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Scale-dependent niche axes of arbuscular mycorrhizal fungi

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Abstract Arbuscular mycorrhizal fungi (AMF) are mutualistic with most species of plants and are known to influence plant community diversity and composition. To better understand natural plant communities and the ecological processes they control it is important to understand what determines the distribution and diversity of AMF. We tested three putative niche axes: plant species composition, disturbance history, and soil chemistry against AMF species composition to determine which axis correlated most strongly with a changing AMF community. Due to a scale dependency we were not able to absolutely rank their importance, but we did find that each correlated significantly with AMF community change at our site. Among soil properties, pH and NO_3 were found to be especially good predictors of AMF community change. In a similar analysis of the plant community we found that time since disturbance had by far the largest impact on community composition.

Introduction

Understanding what factors determine the distribution of organisms is one of the fundamental aims of ecology. While describing the niches of large organisms has occupied researchers for some time, an interest in microbial niches has taken longer to develop. With a growing appreciation of the extensive diversity present in the soil and the many impacts that these microorganisms have on aboveground dynamics and ecosystem processes, microbial niches are now being investigated with greater interest (Crawford et al. 2005; Fitter et al. 2005; Moore et al. 2003; Wardle et al. 2004). In this study we investigate the major niche requirements of a particularly important group of soil- and root-inhabiting microorganisms, arbuscular mycorrhizal fungi (AMF), by using a gradient approach represented by a chronosequence of tallgrass prairie restorations located in the prairie peninsula of North America.

AMF are critical members of terrestrial ecosystems and may account for a major portion of the soil microbial community's biomass in soils associated with tallgrass prairie plants (Allison et al. 2005; Hartnett and Wilson 1999; Miller et al. 1995). They are able to colonize cortical tissue of a majority of plants' root systems and are among the most important soil organisms when it comes to nutrient cycling and plant productivity (Smith et al. 1997). In exchange for fixed C, AMF provide nutrients and water to

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their host usually resulting in improved growth. Even when a biomass response for a host is not evident, these fungi appear to be actively involved with up-regulation of photosynthesis and down-regulation of P uptake in prairie grass (Miller et al. 2002).

Mycorrhizal fungi are increasingly the focus of research into conservation and restoration of natural communities (Bever et al. 2003; Johnson et al. 2006; Klironomos 2002; Renker et al. 2004). For many plant species the mycorrhizal association is obligate for they will not survive without the services provided by their fungal partners (Hetrick et al. 1988; van der Heijden et al. 1998a); and these services can vary depending on the origin of host and soil factors (Schultz et al. 2001; Van der Heijden and Kuyper 2001). AMF have been shown to influence plant communities, through their influence on productivity, composition, and diversity in natural and experimental systems (Hartnett and Wilson 1999; van der Heijden et al. 1998b; Vogelsang et al. 2005). These fungi also influence the soil physical environment in prairie soils via the contributions of their hyphae to the stabilization of soil particles into relatively stable soil aggregates (Jastrow et al. 1998; Miller and Jastrow 1990).

Despite a strong interrelationship of AMF with plant communities we do not yet understand what governs AMF diversity or spatial distribution. The most common explanation for coexistence of multiple species in any system, however, is resource-based niche-partitioning; species are adapted to certain resource conditions and can out-compete other species within such an environment (Gause 1934). Under this assumption, AMF distribution would be best explained by the ability of different species to out-compete others when particular resource conditions are present. For AMF, major niche axes would then likely involve either plant host identity or soil parameters. Indeed, both have already been shown to affect the growth and distribution of AMF (Bever et al. 1996; Johnson et al. 1992; Vandenkoornhuyse et al. 2003).

Spatial niche partitioning is another way to achieve coexistence of multiple species. In this model, species compete for a single resource—space—and plant host identity or soil chemical variation do not affect relative competitive abilities (Amarasekare 2003; Levins and Culver 1971; Tilman 1994). Coexistence of multiple species is made possible through life history tradeoffs. For example, a less competitive species can persist in a system with more competitive species by either being a superior colonizer or a faster exploiter of newly available resources (Amarasekare 2003). In both cases some species are expected to be more prevalent in areas more recently disturbed (less competitive species) and others in areas that have long been stable (more competitive species). In a community governed by spatial niche partitioning, "time since disturbance" is an important variable for predicting the abundance of individual

species in a given location. Spatial niche partitioning as realized by the colonization/competition tradeoff is commonly used to explain succession in plant communities, which are often colonized first by fast-growing high-seed-producing plants and later by slow-growing plants with fewer larger propagules (Tilman 1994).

There are many examples of data that support spatial niche partitioning and its associated life history tradeoffs among AMF. To begin with, AMF exhibit wide variation in life history traits likely involved in spatial niche partitioning, such as speed of growth, propagule size, and propagule number (van der Heijden and Scheublin 2007). Daft and Hogarth (1983) found an inverse relationship between AMF species competitive ability and spore production; this supports the notion that some fungi focus on competition and others on colonization. Also, among fungi in general there is an inverse relationship between spore size and number of spores produced (Chuang and Ko 1983); this also supports a colonization/competition tradeoff as species producing larger spores are expected to be better competitors based on analogous plant seed data (Turnbull et al. 2004). Johnson et al. (1991) found that AMF undergo succession from cropland to old field or prairie, as would occur under this model, but a soil chemistry-independent mechanism was not suggested. The possibility of spatial niche partitioning in these and other studies was advanced by Hart et al. (2001), but there has been no dedicated investigation of this possibility as far as we know. Simple resource-based niche partitioning appears inadequate to explain these observations and a more comprehensive investigation is warranted.

Based on these data we would like to propose plant host, soil, and disturbance as putative niche axes for AMF. We posit for example, that changes in the soil composition will support a different AMF community. Likewise, changes in the plant community or time since disturbance will support a different AMF community. While these three variables are not strictly independent (e.g., disturbance undoubtedly impacts the plant community) we believe they represent independent processes and can be investigated separately. We consider the three proposed axes to have the potential to explain a significant amount of beta, or among-site diversity, but we acknowledge there are likely additional niche axes that constitute the overall hypervolume describing the niche requirements of individual AMF species (Hutchinson 1957). Some of the most important additional axes likely include temporal niche partitioning, partitioning of soil depth, and selective predation (Chesson 2000; Dickie et al. 2002; Pringle and Bever 2002; Reynolds et al. 2005).

Each of the three niche axes we propose—soil, host, and disturbance—have supportive studies. The data presented in these studies are inconclusive, however, because the three theories have never been simultaneously tested and

spatial niches have almost never been considered for AMF. Also, many of the studies that have identified an influence of soil or plants have manipulated soil and plant parameters in artificial ways, which make their results difficult to extend to natural systems (Bever et al. 1996; Johnson et al. 1992; Vandenkoornhuyse et al. 2003). Our study utilized a large-scale observational approach with natural variation in soil and plant abundance combined with variation in time since disturbance created through a restoration chronosequence (i.e., restoration age) to simultaneously test these theories in one coherent approach. Our results suggest that all three niche axes are important for maintaining diversity within the AMF community, but that the appropriate scale of inquiry is needed to find evidence of each.

Materials and methods

Study site

The study utilized data from seven tallgrass prairie reconstructions, which form a restoration chronosequence. They are located within the accelerator ring at Fermi National Accelerator Laboratory (Fermilab), Batavia, Illinois, which is approximately 48 km west of Chicago in DuPage and Kane counties. All plots were last planted with row crops in 1969 and 1970 and were colonized by plant species typical of old field succession until each was plowed, disked, and planted with prairie species. As each plot was restored to prairie, soil preparation, planting times, seed collection, and seed application rates changed and evolved with experience (Betz 1986). From 1976 to 1985, the restored prairie plots were burned annually. Newly planted plots, however, were generally not burned until after their third growing season. Time since disturbance (i.e., restoration age) values are assigned based on the number of years since plowing and seeding and ranged from 2 to 11 years. Soil conditions are summarized at the plot level in Table 1.

Sampling

All samples were collected from 17 to 27 June 1985. Ten sampling replicates were located within each plot using a stratified random design. In each replicate quadrat, live aboveground plant biomass was clipped to within 2 cm of the surface inside a 0.5-m^2 circular quadrat. Following the clipping, biomass samples were sorted by species and dried to constant weight at 65°C in a forced-air oven. After removing rare plants (those present in fewer than five quadrats), biomass of 30 plant species was used in the analysis [see Appendix A for a complete list in the electronic supplementary material (ESM)]. AMF spores were isolated from one 20-cm-deep 4.8-cm-diameter soil core taken within each sampling station. Two 25-g (dry wt) soil aliquots were obtained from the 0- to 10-cm portion of the soil core and were combined with two 25-g aliquots from the 10- to 20-cm-depth portion of the core. Spores were removed from each of the soil aliquots via wet sieving through a 40-μm sieve, followed by centrifugation in a sucrose density gradient (McGraw and Hendrix 1984). Species designations were based on those described by Schenck and Perez (1990). After removing rare AMF (those present in fewer than five quadrats), 11 species of AMF were used in the analysis (Appendix B in ESM). For each AM fungal species, number of spores per square meter of soil from 0 to 20 cm in depth was calculated and used in subsequent analyses. Soil chemical and physical analyses were determined on adjacent soil cores obtained within the same 0.5-m^2 sampling quadrat. All soil chemical and texture analyses were performed at the University of Wisconsin-Madison Soil and Plant Analysis Laboratory. Soil parameters tested included pH, organic C (Walkley Black), available P (Bray-1), exchangeable K, exchangeable Ca, exchangeable Mg, bulk density, % sand, % silt, total N (Kjeldahl), soil C:N based on organic C and total N, soil N:P based on mineral N and available P, extractable soil NO_3-N, and extractable NH_4-N.

Table 1 Summary of soil characteristics in sample plots

Fermi plot ID	Restoration age (years)	pH	Organic C (%)	Total N (%)	N0$_3$-N (mg kg^{-1})	Total P (%)	Bray's P (mg kg^{-1})	K$^+$ (mEq l^{-1})	Ca^{2+} (mEq l^{-1})	Mg^{2+} (mEq l^{-1})	D$_b$ (g cm^{-3})	Sand (%)	Silt (%)
1	11	6.31	4.22	0.33	6.81	0.13	18	131	3,114	23	1.17	27	10
2	10	6.55	5.32	0.42	9.57	0.15	16	113	3,259	25	1.09	24	12
4	8	6.27	3.76	0.31	7.22	0.12	14	98	2,994	22	1.21	25	13
6	6	6.94	5.01	0.37	6.92	0.16	9	103	1,764	13	1.11	23	19
7	5	6.33	3.52	0.28	7.66	0.11	17	100	2,827	21	1.21	22	14
9	3	6.87	3.51	0.26	7.50	0.12	19	122	1,586	12	1.22	24	12
11	2	6.88	4.1	0.37	6.43	0.14	8	87	3,721	29	1.17	25	18

One may question whether AMF spore numbers, as we have used them in this study, are reflective of individual AMF species abundance, which is inferred throughout the paper. Spore numbers, however, are correlated with fungal biomass within roots and have been used in numerous studies as a measure of community composition and individual species abundance (Bever et al. 1996; Declerck et al. 2004; Egerton-Warburton et al. 2007; Johnson et al. 1991). While measuring individual species abundances as hyphae within the root and throughout the soil may be the most desirable metric of AMF species abundance, quantitative analysis of AMF in community roots and soil is currently fraught with problems related to matrix interferences, preferential extraction efficiency and the lack of a universal primer set for AMF. We believe that spore abundance data present a good approximation, albeit a more conservative estimate of AMF abundance.

Statistics

Randomly distributed organisms are believed to follow a Poisson distribution (Pielou 1977). To test for this possibility before exploring more complicated causes of community composition we performed an index of dispersion test on all plant and AMF species used in the study. For a dataset x with n elements, this statistic is $(n - 1) \times \mathrm{var}(x)/\mathrm{mean}(x)$ and its asymptotic distribution is χ^2 with $n - 1$ df (Karlis and Xekalaki 2000; Potthoff and Whitting 1966). A P-value is obtained by determining the cumulative density of the χ^2 $(n - 1)$ distribution to the right of the test statistic, and represents the probability that the observed variance arose by chance from a Poisson distribution.

Mantel tests were used to evaluate the relative influence of the three putative major niche axes on AMF community composition. This statistical tool compares distances among quadrats in multidimensional space for one group of parameters and another (e.g., do quadrats which differ greatly in AMF composition also differ greatly in soil composition?). In order to test for scale dependency of niche axes, we analyzed our data both at the quadrat level and also the plot level. To perform the plot-level analysis we first averaged all species abundances and parameters values to the plot level. Mantel tests were performed on dissimilarity matrices of plant and AMF abundances scaled by species abundance maxima across all plots and then by quadrat totals for AMF and plants, respectively. Both Bray–Curtis and Canberra distances were used to judge the sensitivity of our results to the distance metric used (Legendre and Legendre 1998). Bray–Curtis is a commonly used distance metric in ecology giving semi-metric distances whereas Canberra is a metric distance measurement (Legendre and Legendre 1998). They both have highly desirable properties for measuring ecological distances and are also specifically recommended for analyses discussed later (non-metric mul-

tidimensional scaling) (Faith et al. 1987). When calculating Bray–Curtis distances, we square root transformed our raw fungal and plant abundance data before the other standardizations discussed above as it improved rank order relation to distance along environmental gradients.

Soil parameters, which were significantly non-normal were first square root transformed (organic C, available P, bulk density, NO_3-N, and NH_4-N) and all soil data were scaled relative to parameter maxima. Ratio data (e.g., soil C:N and N:P) was omitted from this analysis. Significance values were assessed through 10,000 random permutations of the data matrix and recalculation of Pearson's correlation coefficient. Mantel tests were implemented using R (version 1.14) (R Development Core Team 2006) and the vegan community ecology package (Okanen et al. 2005).

To evaluate relationships more carefully among the AMF, plant, and soil data sets, nonmetric multidimensional scaling (NMDS) was used (Jongman et al. 1995; Kruskal 1964a, b). NMDS is an ordination technique that attempts to minimize the mismatch between the rank order of distances between pairs of samples and the rank order of distances between the points in the abstract space used to represent them. As for the Mantel tests, we used Bray–Curtis and Canberra distance metrics (Faith et al. 1987). To determine the appropriate number of dimensions to use one starts low and increases the number of dimensions until stress, a measure of the mismatch between the abstract reduced dimensional space and the accurate higher dimensional space, plateaus (Jongman et al. 1995). Three dimensions were adequate for our plant and AMF community data. Standardizations, transformations, and distance calculations were done exactly as for Mantel tests. Vectors of soil, plant, and age were fit to the AMF ordination and soil, AMF, and age were fit to the plant ordination. They were assessed for goodness of fit via Pearson's correlation coefficient and significance via 10,000 random data permutations. To correct for multiple comparisons, a Bonferroni correction was made. Because 45 variables were tested against the AMF ordination a P-value of <0.00111 was necessary for a variable to be considered statistically significant. We tested the plant ordination against 25 variables so a P-value of <0.0020 was necessary. NMDS ordinations were conducted using R (version 1.14) (R Development Core Team 2006) and the vegan community ecology package (Okanen et al. 2005).

Results

Are AMF or plant species randomly distributed?

Species abundance data were tested against a Poisson distribution using the index of dispersion statistical test. All AMF and plant species differed significantly from a Poisson distribution (P-values <0.001).

Table 2 Environmental parameters that contribute to arbuscular mycorrhizal fungi (AMF) community compositional change. A three-dimensional nonmetric multidimensional scaling (NMDS) ordination using Bray–Curtis and Canberra distances was created from AMF community data. All soil variables, plant species abundances, and restoration age values were then tested against this ordination using least squares regression. Significance values were generated using 10,000 permutation tests. Only those parameters with P-values <0.00111 (shown in *bold*) are regarded as significant due to a Bonferroni correction for multiple tests. All variables with a $P < 0.05$ are reported
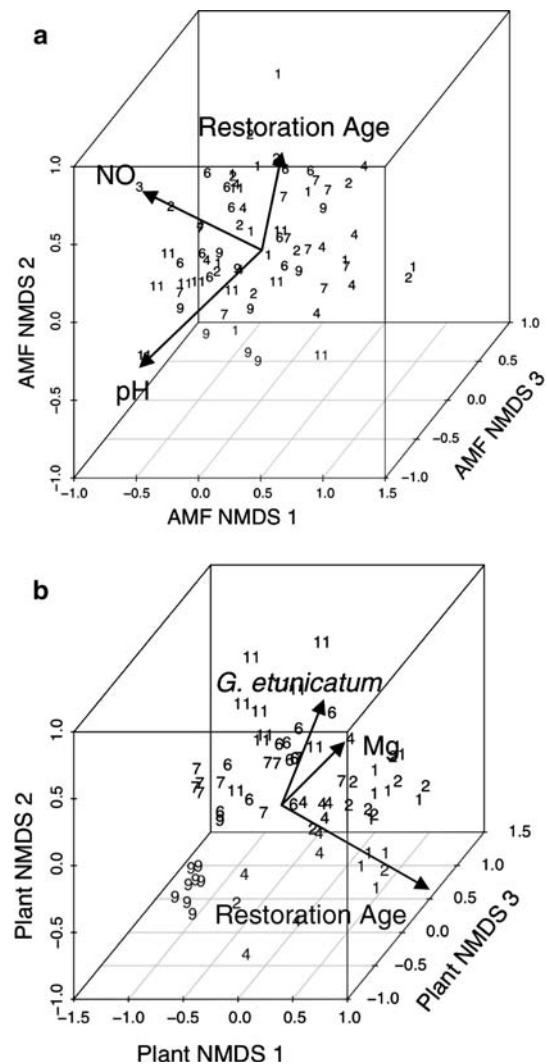
Factors	Bray–Curtis distance		Canberra distance	
	R^2	P-value	R^2	P-value
pH	**0.411**	**<0.0001**	**0.450**	**<0.0001**
NO$_3$-N	**0.325**	**<0.0001**	**0.308**	**0.0001**
Restoration age	**0.225**	**0.0010**	**0.210**	**0.0013**
Total N	**0.223**	**0.0005**	0.182	0.0050
Bulk density	0.188	0.0016	0.136	0.0190
% Sand	0.183	0.0036	0.086	0.1129
Soil organic C	0.179	0.0030	0.162	0.0082
Exchangeable Mg	0.169	0.0086	0.143	0.0185
% Silt	0.156	0.0125	0.118	0.0425
Exchangeable Ca	0.146	0.0156	0.109	0.0547
Cirsium arvense	0.095	0.0857	0.139	0.0179
Available N:P	0.132	0.0275	0.165	0.0090
Taraxacum officinale	0.073	0.1699	0.127	0.0313
Soil C:N	0.123	0.0312	0.050	0.3303
Bromus japonicus	0.117	0.0401	0.155	0.0093
Aster simplex	0.114	0.0465	0.133	0.0236

Fig. 1 Plant and arbuscular mycorrhizal fungi (*AMF*) nonmetric multidimensional scaling (*NMDS*) ordinations with significant predictor variable. NMDS ordinations were created for both **a** AMF and **b** plants. All environmental, disturbance, and community data vectors were fit to the three-dimensional AMF and plant community ordinations. *Arrows* Length is proportional to strength of correlation and they point in the direction of maximum change. *Numbers* correspond to plot ID. *G. etunicatum Glomus etunicatum*

What factors most influence AMF distribution?

All plant and soil factors that significantly contribute to AMF distribution using both Bray–Curtis and Canberra distances for the NMDS ordination are listed in Table 2. The results of the two analyses are basically the same with only small changes in correlation and P-values, so Canberra results will not be discussed further. Using Bonferroni adjusted P-values, no individual plant species significantly affected the AMF community. Soil pH, time since disturbance, total N, and NO$_3$-N all were able to significantly explain the NMDS variation. These parameters are shown as vectors fit to the AMF NMDS space in Fig. 1. For clarity, total N was omitted from the figure because it is highly correlated with NO$_3$-N, and NO$_3$-N has a higher R^2. Restoration age and pH are weakly correlated with each other ($R^2 = 0.17$, $P = 0.004$).

What factors most influence plant distribution?

All soil factors and AMF species that significantly contribute to plant distribution using Bray–Curtis and Canberra distances for the NMDS ordination are listed in Table 3. The results of the analyses are very similar and only the

Bray–Curtis results will be discussed further. Restoration age, the AMF species *Glomus etunicatum* (W.N. Becker & Gerdeman), exchangeable Mg, and exchangeable Ca were able to significantly explain variation in plant NMDS space. Restoration age was by far the most important predictor of plant community composition ($R^2 = 0.85$, $P < 0.0001$). The parameters are shown as vectors fit to the plant ordination space in Fig. 1. Exchangeable Ca was omitted because of its strong correlation with Mg and smaller R^2.

In order to determine whether a specific plant or group of plants was driving the strong *G. etunicatum* relationship, *G. etunicatum* abundance was compared to plants that

Table 3 Environmental parameters that contribute to plant community compositional change. A three-dimensional nonmetric multidimensional scaling (NMDS) ordination using Bray–Curtis and Canberra distances was created from plant community data. All soil variables, arbuscular mycorrhizal fungi (AMF) species abundances, and restoration age values were then tested against this ordination using least squares regression. Significance values were generated using 10,000 permutation tests. Only those parameters with P-values <0.002 (shown in **bold**) were considered significant due to a Bonferroni correction for multiple tests. All variables with a $P < 0.05$ are reported

Factors	Bray–Curtis distance		Canberra distance	
	R^2	P-value	R^2	P-value
Restoration age	**0.849**	**<0.0001**	**0.843**	**<0.0001**
Glomus etunicatum	**0.331**	**<0.0001**	**0.316**	**<0.0001**
Exchangeable Mg	**0.291**	**<0.0001**	**0.284**	**<0.0001**
Exchangeable Ca	**0.230**	**0.0006**	**0.246**	**<0.0001**
Available P	0.193	0.0024	**0.157**	**0.0002**
Exchangeable K	0.184	0.0029	**0.155**	**0.0109**
Gigaspora gigantea	0.165	0.0050	**0.116**	**0.0109**
Available N:P	0.157	0.0088	0.105	0.0381
pH	0.124	0.0309	0.148	0.0629

themselves showed a strong correlation with the plant NMDS space. We found *Setaria* spp. to show a very strong correlation with both the plant NMDS space ($R^2 = 0.45$, $P < 0.0001$) and *G. etunicatum* abundance ($R^2 = 0.25$, $P < 0.0001$).

Contrasting niche axes

In order to directly compare the effects of soil, host, and disturbance on AMF community composition within the same statistical framework, Mantel tests of the data were performed. As in the previous section, Bray–Curtis and Canberra distances were used, but since the results are in broad agreement we will not discuss the Canberra results

further except to point out where they differ. The plant and AMF results appear in Table 4 with the results for AMF shown graphically in Fig. 2 and those for plants in Fig. 3. We found at the quadrat level that soil and disturbance correlate with a changing AMF community. Soil differences explained the most variation ($R = 0.15$, $P = 0.005$) followed by restoration age ($R = 0.10$, $P = 0.015$). When analyzing plant community composition, restoration age was the only significant niche axis and its ability to explain the variation was high ($R = 0.65$, $P < 0.001$). Using Canberra distances, change in soil was significantly correlated with changes in plant community composition ($R = 0.12$, $P < 0.001$) and change in the AMF community was significantly correlated with plant community change ($R = 0.08$, $P = 0.048$).

Results differed when the data were pooled to the plot level and re-analyzed. At the plot level soil was not found to be a significant predictor of AMF community composition. However, the plant community became a significant predictor of AMF community change and restoration age became more important (plant, $R = 0.48$, $P = 0.021$; restoration age, $R = 0.46$, $P = 0.035$). This trend was mirrored in the plant community plot level analysis: soil not significant with restoration age and AMF becoming more significant ($R = 0.85$, $P < 0.001$). Using Canberra distances caused correlations between AMF and restoration age and AMF and plant species composition to become marginally non-significant ($R = 0.34$, $P = 0.052$; $R = 0.33$, $P = 0.070$).

Discussion

Our results support the recognition of restoration age or time since disturbance as an independent factor contributing to AMF community composition. This was demonstrated by both the Mantel and NMDS analyses and by the lack of a large correlation with other important soil characteristics. In this sense, AMF are more similar to their

Table 4 Mantel tests comparing AMF and plant community composition to changes in soil, disturbance, and symbiont. Bray–Curtis and Canberra distance measures of AMF community composition were compared to distances for time since disturbance, soil, and plant community composition. Similar comparisons were made between plant communities and changes in soil and time since disturbance. Analyses were done at both the quadrat- and plot-level scales. P-values were generated via 10,000 randomization tests. Significant values are shown in bold. Results for Bray–Curtis distances are shown graphically in Figs. 2 and 3

Comparison	Bray–Curtis distance				Canberra distance			
	Quadrat		Plot		Quadrat		Plot	
	R	P-value	R	P-value	R	P-value	R	P-value
AMF and disturbance	**0.10**	**0.015**	**0.47**	**0.028**	**0.03**	**0.021**	0.34	0.052
AMF and plant	0.05	0.121	**0.48**	**0.022**	**0.08**	**0.048**	0.33	0.070
AMF and soil	**0.15**	**0.005**	0.23	0.205	**0.12**	**0.005**	0.26	0.156
Plant and soil	0.06	0.079	0.17	0.253	**0.12**	**<0.001**	0.06	0.366
Plant and disturbance	**0.65**	**<0.001**	**0.86**	**<0.001**	**0.53**	**<0.001**	**0.83**	**<0.001**

Fig. 2 Mantel plots of AMF community versus potential niche axes at different scales. Bray-Curtis distance matrices for the AMF community were compared to distance matrices for time since disturbance, plant community and soil community. Comparisons were made at the quadrat (**a, c, e**) and plot level (**b, d, f**). Mantel correlation and significance values are reported in Table 3. Least squares regression lines are added to plots that show a significant relationship
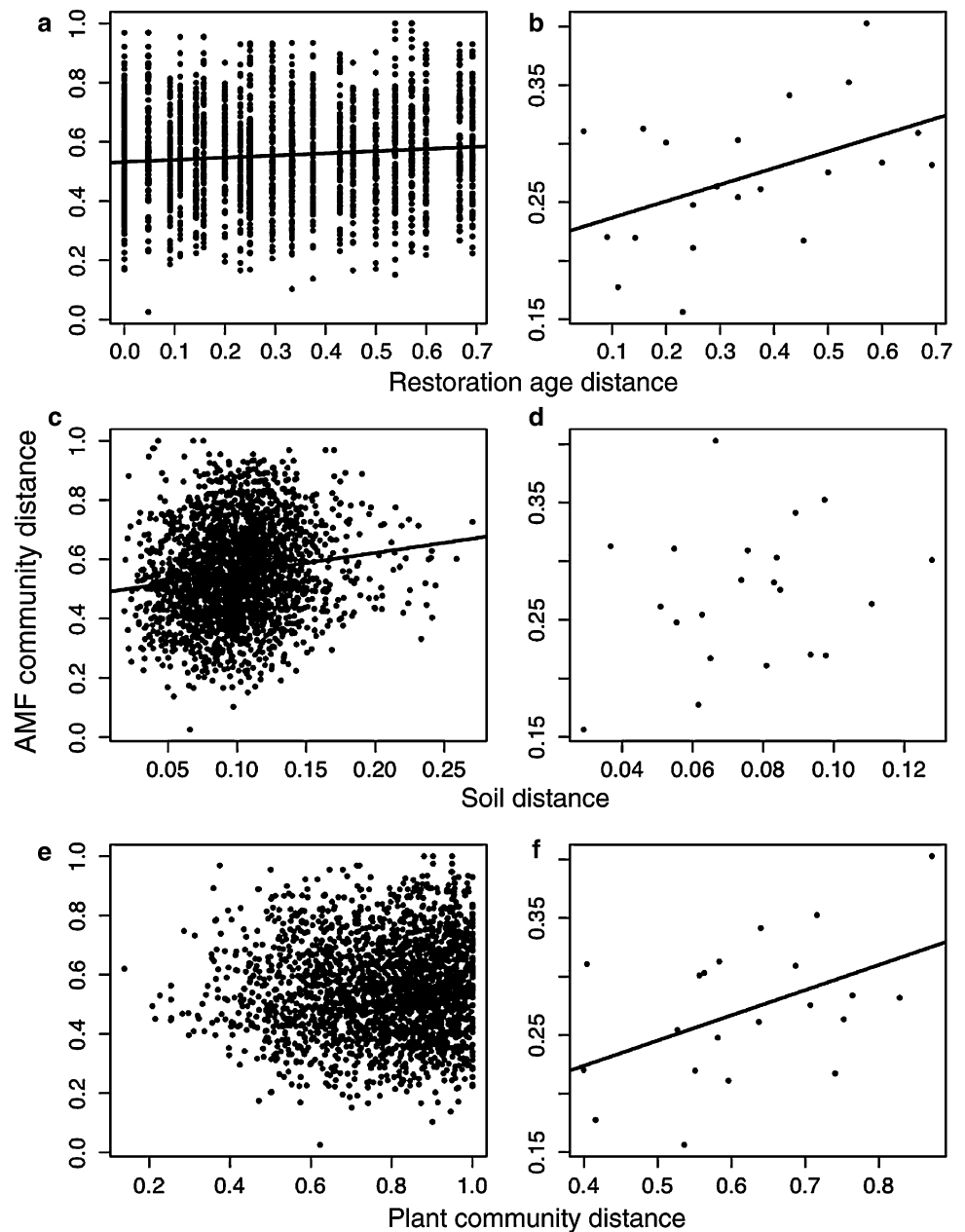

symbionts, plants, than one might have expected; both undergo succession. While our study used large-scale plot-level disturbances to uncover the influence of disturbance, it is our belief that disturbance at finer scales in natural systems may be important as well (Hole 1981).

As was anticipated, soil and plant composition are also important predictors of AMF community composition, but identifying their effect requires examining the data at the appropriate scale. In the Mantel tests, the influence of plants is only apparent if one examines data at the plot level and soil data are only significant when examined at the quadrat level. The trends are the same using Canberra distances if one examines the correlation coefficients and does

not interpret $P = 0.05$ as a firm cut off for significance. We suggest that the scale dependency of plant/AMF interactions is likely due to the much greater mobility of plants compared with AMF. Plants, because of their seed dispersal capability, are much more mobile than AMF (which sporulate underground), allowing for their abundance to exhibit greater point-to-point changes from year to year. Additionally, spores may persist in the soil for multiple years further clouding the plant/AMF interaction signal. This suggests that even if plant identity causes variable growth rates among AMF species, which it certainly does, measures of plant biomass for a specific point will not necessary correlate with AMF species abundance for that position. At the

Fig. 3 Mantel plots of plant community versus potential niche axes at different scales. Bray-Curtis distance matrices for the plant community were compared to distance matrices for time since disturbance and soil community. Comparisons were made at the quadrat (**a, c**) and plot level (**b, d**). Mantel correlation and significance values are reported in Table 3. Least squares regression lines are added to plots that show a significant relationship. AMF and plant interactions are shown in Figure 2
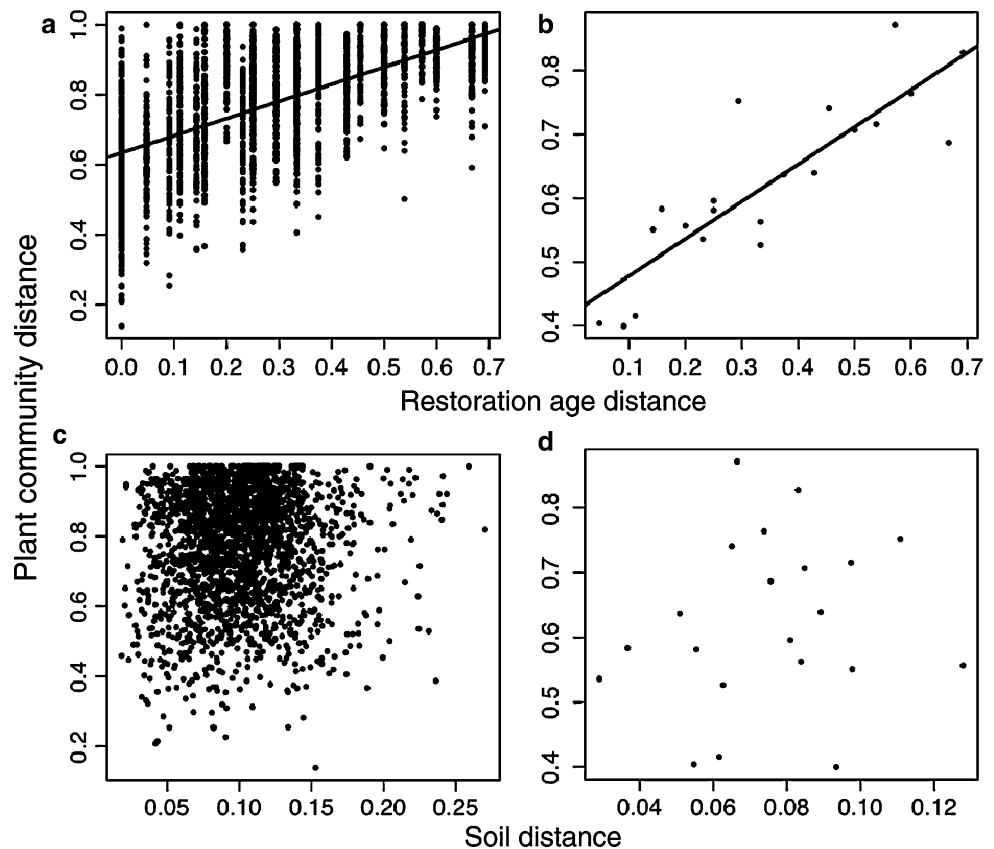
plot level, however, these differences are smoothed over and the significance of plant identity may be more readily revealed. Soil composition, on the other hand, does not vary widely from year to year and thus one can find the signal of interaction at each individual sampling point, but not at the plot level due to intraplot variation.

We found that the factors influencing plant composition were different from those affecting AMF composition. Restoration age is far more important in predicting plant community change. The stronger effect of disturbance on plant composition is likely because the abundance of weedy plant species decreases with time as more competitive, native plant species increase in abundance. The restored prairie plots were initially planted with what is referred to as a "prairie matrix" seed mix (Betz 1986), composed of easy to establish plants so, unlike AMF, plants begin with a suite of competitive species, which with time and appropriate management (e.g., burn regime) take over. More competitive AMF species are also likely increasing in abundance as restoration age increases, but the most competitive species may be entirely absent from the system because the restoration plots started with only those AMF that persisted through a century of agriculture. This then puts finding the signal of succession for AMF at a great disadvantage relative to plants. Because of this our study is likely underestimating the influence of disturbance on AMF community composition.

Unlike AMF, plants responded less to changes in soil conditions. While the influence of Mg and Ca is apparent from the NMDS ordination, overall soil changes did not correlate to a changing plant community at the quadrat or plot scales as observed by the Mantel tests. Results using Canberra distances show plants and AMF communities responding more similarly, however. This observation may be due to the overwhelming influence of restoration age and successional processes on plant community development in our restorations. If we were to look at more mature prairie systems, we would expect to find a stronger influence of soil on plant composition.

We used NMDS analysis to determine which soil factors and plant species specifically contributed to AMF community change and to verify the impact of restoration age. This was to give us an idea of how the soil or plant niche was further partitioned. While changes in plant community affected AMF composition as demonstrated in the Mantel tests, no single plant appeared to significantly affect the AMF community. Since the plant community is fairly diverse, it should not be surprising that the impact of individual species would not be detected. Soil parameters on the other hand, when examined individually, had a much larger effect than was obvious from the Mantel test of soil influence; soil pH, for instance, explained over 40% of the variation in the NMDS ordination. Since pH is known to control the availability of a suite of soil chemicals, it is not

surprising that this factor should be a crucial parameter affecting community composition. It has also been shown in separate studies at this site and by other researchers to be an important predictor of microbial community composition (Allison et al. 2005; Fierer and Jackson 2006). Levels of various forms of N also appeared important in determining AMF community composition (e.g., NO_3-N and total N). That N and not P, which is thought to be the main contribution of AMF to plant fitness, appeared significant is somewhat surprising. The lack of a response to available P for both AMF and plant communities is most likely the result of elevated P levels associated with past agricultural practices. Previous studies using soil from the site indicate, at least for *Andropogon gerardii*, that P is not limiting to host growth (Miller et al. 2002; Schultz et al. 2001). However, similar results for AMF have been reported for remnant oak savannas (Landis et al. 2004). While the role of AMF in relieving N deficiency in plants is not straightforward, it may yet affect the relative fitness of AMF species (Reynolds et al. 2005). Finally, the NMDS analysis supported the role of disturbance in structuring the AMF community.

The plant NMDS analysis revealed some similarities with AMF and some notable differences. Restoration age appears to affect both communities, but different soil parameters influence their distribution. Plants are influenced by exchangeable Mg and Ca and not pH as with AMF. While Mg is an important component of plant nutrition, it is not immediately clear why this element would be the most important soil predictor of plant community change. More interestingly perhaps, a species of AMF, *G. etunicatum*, was strongly correlated with the plant NMDS ordination. We believe this may be due to *G. etunicatum*'s relationship with *Setaria* spp*.,* a non-native grass, which, in turn, strongly and significantly correlates with the plant NMDS ordination. It is probably unwise to speculate which partner is driving the association. Another possibility is that both *G. etunicatum* and *Setaria* spp. are simply early successional species. While present throughout the chronosequence, *G. etunicatum* is most abundant in the plot with lowest restoration age.

The scale dependency uncovered in this study has significance beyond that of AMF and plants. It is important to consider this in future studies attempting to assess interactions among species that disperse at different rates. We are not the first to suggest that choosing the appropriate scale of study is important in ecology, but our study gives a concrete example of when this is crucial to discern an underlying pattern (Levin 1992; Molofsky et al. 2002). Ours is also not the first study to examine the effect of scale on AMF and plant interactions, but it is the first to find a difference. Landis et al. (2004) did a similar analysis, but they recovered nearly identical plot- and quadrat-level correlations in Mantel tests between AMF and plant communities. Since

their sites are older than the primarily early successional prairie plots used in this study, the plant community is likely composed of more perennial species and the plant quadrat composition is thus more stable from year to year. Scale may thus be a more important consideration in communities that have not yet reached equilibrium.

The results of our study have implications for restoration of natural communities. There is an increasing amount of research linking heterogeneity in disturbance to diversity (Fuhlendorf and Engle 2004; Ostfeld et al. 1997; Rosenzweig 1995). The fact that disturbance structures AMF communities and that AMF community composition affects the fitness of plants offers an intriguing second-order mechanism for the effect of disturbance on plant community composition and succession. Our findings suggest that restoration and conservation management plans directed at increasing plant diversity should therefore consider the role of disturbance not only for its direct impact on plant community composition, but also through indirect impacts on the soil symbiotic community. The same argument applies to differences in soil chemistry and its direct and indirect effects on plant community composition.

There are some limitations of this study, which should be addressed in further work. The largest perhaps is that our study is observational and not experimental in nature. This makes it difficult to completely isolate one effect from another. For instance, plant composition and restoration age are so closely related, especially at the plot level, that it is impossible to isolate one factor from another when making comparisons to the AMF community; this is why we did not attempt partial Mantel tests. Also, causation is difficult to infer from the NMDS analysis. Without further study, for example, we cannot determine a mechanism for why *G. etunicatum* was such a good predictor of plant community change. While further experimentation is necessary to validate our conclusions, we have generated testable hypotheses that were not obvious a priori and should form the basis of experimental work in the future.

Another limitation concerns the length of the chronosequence; it spans only 10 years. Since we have uncovered a restoration age effect over this short duration then likely the effect will be much stronger over a greater time scale, but this remains to be proved. A longer chronosequence would also enable one to determine whether the scale dependency between AMF and plants lessens in later successional prairies as we have suggested. Finally, the morphological species concept employed may be obscuring or altering important trends and relationships. AMF have very low morphological diversity and molecular techniques have increasingly become the standard for species level identification (Clapp et al. 1995; Helgason et al. 1998; Vandenkoornhuyse et al. 2002). Such techniques allow one to differentiate among many species that closely resemble one

another and should probably be used in further studies of this nature.

Conclusion

We believe this study has shed light on the niche space of AMF. We have shown that disturbance, in addition to soil and plant host differences, is a strong influence on AMF community composition. We have also further partitioned the soil niche axes into variation in NO_3-N levels and pH. Additionally, the study demonstrates an important scale dependency which needs to be considered in future research focusing on interactions between organisms with different dispersal abilities; annual plants and soil fungi are an excellent example of this disparity. Because of the scale dependency, it is not possible to rank disturbance, soil, and plants in order of importance as niche axes as was our original intent, but they each appear to have a significant influence on AMF community composition. Hopefully, our findings will encourage work regarding the effect of natural disturbance on AMF and plant communities and the effect of pH and NO_3-N on the fitness of AMF.

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