Growth and Arbuscular Mycorrhizal Fungal Colonization of Two Prairie Grasses Grown in Soil from Restorations of Three Ages

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Abstract
I compared growth and arbuscular mycorrhizal fungal (AMF) colonization of two prairie grasses (Wild rye [Elymus canadensis] and Little bluestem [Schizachyrium scoparium]), an early- and a late-dominating species in prairie restorations, respectively, grown in soil from restored prairies of differing age, soil characteristics, and site history. There were no consistent patterns between restoration age and soil inorganic nutrients or organic matter. The oldest restoration site had higher soil mycorrhizal inoculum potential (MIP) than 2- and 12-year-old restorations. However, MIP did not translate into actual colonization for two species grown in soils from the three restorations, nor did MIP relate to phosphorus availability. There were significant differences in root mass and colonization among Wild rye plants but not among Little bluestem plants grown in soils from the three restorations. Wild rye grown in 2-year-old restoration soil had significantly higher AMF colonization than when it was grown in soils from the 12- and 17-year-old restorations. Wild rye grown in 2-year-old restoration soil also had higher colonization than Little bluestem grown in 2- and 12-year-old restoration soils. Little bluestem had no significant correlations between shoot biomass, root biomass or colonization, and concentrations of soil P, total N, or N:P. However, for Wild rye, total soil N was positively correlated with root mass and negatively correlated with colonization, suggesting that in this species, mycorrhizae may affect N availability. Collectively, these results suggest that soil properties unrelated to restoration age were important in determining differences in growth and AMF colonization of two species of prairie grasses.

Key words: arbuscular mycorrhizal fungi, Elymus canadensis, inorganic nutrients, N:P, prairie restoration, Schizachyrium scoparium.

Introduction
Restored native plant communities often are not comparable in species diversity and other attributes, such as wildlife supported, to the undisturbed remnant communities they are designed to represent (Galatowitsch & van der Valk 1996; Zedler 1996; Kindscher & Tieszen 1998; Sluis 2002). In the Midwestern United States, restored native tallgrass prairies often have low species diversity and are dominated by warm-season C4 grasses and early-dominating, aggressive, coarse, late summer, or early fall flowering forbs (Warkins & Howell 1983; Betz 1986; Warkins 1988; Kindscher & Tieszen 1998). Many of the less aggressive forbs associated with high-quality remnant prairies fail to become established (Betz 1986; Schramm 1990). Several reasons have been offered to explain the low forb diversity and high grass dominance of these restored prairies, including the following: (1) grasses are planted too densely, and they suppress the growth of forbs (Schramm 1978; McClain 1997); (2) fire frequency and timing, especially dormant season burns (e.g., late winter or early spring), that encourage dominance of C4 grasses and suppress forbs (Howe 1995; Collins et al. 1998); (3) absence of large herbivores (Collins et al. 1998; Knapp et al. 1999; Anderson 2006); and (4) high soil fertility on the restoration site (Baer et al. 2004).

Additionally, a large body of literature suggests that, in an environment such as a restoration site, mycorrhizal fungal associations could influence growth and competitive ability of plant hosts (Allen et al. 1984; Allen & Allen 1986; Grime et al. 1987; Hartnett et al. 1993). Furthermore, mycorrhizal fungi have been shown to improve soil structure during prairie restoration, which may encourage establishment of some prairie species (Jastrow 1987; Miller & Jastrow 2000; Allison et al. 2005). Similarly, Baer et al. (2002) reported that across a chronosequence (2–12 years) of restored grasslands (Conservation Reserve Program), bulk density in the upper 10 cm of soil decreased and total C, microbial biomass C, and C mineralization rates increased over time. They concluded that restoration establishment of native C4 grasses drives the trajectory of ecosystem processes in the direction of original tallgrass prairie. However, few studies have examined how soil conditions affect the trajectory of change in ecosystem processes during restoration and how early- and late-dominating...
species in prairie restorations respond to varied soil conditions (Baer et al. 2004). In this study, I compared biomass production and arbuscular mycorrhizal fungal (AMF) colonization of two prairie grasses grown in soils from prairie restoration of different ages. One of these (Wild rye [Elymus canadensis]) dominates early in prairie restorations (Liegel & Lyon 1986; Bradley 1987), and the other (Little bluestem [Schizachyrium scoparium]) is known to dominate later stages of restoration (Schramm 1990; Betz et al. 1996). Wild rye has been recommended as a cover crop for prairie restorations because it provides a short-lived perennial cover that competes well with weedy species and declines in dominance after 5–8 years as slower establishing native species increase in abundance (Morgan 1997). Little bluestem increases in dominance in the later stages of prairie restoration and can persist with competition from other perennial prairie species (Schramm 1990). Little bluestem reaches its highest abundance on dry-mesic and dry prairie sites, whereas Wild rye is more abundant on wet-mesic and wet sites (Weaver 1968; Curtis 1971). Little bluestem is strongly dependent upon mycorrhizal associations under conditions of low phosphorus; however, Wild rye shows no dependency on mycorrhizae even under conditions of low phosphorus availability (Anderson & Liberta 1992; Hartnett et al. 1993; Anderson et al. 1994; Noyd et al. 1995).

I predicted that when grown in soil from the restorations of various ages (1) Wild rye would have its highest colonization and biomass production when grown in soil from the youngest restoration and (2) Little bluestem would have its highest colonization and biomass production when grown in soil from the oldest restoration. These predictions would be in agreement with the known differences between the two species in terms of time taken to reach dominance during prairie restorations.

Methods

Soils were obtained from three restored prairies that were 2, 12, and 17 years old, occurring on the Fermilab National Laboratory (Batavia, IL) on 17 May 1995. The three prairie restorations occurred on sites with similar topography and soils and were in row crops (corn and soybeans) prior to restoration. The soil types on the sites were Mundelein silt loam (fine silty, mixed mesic Aquic Argiudoll) and Drummer silty clay loam (fine silty, mixed mesic Typic Haplauvol). Information is not available about the species composition of the seed mixture sown on the three sites. Although the sites were selected because of differences in age and the similarity of their soils, there were several extrinsic factors at each site that made it difficult to consider these sites as a chronosequence. Two of the sites (12 and 17 years old) occurred within the accelerator ring at the Fermilab where a channel around the ring creates an artificially high water table. The 2-year-old restoration site was situated outside the ring. Additionally, crop rotations and fertilization regimes during the farming era were almost certainly variable among the three sites.

Fifteen soil samples were collected from each site using a stratified random sampling procedure. At each site, five points were located at 8-m intervals along a 40-m line in an area that was relatively uniform in terms of vegetative cover and topography. From each point, three soil samples were collected along separate sampling lines that were located at randomly selected 10° azimuth intervals and at random distances that varied from 0 to 10 m. The area sampled at each site was approximately 1,200 m². At each sampling point, approximately 1 L of soil was collected from the upper 0–10 cm, and all plant species within 30 cm of the center of the area from which soil samples were collected were recorded. Soil from six randomly selected samples per site was air-dried and analyzed for organic matter, total nitrogen, calcium, magnesium, potassium, and phosphorus by the Plant and Soil Analysis Laboratory at the University of Wisconsin–Madison.

The 15 soil samples collected from each of the restoration sites were mixed with perlite (3:1) to maintain soil porosity and aid drainage, and 167 cc of the mixture from each sample was placed in a separate plastic conical pot (cone-tainer). To assess the initial AMF inoculum potential of soil from the three restoration sites, on 19 May, a single, pregerminated surface-sterilized corn kernel was planted in each cone-tainer and grown for 15 days (Moorman & Reeves 1979). Cone-tainers were placed in a growth chamber, and at the end of the 15 days, roots were harvested and scored for mycorrhizal colonization as described below for Wild rye and Little bluestem.

On 4 May, Wild rye seeds that were locally collected and seeds of Little bluestem purchased from a commercial source (Stock Seed Farms, Murdock, NE, U.S.A.) were sown on moistened ProMix potting soil and placed under a mist in the greenhouse. Seedlings of Wild rye and Little bluestem were transplanted into separate 10-cm-diameter clay pots containing 450 cc of the 15 mixed soil–perlite replicates from each of the three sites (n = 15/site/species) on 19 May. Plants were grown for 60 days under greenhouse conditions. At the end of the growth period, the number of tillers per plant was counted, the height of each tiller was measured, aboveground and belowground tissues were harvested, and a 0.25 g fresh weight subsample of roots from each plant was excised from the root mass. Excised roots were cut into 1-cm segments, cleared with KOH, and stained (Phillips & Hayman 1970), and all segments were scored for mycorrhizal colonization using the gridline intersect method (Giovannetti & Mosse 1980). The remaining roots and shoots were oven-dried for 48 hours and weighed. During the experiment, 1 Wild rye plant died in the 17-year-old restoration, and 4, 6, and 1 Little bluestem plants died in the 2-, 12-, and 17-year-old restorations, respectively.

Data were analyzed using multivariate analyses of variance (MANOVAs) and protected analyses of variance...
Results

Vegetation at the Restoration Sites

The four leading taxa and their frequencies, in parentheses, sampled at each of the three restoration sites were as follows: 2-year-old site: Andropogon gerardii (53.3%), Sorghastrum nutans (40.0%), Monarda fistulosa (33.3%), and Melilotus spp. (26.7%); 12-year-old site: Solidago juncea (86.7%), An. gerardii (80%), Sol. canadensis (73.3%), and Poa pratensis (66.7%); 17-year-old site: Ratibida pinnata (66.7%), An. gerardii (60.0%), Po. pratensis (46.7%), and Silphium integrifolium (46.7%). Species richness for all species and prairie forbs was highest for the 17-year-old site (20 and 14, respectively) for the 2-year-old site (9 and 4, respectively) and 2-year-old site (12 and 4, respectively). The 17-year-old site had several species of forbs that were not sampled at the other two sites: Aster laevis, Coreopsis tripteris, Eryngium yuccifolium, Helianthus mollis, Si. laciniatum, Si. terebinthinaceum, Sol. rigida, and Veronicastrum virginicum. At the 17-year-old and 2-year-old sites, two prairie grasses were represented in the sample (An. gerardii and Sor. nutans), and at the 12-year-old site, one prairie grass was sampled (An. gerardii). The 17-year-old site had the highest diversity (H’ = 2.62), followed by the 2-year-old site (2.18), and the 12-year-old site had the lowest diversity (1.88). Evenness (J) was similar among the sites and was 0.88, 0.87, and 0.85 for the 2-, 17-, and 12-year-old restoration sites, respectively.

Soil Analysis

For inorganic nutrients, MANOVA indicated significant effects owing to site, and all one-way ANOVAs for individual nutrients indicated significant site effects. Both eigenvectors of the one-way MANOVA were significant. However, the first eigenvector accounted for nearly all the retained variance (97.63%). The variables with the highest standard canonical coefficients for the first eigenvector were total nitrogen (7.859), magnesium (6.233), and phosphorus (3.272). The 12-year-old restoration site had higher levels of most measured soil variables than the other sites except for phosphorus and potassium. The 12-year-old site had significantly lower levels of phosphorus than the other sites; available potassium was highest in the 17-year-old site. The 2-year-old site had significantly less total N and available calcium than the other two sites (Table 1).

MANOVA and ANOVA Results. For root and shoot mass and AMF colonization, two-way MANOVA indicated significant differences owing to site, species, and two-way interactions (p < 0.001 for both the main effects and the interactions). Standard canonical coefficients indicated that root mass was the most important variable causing differences among main effects. Root mass was negatively correlated across main effects and interactions with shoot mass and percent colonization. Follow-up, univariate two-way ANOVAs indicated significant two-way interactions for root mass and percent colonization but only significant site effects for shoot mass.

Table 1. Comparison of measured soil variables for soils from three restoration sites.

<table>
<thead>
<tr>
<th>Soil Variable</th>
<th>2 Years</th>
<th>12 Years</th>
<th>17 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen (%)</td>
<td>0.19 ± 0.003a</td>
<td>0.36 ± 0.008b</td>
<td>0.26 ± 0.006c</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>4.9 ± 0.1a</td>
<td>8.5 ± 0.2b</td>
<td>6.5 ± 0.2c</td>
</tr>
<tr>
<td>Calcium</td>
<td>1,717 ± 51.1a</td>
<td>3,300 ± 38.7b</td>
<td>2,175.0 ± 123.7c</td>
</tr>
<tr>
<td>Potassium</td>
<td>140.8 ± 0.4a</td>
<td>129.2 ± 2.3ab</td>
<td>179.2 ± 14.7b</td>
</tr>
<tr>
<td>Magnesium</td>
<td>615.0 ± 27.7a</td>
<td>1,123.3 ± 20.2b</td>
<td>706.7 ± 35.6a</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>25.8 ± 1.2a</td>
<td>12.0 ± 1.8b</td>
<td>20.9 ± 4.0a</td>
</tr>
</tbody>
</table>

Total nitrogen and organic matter are given in percentage, and the available ions are presented as μg/g. Values are means ± SE. Within a row, means with the same letter are not significantly different. For each mean, sample size was 6. Ryan–Einot–Gabriel–Welsch multiple range tests, which corrects for experimentwise error, were used for all comparisons.
Root Biomass. Wild rye had significantly higher root biomass in the 12-year-old restoration than when it was grown in soil from the other restorations and Little bluestem grown in soil from the 2- and 12-year-old restorations (Fig. 1). There were no significant differences in root mass among Little bluestem grown in the three soils.

Colonization. AMF colonization was higher for Wild rye in the 2-year-old restoration soil than Little bluestem grown in soil from the 2- and 12-year-old restorations. However, when grown in the 17-year-old restoration soil, Little bluestem had higher colonization than Wild rye. Across restorations, there were no significant differences in colonization for Little bluestem, whereas Wild rye had significantly higher colonization in the 2-year-old restoration soil than in the 12- or 17-year-old restoration soils (Fig. 2).

Tillers and Shoot Mass

Two-way MANOVA indicated that there were significant effects owing to site and species for number of tillers and sum of tiller heights, but the two-way interactions were not significant. For sites, only the first eigenvector was significant, and it accounted for 90% of the retained variance. Sum of tiller height had a larger standard canonical coefficient (0.954) than number of tiller (0.460) for the first eigenvector. For the single eigenvector for species, number of tillers had a larger standard canonical coefficient (1.886) than sum of tiller heights (−0.414), and the two variables were negatively correlated across species.

For Wild rye, mean number of tillers and sum of tiller height were 1.5 ± 0.10 and 3.4 ± 0.2 cm, respectively, and the same values were significantly larger for Little bluestem (3.9 ± 0.3 and 6.6 ± 0.5 cm, respectively). Plants growing in the 12- and 17-year-old restorations had significantly more tillers and greater sum of tiller heights than plants growing in 2-year-old restoration soil (Table 2). Plants grown in the soil from the 12-year-old restoration had significantly greater shoot mass than those grown in

the soil from the 2-year-old restoration (Fig. 3). Shoot mass was significantly ($p < 0.001$) correlated with number of tillers ($r = 0.61$) and sum of till heights ($r = 0.75$).

Nutrients and Plant Response

For Little bluestem, there were no significant correlations between shoot or root biomass, colonization, soil P, total N (arcsine transformed), or N:P across sites. However, for Wild rye, arcsine-transformed total N was positively correlated with root mass ($r = 0.71$, $p = 0.0009$, $df = 16$) and negatively correlated with colonization ($r = −0.49$, $p = 0.0351$, $df = 16$).

Discussion

The prairie restorations studied ranged in age from 2 to 17 years, but the sites also varied in soil inorganic nutrients, which is likely due to past farming practices (Allison et al. 2005). Differences in the responses of the two prairie grasses grown in soils from the three sites appear to be largely due to differences in soil properties, which did not

Table 2. Measured ($\bar{X} \pm SE$) plant growth variates for plants grown in soil from prairie restorations of various ages.

<table>
<thead>
<tr>
<th>Plant Growth Variates</th>
<th>Restoration Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 Years</td>
</tr>
<tr>
<td>Shoot weight (g)</td>
<td>$0.29 \pm 0.03a$</td>
</tr>
<tr>
<td>Number of tillers</td>
<td>$2.1 \pm 0.3a$</td>
</tr>
<tr>
<td>Sum of tiller shoot height (cm)</td>
<td>$3.9 \pm 4.0a$</td>
</tr>
</tbody>
</table>

Within a row, means followed by the same letter are not significantly different (Ryan–Einot–Gabriel–Welsch multiple range tests).
2-year-old site. had significantly greater shoot weight than plants grown in soil from

sites. The plants grown in soil from the 12-year-old site of three ages. For shoot weight, there were only significant differen-

tions among sites. The plants grown in soil from the 12-year-old site had significantly greater shoot weight than plants grown in soil from the 2-year-old site.

show any consistent patterns with respect to restoration age. Perhaps because of this unexpected difference in soil characteristics, none of the predictions proposed in this study were supported except for the predicted mycorrhizal colonization of Wild rye.

Wild rye had its highest colonization in the 2-year-old restoration, which is consistent with its status as a species that does well in the early stages of restoration (Betz 1986; Schramm 1990). However, its high colonization in this restoration may have been due to soil factors unrelated to restoration age. For example, N:P has been shown to influence levels of AMF colonization (Anderson & Liberta 1992; Eom et al. 1999; Johnson et al. 2003). Several studies concluded that nitrogen addition to prairie sites reduced AMF structures at sites with low N:P ratios and increased AMF structures at sites with high N:P ratios. This observed increase in AMF structures results from adding N to soil with high N:P ratios because the added N only exacerbates P limitation, and, therefore, the importance of mycorrhizae to plants for P acquisition is enhanced (Eom et al. 1999; Johnson et al. 2003; Egerton-Warburton et al. 2007).

Nevertheless, I found that for both species examined in my study, root and shoot masses and colonization were not significantly correlated with soil P or N:P across all sites. However, colonization of Wild rye was negatively correlated with total soil N, and root biomass was positively correlated with total soil N. These results suggest that Wild rye may use the mycorrhizal association to obtain an additional source of N at low levels of soil N. Other studies have concluded that mycorrhizal effects on nitrate assimilation may be due to improved N uptake and translocation and not mediated by improved P nutrition (Faure et al. 1998; Subramanian & Charest 1998). Adequate availability of nitrogen, Wild rye may increase in-

vestment in roots to obtain other inorganic nutrients (Anderson & Menges 1997), which would explain the increased root growth in soil from the 12-year-old site, which had the highest level of total N of the three restorations.

Availability of soil P had no relationship to mycorrhizal inoculum potential (MIP) of the soils of the three restorations. Levels of AMF colonization in the two native prairie grasses and their biomass production were also unrelated to P availability. The 12-year-old restoration soil had significantly lower levels of phosphorus than soil from the other two restorations. However, despite high levels of P in the 2-year-old restoration soil, Wild rye, unexpectedly, had significantly higher colonization when grown in this soil than when it was grown in soil from the 12- and 17-year-old restorations. The 17-year-old restoration soil had a significantly higher MIP than the 2- and 12-year-old restoration soils. Nevertheless, shoot mass tended to be higher, and Wild rye root mass was significantly higher in the 12-year-old restoration soil than when the plants were grown in soil from the other two restorations. These results likely occurred because phosphorus was not a limiting factor in these soils (Schultz et al. 2001), and the generally higher fertility level of the soil from the 12-year-old restoration increased growth of plants compared to their growth in soil from the other two restorations. The lower nutrient status of the 2-year-old site, except for phosphorus, may account for the generally reduced shoot and root growth and tiller development of the two species of grass grown in soil from the 2-year-old restoration site compared to their growth in the soils from the older restorations.

In prairie restoration, nonmycorrhizal or facultative mycorrhizal species are thought to dominate early in restorations and are replaced by obligate mycorrhizal species later (Smith et al. 1998). Hart and Reader (2002) showed that among arbuscular mycorrhizal fungi, there is separation among taxonomic groups related to colonization strategies, including the rate and extent to which they colonize roots, with most extensive colonization associated with the fastest colonizers, and the proportion of fungal biomass in roots or soil. However, the authors concluded that there is no evidence that these functional AMF groups benefit host plants differently. Thus, there is an undocumented potential for a correspondence to occur between arbuscular mycorrhizal fungi and host plants dominating a restoration site (Koide 2000; Bever et al. 2003). Nevertheless, initial soil conditions can dictate how mycorrhizal associations influence restoration and modifying initial conditions may aid restoration (Cuenca et al. 1998). Modifying substrate conditions to maximize the beneficial effects of mycorrhizae during restoration requires knowledge of initial substrate conditions including availability of inorganic nutrients and type and amount of mycorrhizal inoculum and plant response to these conditions. Restoration sites should have a high diversity of arbuscular mycorrhizal fungi because of the generally positive relationship between host plant diversity and AMF diversity (Anderson et al. 1984; Bever et al. 2001, 2003;}

![Figure 3. Mean ± SE shoot weight (g) for Little bluestem (Schizachyrium) and Wild rye (Elymus) grown in soil from prairie restorations of three ages. For shoot weight, there were only significant differences among sites. The plants grown in soil from the 12-year-old site had significantly greater shoot weight than plants grown in soil from the 2-year-old site.](image-url)
Implications for Practice

- Several studies have found that changes in soil conditions in restored prairies over time could facilitate changes in prairie species composition and abundance. However, my study indicates that soil properties unrelated to restoration age were important in determining differences in growth and AMF colonization of an early- and a late-dominating plant species in prairie restorations.
- These results suggest that trajectory of plant responses in prairie restoration may be strongly dependent upon initial soil conditions rather than being largely determined by changes in substrate conditions over time on a restoration site.
- Establishing initial soil conditions and resolving how to modify restoration plans based on this information may be an important step in increasing restoration success.

Acknowledgments

I thank Fermi National Accelerator Laboratory for permitting soils to be collected from their restored prairies. J. Jastrow, Argonne National Laboratory, Argonne, Illinois, assisted me in identifying study sites on the Fermilab. M. Simone and J. Laatsch provided technical assistance by scoring of roots for AMF colonization.

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