronosequence. *Silphium terebinthinaceum* growth was largely unresponsive to the restoration chronosequence with the only substantial depression of growth in old restoration soils. Overall, there was no clear pattern along the restoration chronosequence with little variation in biomass production. This species has large seeds (21.47 mg on average from a sample of 50 seeds), which may have buffered it from inhibitory impacts of soil microbes (Westoby 1998).

Baptisia leucantha performance across the chronosequence largely reflected the ability of legumes to form nodules with nitrogen fixing bacteria—a strong positive plant–soil feedback. The benefit of nodules explains the consistent increase in growth in all live soil, regardless of chronosequence position. Larson and Siemann (1998) found legume abundance was unrelated to field age and soil nitrogen content, but was dependent on whether specific rhizobia were present to form symbiosis with the legume host. Our results differ somewhat in that there was an initial depression of nodule formation that recovered with successional development of the restorations. The initial benefits

of the symbiosis may disappear as negative feedbacks develop later in succession (van der Putten et al. 2013). This can be seen in the reduced benefit of nodules in old restoration and remnant soils. These results suggest that the microbial community became more antagonistic later in the chronosequence, which should promote diversity and coexistence among forbs (Mills & Bever 1998; Reynolds et al. 2003).

Potential Applications

Plant–microbe interactions play a critical role in driving succession and in maintaining the diversity of natural prairies (Reynolds et al. 2002; Fitzsimons & Miller 2010), which can be exploited in combination with traditional restoration tools. Although positive feedbacks tend to occur early in succession and allow the system to become dominated by a few species, they later give way to negative feedbacks, which promote species diversity (Reynolds et al. 2003; Petermann et al. 2008; Bever et al. 2010; Reinhart 2012). Overall, this experiment showed strong site variability, representing patchiness in plant–microbe interactions, though older soils consistently had the strongest inhibitory effects. Encouraging the accumulation of late successional soil microbes might be beneficial during restoration by jump starting negative feedbacks and minimizing dominance (Fitzsimons & Miller 2010; Middleton & Bever 2012). Although species varied individually, the overall analysis suggested that the net effects of soil microbial communities in restorations were largely similar to remnants after 20 years.

Soil inoculations have been used to increase the performance of late successional species (Carbajo et al. 2011; Middleton & Bever 2012) and increase legume density and species richness (Beyhaut et al. 2014). Our results indicate that target soil microbes would likely be inhibitory toward plant performance, potentially reducing the growth of all species. Arbuscular mycorrhizal (AM) fungal inoculates have been advocated to provide native grasses with a competitive advantage over weedy species (Allen & Allen 1984; Smith et al. 1998). However, the competitive advantage that AM fungi provide may allow grasses to become dominant at the expense of forbs (Smith et al. 1998). Alternative restoration strategies for places where grass dominance can be problematic would be reducing AM fungi in order to reduce the competitive advantage of grasses and promote forb diversity (Gange et al. 1993) or inoculation with forb-specific AM to provide differential benefits to forbs. A passive strategy utilizing the natural successional development of soil microbial communities would be to delay introducing grasses until later in the restoration process. Once negative feedbacks developed in a site, grasses would no longer have the temporal opportunity to become dominant and displace established forbs. Alternatively, manipulating microbial communities through controlled inoculations or cultural conditions to delay grass establishment until the microbial community becomes established may be useful.

Overall, there is a dearth of knowledge about how soil microbial communities change in restoration. During succession, AM fungi increase in abundance and experience compositional shifts (Allen & Allen 1984; Johnson et al. 1991; Sikes et al. 2012) that may result in functional shifts (Chagnon et al. 2013). The

dynamics of other major groups of soil microbes in succession and whether active restoration efforts also generate these changes is unknown. Our results provide evidence that microbial communities have potential as a prairie restoration tool. Further studies need to focus on the response of plant functional groups to biotic feedbacks and include more species before this can be fully utilized in prairie restoration. This information may provide the ability to target specific restoration goals and would determine the range of species responses that should be expected. More detailed study on the composition and function of the microbial community is needed to determine the responses of different microbial groups to restoration. The net effects documented in this study may mask complex dynamics of individual mutualistic or antagonistic microbes.

Acknowledgments

This work was supported by the Illinois Department of Natural Resources, Wildlife Preservation Fund, grant number 13-024 to S.J.M., and undergraduate research grants from the Eastern Illinois University Department of Biological Sciences and Honor's college to A.J.H.

LITERATURE CITED

- Allen EB, Allen MF (1984) Competition between plants of different successional stages: mycorrhizae as regulators. *Canadian Journal of Botany* 62:2625–2629
- Anderson RC (2008) Growth and arbuscular mycorrhizal fungal colonization of two prairie grasses grown in soil from restoration of three ages. *Restoration Ecology* 16:650–656
- Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist* 157:465–473
- Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M (2010) Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology and Evolution* 25:468–478
- Beyhaut E, Larson DL, Allan DL, Graham PH (2014) Legumes in prairie restoration: evidence for wide cross-nodulation and improved inoculant delivery. *Plant and Soil* 377:245–258
- Carbajo V, den Barber B, van der Putten WH, De Deyn GB (2011) Enhancement of late successional plants on ex-arable land by soil inoculations. *PLoS ONE* 6:e21943
- Chagnon P, Bradley RL, Maherali H, Klironomos JN (2013) A trait-based framework to understand life history of mycorrhizal fungi. *Trends in Plant Science* 18:484–491
- Collins SL, Knapp AK, Briggs JM, Blair JM, Steinauer EM (1998) Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science* 280:745–747
- Connell JH (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Pages 298–310. In: de Boer PJ, Gradwell GR (eds) *Dynamics of populations*. PUDOC, Wageningen, the Netherlands
- Faber S, Markham J (2012) Biotic and abiotic effects of remnant and restoration soils on the performance of tallgrass prairie species. *Ecological Restoration* 30:106–115
- Fitzsimons M, Miller R (2010) The importance of soil microorganisms for maintaining diverse plant communities in tallgrass prairie. *American Journal of Botany* 97:1937–1943
- Gange AC, Brown VK, Sinclair GS (1993) Vesicular-arbuscular mycorrhizal fungi: a determinant of plant community structure in early succession. *Functional Ecology* 7:616–622

- Goldblum D, Glaves BP, Rigg LS, Kleiman B (2013) The impact of seed mix weight on diversity and species composition in a tallgrass prairie restoration planting, Nachusa Grasslands, Illinois, USA. *Ecological Restoration* 31:154–167
- Hansen MJ, Gibson DJ (2013) Use of multiple criteria in an ecological assessment of a prairie restoration chronosequence. *Applied Vegetation Science* 17:63–73
- van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11:296–310
- Hodge A, Fitter A (2013) Microbial mediation of plant competition and community structure. *Functional Ecology* 27:865–875
- Janzen DH (1970) Herbivores and the number of species in tropical forest. *American Naturalist* 104:501–528
- Johnson EA, Miyanishi K (2008) Testing the assumptions of chronosequences in succession. *Ecology Letters* 11:1–13
- Johnson NC, Zak DR, Tilman D, Pflieger FL (1991) Dynamics of vesicular-arbuscular mycorrhizae during old field succession. *Oecologia* 86:349–358
- Kardol P, Cornips N, van Kempen M, Bakx-Sxhotman T, van der Putten W (2007) Microbe-mediated plant-soil feedbacks cause historical contingency effects in plant community assembly. *Ecological Monographs* 77:147–162
- Kardol P, De Deyn GB, Lalilberte E, Mariotte P, Hawkes CV (2013) Biotic plant-soil feedbacks across temporal scales. *Journal of Ecology* 101:309–315
- Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84:2292–2301
- Larson JL, Siemann E (1998) Legumes may be symbiont-limited during old-field succession. *American Midland Naturalist* 140:90–95
- Lawson D, Inouye RS, Huntly N, Carson WP (1999) Patterns of woody plant abundance, recruitment, mortality, and growth in a 65 year chronosequence of old-fields. *Plant Ecology* 145:267–279
- McCain KNS, Baer SG, Blair JM, Wilson GWT (2010) Dominant grasses suppress local diversity in restored tallgrass prairie. *Restoration Ecology* 18:40–49
- Middleton EL, Bever JD (2012) Inoculation with a native soil community advances succession in a grassland restoration. *Restoration Ecology* 20:218–226
- Mills K, Bever J (1998) Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. *Ecology* 79:1595–1601
- Pendergast TH, Burke DJ, Carson WP (2013) Belowground biotic complexity drives aboveground dynamics: a test of the soil community feedback model. *New Phytologist* 197:1300–1310
- Petermann J, Fergus A, Turnbull L, Schmid B (2008) Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* 89:2399–2406
- Pickett STA (1989) Space-for-time substitutions as an alternative to long-term studies. Pages 110–135. In: Likens GE (ed) *Long-term studies in ecology*. Springer-Verlag, New York
- van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, et al. (2013) Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology* 101:265–276
- Reinhart KO (2012) The organization of plant communities: negative plant-soil feedbacks and semiarid grasslands. *Ecology* 93:2377–2385
- Reynolds H, Packer A, Bever J, Clay K (2003) Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology* 84:2281–2291
- Shannon S, Flory SL, Reynolds HL (2012) Competitive context alters plant-soil feedback in an experimental woodland community. *Oecologia* 169:236–243
- Sikes BA, Maherali H, Klironomos JN (2012) Arbuscular mycorrhizal fungal communities change among three stages of primary sand dune succession but do not alter plant growth. *Oikos* 121:1791–1800
- Smith MR, Charvat I, Jacobson RL (1998) Arbuscular mycorrhizae promote establishment of prairie species in a tallgrass prairie restoration. *Canadian Journal of Botany* 76:1947–1954
- Vankat JL, Snyder GW (1991) Floristics of a chronosequence corresponding to old field-deciduous forest succession in southwestern Ohio. I. Undisturbed vegetation. *Bulletin of the Torrey Botanical Club* 118:365–376
- Walker LR, Wardle DA, Bardgett RD, Clarkson BD (2010) The use of chronosequences in studies of ecological succession and soil development. *Journal of Ecology* 98:725–736
- Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil* 199:213–227

Coordinating Editor: Purushothaman C. Abhilash

Received: 15 September, 2014; First decision: 12 November, 2014; Revised: 12 November, 2014; Accepted: 13 November, 2014