5-2014

Plant-Microbial Interactions Change Along a Prairie Restoration Chronosequence

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by

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HONORS THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE IN BIOLOGICAL SCIENCES WITH HONORS

AT EASTERN ILLINOIS UNIVERSITY

CHARLESTON, ILLINOIS

May 2014

I hereby recommend that this Honors Thesis be accepted as fulfilling this part of the undergraduate degree cited above:

Thesis Director _____ Date 5/29/14

Honors Program Director _____ Date 29 Sept 2014

Abstract

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 prairie restorations. Specifically, I 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*, produced larger biomass 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. The forb, *Silphium terebinthinaceum* was largely unaltered by the inoculation or position along the restoration chronosquence. *Baptisia leucantha* growth appeared limited by nodule formation in agricultural soils, peaked in young restoration soils along with module formation, but decreased in older soils as the microbial community became more antagonistic. Overall, this experiment showed strong site variability, representing patchiness in microbial interactions, though older soils consistently had the strongest inhibitory effect on growth. Negative feedbacks tended to be less important in the beginning stages of succession in these restorations but appear important in remnant and restored prairies. My results provide evidence that it maybe advantageous for management practices to take negative feedbacks into consideration when trying to recreate the diversity of prairies.

Key words: soil microbial communities, negative feedbacks, tall grass prairie, root nodules.

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; Sikes et al. 2012 Middleton & Bever 2012). However, microbial community composition is critical to the development, abundance and diversity of the above ground plant community. Plant species differ widely in response to individual microbial species with positive and negative effects often being host specific, with the net microbial community effect impacting plant performance (van der Heijen et al. 2006; 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. These net interactions can facilitate or inhibit further growth of both the plant community and the soil microbial community. (Kardol et al. 2007; Bever et al. 2010; Sikes et al. 2012). Plant soil feedbacks are generated first by plants inducing changes in the composition of their soil microbial community, which then affects plant for performance (Bever 2003; Bever et al. 2010). If changes in soil biota increase plant performance

relative to other plants, the positive feedback may generate increased abundance and maintain dominance of the species in the community (Reynolds et al. 2003; 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) that increases with root colonization. Evidence suggests that positive feedbacks can lead to the development of plant monocultures and slow successional replacement (Kardol et al. 2007). 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. (Kardol et al. 2007; Petermann et al. 2008; Fitzsimons and Miller 2010; Mills & Bever. 1998. While 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 (Reynolds et al. 2003 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 and Bever 2012; Hansen and Gibson 2014. In the Midwestern US, 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, which are similar to the crops that were historically grown, 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 (McCain et al. 2010; Goldblum et al. 2013), the timing of management fires that enhance C_4 plant growth (Collins et al. 1998), the absence of grazing animals, residual fertility from agricultural amendments (Anderson 2008; Goldblum et al. 2013) and the lack of established microbial feedbacks that are needed to maintain diversity (Fitzsimons & Miller 2010). Species which 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 success of the restoration (Anderson 2008). As diversity within a restored prairie is critical to providing a 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 I examined the performance of two dominant, C_4 grasses and two less abundant prairie forbs (including one legume) in soils from a prairie restoration chronosequence. My goal was to determine whether the dominance of grasses in prairie restorations was caused by species' interactions with the soil microbial community. 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) Is the dominance of grass species over less abundant forbs driven by the strength of soil microbial effects? 3) How does the presence of nitrogen fixing bacteria alter legume response to the microbial community along the restoration

chronosequence? The overarching goal of this work was to understand the effect of microbial communities have on prairie community dynamics 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 15 ha with several smaller fragments that has 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.

I 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 now 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 observation*). Seeds were collected from the RWF property to ensure the appropriateness of the plant-microbe interactions. All seed was stored dry at 4°C before usage.

Silphium terebinthinaceum was cold-moist stratified at 4°C for 60 d and *Baptisia leucantha* for 10 d following scarification with sandpaper to break dormancy.

Experimental design –I selected 8 different sites at RWF, two of each from four age classes along a restoration chronosequence: 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 caused by differences in soil type, I selected locations within each site 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 10 cm using a 7 cm diameter soil auger. 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 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, Tangent OR, USA) 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. After 60 days they were harvested, dried and weighed. I used analyses of variance (ANOVA) to determine the overall impacts of microbial communities and chronosequence position on plant performance. In these analyses, site identity was nested within chronosequence position to account for variation within each age class.

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, I also quantified mutualists on plant roots. When the above experiment was harvested, *Baptisia* root tissues were also collected. Roots were cleaned and examined to determine the whether the plant was colonized 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, the *Baptisia* growth was compared between colonized and uncolonized plants (live soils only) in a nested ANOVA as described above.

RESULTS

All species responded to both 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 chronosequence position, soil sterilization and their interaction (Figure 1A). 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 1B; 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 suppression 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, 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 (Figure 1C). There was a 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 1D). Live remnant soils produced the least benefit to *B. leucantha* growth.

Looking across sites, I found the strongest microbial inhibition (or least benefit) to growth in the remnant or old restoration soils. Similarly, I 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 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 the agricultural soils, and lowest in remnant prairie soils ($\chi^2 = 28.4$, df=3, $P < 0.001$; Figure 2). 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 *Baptisia leucantha* was always higher in colonized plants compared to uncolonized and there was variation with chronosequence position (Fig. 3). Though the biomass difference between colonized and uncolonized plants disappeared in remnant soils, there was no age × colonization interaction.

DISCUSSION

Chemical, physical and biological properties help to shape the nature of soil, determining the growth, productivity and reproductive success of individual and coexisting plant species (Sikes et al. 2012; van der Putten et al. 2013). I used a restoration chronosequence to represent the temporal dynamics of plant-soil community interactions. Although there are limitations (Pickett & Likens 1989 Johnson & Miyanishi 2008), the chronosequence approach has been quite useful in studies that measure plant and soil communities' temporal changes (Vankat & Snyder 1991; Lawson et al. 1999; Walker et al. 2010). This experimental design allowed me to

examine the development of soil microbial communities during restoration to determine if they have the potential to regulate restoration success.

Although sites were selected based on similarity of soil and topographic structure, I observed site variation in sterilized soils that might be attributed to chemical and physical differences among the sites (Kardol et al. 2007; Anderson 2008). In the sterilized controls, I observed similar performance patterns for *A. 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 indicates 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 that might 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 while *Silphium terebinthinaceuma* growth slightly peaked in sterilized soil from old restored sites. This variation among sites and species could be caused by changes in soil characteristics or species-specific interactions (Middleton & Bever 2012). Shifts in plant performance associated with abiotic soil properties are not uncommon in such studies. In a survey of two prairie grasses grown in soils from three different restoration ages, Anderson (2008) found differences in plant success caused by soil nutrient levels, but these were not directly related to restoration age. Similarly, Faber and Markham (2012) found that *A. gerardii* had higher biomass in restored sites than remnant sites, and attributed this effect to agricultural fertilizer residues.

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 *Sorghastrum nutans*, reflects its fast establishment (Smith et al. 1998; Anderson 2008) that might make it vulnerable to negative feedbacks (Reynolds 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 C_4 grasses likely because they are similar physiologically to cultured species such as corn (Reynolds et al. 2003, 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).

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 (Kardol et al. 2007). 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). Restored prairies may become

dominated by grasses because the altered soil microbial communities of post-agricultural restorations initially favor dominant matrix grasses at the expense of forbs. Similar to my findings, Faber and Markham (2012) found differences in the feedbacks associated with remnant and restored prairies. The microbial community of remnant sites in that they produced positive feedbacks on *A. gerardii* growth, however, which differs from the negative feedbacks produced by my live remnant soil inoculates. Carbajo et al. (2011) also found that late successional plants benefit from late successional soil inoculates.

Dominance by C_4 grasses can be problematic in restorations because of their aggressive nature and persistence. When dominant grasses such as *A. gerardii* are removed, light availability, forb production and diversity increase (McCain et al. 2010). Similarly, frequent fires are clearly linked with increased C_4 grass cover and a decrease in forb richness unless competitive hierarchies are disrupted (Collins et al. 1998). Problems of grass dominance are not ubiquitous, as Hansen and Gibson (2014 found that while C_4 grasses tend to become dominant in prairie restorations, forb cover remained constant over an 18-y restoration chronosequence. However, forb richness did decline during this period, suggesting that the grass expansion did have some negative effects.

One of the major components of soil microbial communities are arbusuclar mycorrhizal (AM) fungi. These mutualists are associated closely with C_4 grasses and increase nutrient uptake, drought tolerance and protects plant roots from pathogens (Smith et al. 1998; Sikes et al. 2012; Gange et al. 1993. During succession AM fungi increase in abundance while also experiencing compositional shifts (Allen & Allen 1984; Johnson et al. 1991 Sikes et al. 2012). A review by Chagon et al. (2013) that applied Grime's (2006) CSR perspective to AM community dynamics further argues for large changes in AM communities with succession. Ruderal AM

fungi that are tolerant of frequent plowing disturbance function more in protecting plants against pathogens than P uptake. Ruderal AM fungi are replaced by competitive types with improved carbon acquisition and P uptake abilities and then give way to stress tolerant AM when demand for resources exceeds supply (Chagon et al. 2013). Such functional shifts and the species-specific nature of AM interactions (Klironomos 2003) provide a mechanism for the changes in plant response to microbial communities over the restoration chronosequence.

Forb responses to the microbial chronosequence

The target of a successful prairie restoration focuses on forb diversity, which provides benefits such as increased nutrient retention 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), which may have buffered it from inhibitory impacts of soil microbes (Westoby 1998).

Baptisia leucantha performance across the chonosequence largely reflected the ability of legumes to form nodules with nitrogen fixing bacteria – a strong positive plant-soil feedback. The value of this symbiosis is greatest on nitrogen poor soils (van der Heijden et al. 2008; Hodge & Fitter 2013). The benefit of nodules explains the consistent beneficial response of *B. leucantha* to all live soil, regardless of chronosequence position. According to Larson & Siemann (1998), legume abundance is unrelated to field age and soil nitrogen content but is dependent on if specific rhizobia are present to form symbiosis with the legume host. My results differ 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 decreased growth benefit of nodules in old restoration and remnant soils. These results indicate 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.

Implications for application

Plant-microbe interactions play a role in driving succession and in maintaining the diversity of natural prairies (Reynolds 2003 Fitzsimons & Miller 2010), which can be exploited in combination with traditional restoration tools. While 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 promotes species diversity (Reynolds 2003 Petersmann et al. 2008; Bever 2003 Reinhart 2012). Overall, this experiment showed strong site variability, representing patchiness in plant-microbe interactions, though older soils consistently had the strongest inhibitory effect. 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).

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). My results indicate that target soil microbes would likely be inhibitory towards plant performance, however, reducing the growth of all species. AM fungi inoculates have been advocated to provide native grasses with a competitive advantaged over weedy species (Allen & Allen 1984; Smith et al. 1998). The competitive advantage that AM fungi

provide allow grasses to become dominant in restored prairies at the expense of forbs (Smith et al. 1998). An alternative restoration strategy for places where grass dominance can be problematic would be reducing AM fungi in order to level the advantage of the grasses and promote forb diversity (Gange et al. 1993. 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 forbs. Alternatively, manipulating microbial communities through controlled inoculations or cultural conditions to delay grass establishment until the microbial community becomes established may be useful.

My results provide strong 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. Studies that evaluate 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 are also necessary.

Acknowledgments

I would like to thank Dr. Scott Meiners, Paula Butts and Daniel Armstrong for their assistance throughout the duration of this research project. I would also like to thank Brian Towey for allowing me to use Richardson Wildlife Foundation to collect media for the project. This work was supported by the Illinois Department of Natural Resources, Wildlife Preservation Fund; grant number 13-024 to S. Meiners, and undergraduate research grants from the Eastern Illinois University Department of Biological Sciences and Honor's college to AJH.

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Table 1. 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	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	
Sterilization	1	187786.11	8.54	0.0037	
Age × sterilization	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	
Sterilization	1	10797.47	0.75	0.3865	
Age × sterilization	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	
Sterilization	1	5389.21	0.62	0.4309	
Age × sterilization	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	
Sterilization	1	2048312.94	134.31	<0.0001	
Age × sterilization	3	130269.05	8.54	<0.0001	
Error	289	15250.95			

Table 2. Growth 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
Colonization	1	272550	17.40	<0.0001	0.346
Age	3	81820	5.22	0.0019	
Col x Age	3	24258	1.55	0.2048	
Site(Age)	4	27856	1.78	0.1367	
Error	141	16010.92			

Figure Headings

Figure 1. Above ground biomass (mg) responses to live and dead soil along a restoration chronosequence: (A) *Andropogon geradii*; (B) *Sorgastrum nutans; Silphium terebinthinaceum*; (D) *Baptisa leucantha.* Bars are mean ±1 standard error.

Figure 2. 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 ±1 standard error.

Figure 3. 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 ±1 standard error.

Figures

Figure 1.

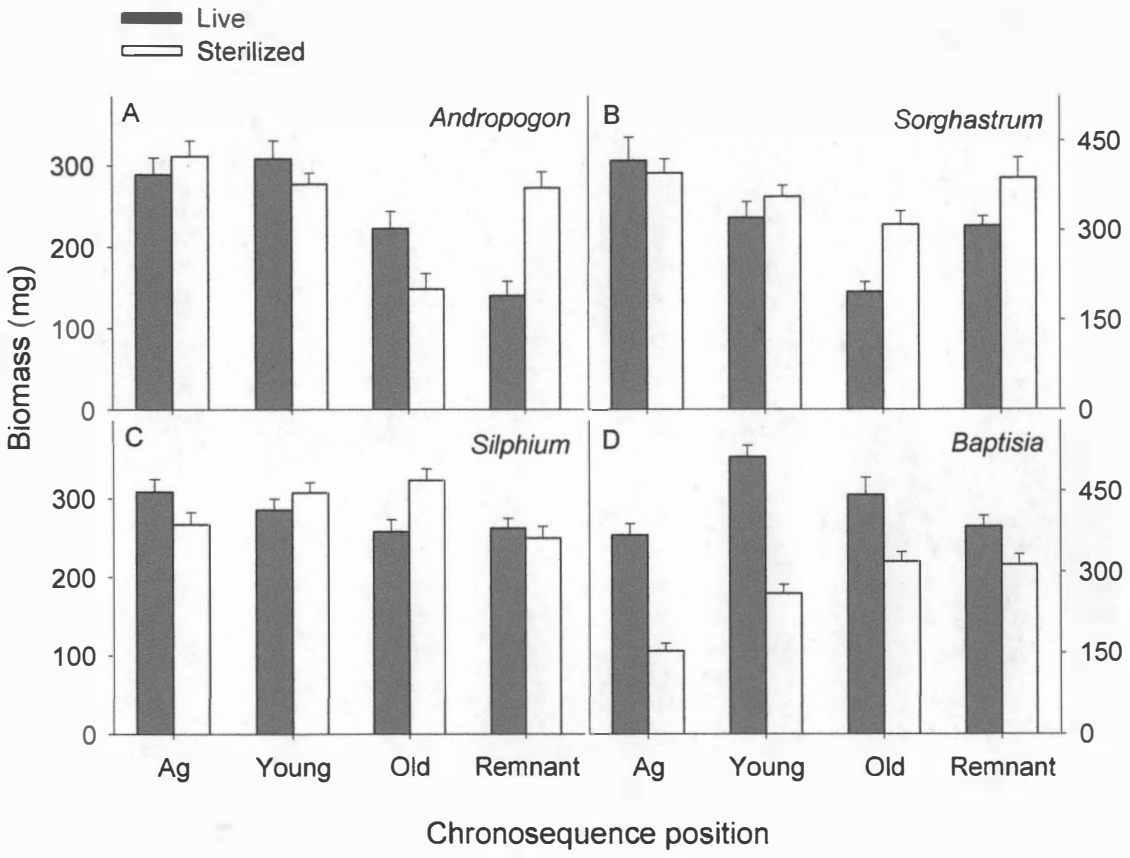


Figure 2.

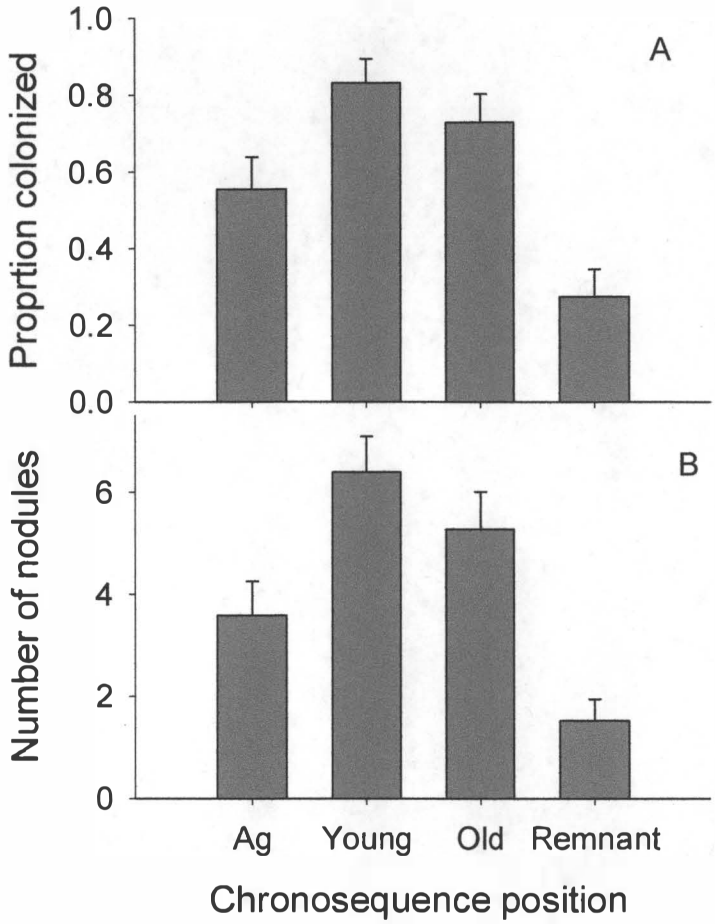
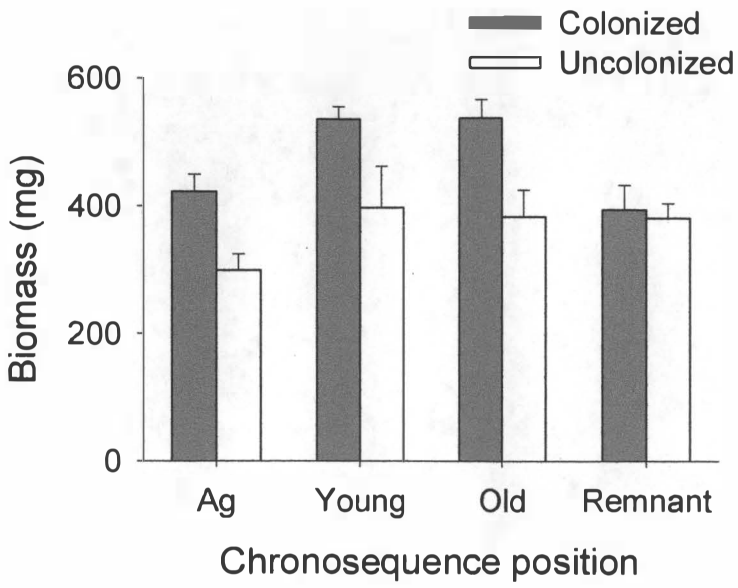

Proprtion colonized
1.0
0.8
0.6
0.4
0.2
0.0
A
Number of nodules
6
4
2
0
B
Ag
Young
Old
Remnant
Chronosequence position

Figure 3.


Colonized
Uncolonized
600
400
200
0
Biomass (mg)
Ag
Young
Old
Remnant
Chronosequence position