COMMUNITY ECOLOGY - ORIGINAL PAPER

Comparisons of AM fungal spore communities with the same hosts but different soil chemistries over local and geographic scales

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Abstract Arbuscular mycorrhizal (AM) fungi are ubiquitous and ecologically important microbes in grasslands. Both the host plant species and soil properties have been suggested as potentially important factors structuring AM fungal communities based on studies within local field sites. However, characterizations of the communities in relation to both host plant identity and soil properties in natural plant communities across both local and broader geographic scales are rare. We examined the AM fungal spore communities associated with the same C_4 grasses in two Eastern serpentine grasslands, where soils have elevated heavy metals, and two Iowa tallgrass prairie sites. We compared AM fungal spore communities among host plants within each site, looked for correlations between fungal communities and local soil properties, and then compared communities among sites. Spore communities did not vary with host plant species or correlate with local soil chemical properties at any site. They did not differ between the two serpentine sites or between the two prairie sites, despite geographic separation, but they did differ between serpentine and prairie. Soil characteristics are suggested as a driving force because spore communities were strongly correlated with soil properties when data from all four sites are considered, but climatic differences might also play a role.

Communicated by Catherine Gehring.

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Introduction

Quantifying local and regional patterns of biodiversity can offer important insights into the biotic and abiotic factors shaping that diversity. Local community structure may be driven by species interactions and small-scale habitat variation, while community composition differences at larger geographic scales may reflect greater differences in habitat—including climate, historical factors, and dispersal barriers (Ricklefs 2008). Understanding how diversity varies across different spatial scales is a long-standing goal of ecology.

Interest in spatial patterns of biodiversity has only recently extended to communities of soil microbes (Ettema and Wardle 2002; Fierer et al. 2009; Decaens 2010), including arbuscular mycorrhizal (AM) fungi, which form symbiotic associations with the majority of vascular plants (Smith and Read 1997). One view is that many of the 200 or more AM fungal species (http://www.lrz.de/~schuessler/amphylo/) have worldwide distributions, potentially living with a broad range of host plant species in a remarkable variety of soil environments (Brundrett 1991; Fitter and Moyersoen 1996; Douds and Millner 1999). Endemism has not been identified for any species (Morton et al. 2005). Yet, AM fungal species vary both structurally and functionally (Schenck and Pérez 1990; Dodd et al. 2000; Hart and

Reader 2002), setting the stage for differences in ecological preferences. Moreover, field surveys typically find only 5–30 species in a particular plant community (Douds and Millner 1999; Wang and Qiu 2006). Understanding how AM fungal community composition varies both within and among plant communities, and the abiotic and biotic factors that impact that diversity, are fundamental issues in mycorrhizal ecology.

Edaphic factors are expected to impact AM fungal community composition because the fungi's extraradical hyphae interact closely with soil, serving as conduits for the uptake of water, P, N and other nutrients. Evidence from a few correlative studies, such as one involving an anthropogenic N deposition gradient (Egerton-Warburton and Allen 2000) and another from sites varying in nutrient levels (Johnson 1993), shows changes in AM fungal community composition with large-scale soil heterogeneity.

Studies seldom examine AM fungal community structure as a function of soil properties and host plant species concurrently, despite the potential importance of the latter (Eom et al. 2000; Castelli and Casper 2003; Lovelock et al. 2003). Root anatomy and the physiological status of the host plant, the fungi's sole source of carbon, can also influence AM fungal root colonization, extraradical hyphal development, and sporulation (Sanders 1993; Bever et al. 1996). Thus, differences in plant traits, including phenology (Hetrick et al. 1992; Brundrett 2002) and their ability to influence soil properties (Ehrenfeld et al. 2005), might also lead to differences in associated AM fungal communities. Nevertheless, there is little evidence suggesting specificity of AM fungi among host plant species, at least as defined by fungal species presence or absence (Smith and Read 1997; Sanders 2002).

Understanding the relative importance of local and geographic influences on AM fungal community composition requires investigations at multiple spatial scales, which is the aim of the present study. We examine the compositional structure of AM fungal communities associated with the same four grass species within each of four plant communities as a function of local variation in soil properties and host plant species, between geographically separated plant communities with a similar parent soil substrate and climate, and between communities of greater geographic separation and with stronger differences in substrate and

climate. Two sites are Eastern serpentine grasslands, where soils are elevated in Ni, Cr, and Mg, and two are remnants of tallgrass prairie in Iowa. Three of the four grasses occur at all four sites, giving us the same host plant comparisons replicated among sites, and standardizing host species when making intersite comparisons.

Materials and methods

Study sites

The serpentine sites, Nottingham County Park (S-NOT), Chester County, Pennsylvania and Soldiers Delight Natural Environment Area (S-SOL), Baltimore County, Maryland, are separated by 77 km. Hayden Prairie (P-HAY), Howard County, Iowa and Anderson Prairie (P-AND), Emmet County, Iowa are 500 km apart. Due to their geographic locations, the serpentine sites are warmer and receive greater annual precipitation than the prairie sites (Table 1).

All four are well-established preserves with climax grassland communities where conservation is a concern. Eastern serpentine grasslands are experiencing encroachment by woody vegetation and vines (Latham 1993), and the prairie sites are remnant islands of the once extensive Midwestern tallgrass prairie. As a restoration practice, controlled burning is repeated every 3–5 years at two serpentine sites and every 3–5 years at two prairie sites. All are hotspots for rare, native plant species (Brooks 1987; Knapp et al. 1998). The four C_4 grasses in this study, *Andropogon gerardii* (Vitm.), *Sorghastrum nutans* [(L.) Nash], *Schizachyrium scoparium* [(Michx.) Nash], and *Sporobolus heterolepis* [(A. Gray) A. Gray] are among the dominant species at all four sites, except that *S. heterolepis* is absent from S-SOL. All grasses grow as distinct bunch grasses in serpentine but are larger and intermingle with a more diverse assortment of subdominant warm- (C_4) and cool-season (C_3) grasses, forbs and woody species in the prairie.

At S-NOT and S-SOL the soils are Alfisols, with the former classified as fine-silty or fine-loamy, mostly serpentinic, mesic Typic Hapludalfs or Lithic Hapludalfs, depending on the depth to bedrock (Rabenhorst et al. 1982). The latter soils are classified as fine, mixed, superactive, mesic Typic Hapludalfs or fine, smectitic, mesic Typic Albaqualfs

Table 1 Location and characteristics of the field sites

	S-NOT	S-SOL	P-HAY	P-AND
Coordinates	39°44′N, 76°02′W	39°25′N, 76°50′W	43°26′N, 92°23′W	43°26′N, 94°52′W
Mean annual temperature[a]	11.2°C	12.5°C	6.9°C	7.5°C
Mean annual precipitation[a]	1,290 mm	1,160 mm	890 mm	720 mm
Soil order	Alfisols	Alfisols	Mollisols	Mollisols

[a] 20-year means (1988–2007) from the closest weather stations listed by the National Climatic Data Center (http://www.ncdc.noaa.gov/oa/ncdc.html)

(Reybold and Matthews 1976). At P-AND and P-HAY, the soils are predominantly Mollisols, classified as fine-loamy, mixed, superactive, mesic Typic or Aquic Hapludolls or Typic Eutrudepts (Knapp et al. 1998). In addition to important differences in soil chemistry, the more shallow serpentine soils also have lower water holding capacities (Brady et al. 2005).

Sampling schemes

Soil chemical properties and the AM fungal spore community composition were examined at each site using the following sampling scheme: 15 widely separated (>30 m) collection points were arbitrarily selected in June and early July, a time of active plant growth, in 2004. At each point, we chose the closest individual plant of each species that was large enough for our soil sample requirements. We collected topsoil under each individual, including roots, to a depth of 15 cm, except that some samples were shallower in the rocky serpentine. This yielded 60 soil samples (4 host plants at 15 collection points) from each of S-NOT, P-HAY, and P-AND and 45 samples (3 host plants at 15 collection points) at S-SOL, where *Sporobolus* was absent. Soils were transported in coolers and stored at 4°C.

Each soil sample was divided into several subsamples, including one set aside for an experiment on plant–soil feedback and not included in this study (Casper et al. 2008). The remaining soil was homogenized and divided into three parts, one (approximately 200 g) for setting up trap cultures (Bever et al. 2001), one (approximately 50 g) for a direct assessment of the AM fungal spore community, and one (approximately 10 g) for soil chemical analysis. Trap cultures were set up (details below) with soil samples from under each plant at each of the 15 collection points per site (60 samples each from S-NOT, P-HAY, and P-AND, and 45 samples from S-SOL, 225 samples in total). Soil chemical analyses were performed on soils collected under each plant from 10 collection points per site (40 samples each from S-NOT, P-HAY, and P-AND, and 30 samples from S-SOL, 150 samples in total). From five of these same 10 collection points, spores were extracted from 50 ml of field soil from under each plant (20 samples each from S-NOT, P-HAY, and P-AND, and 15 samples from S-SOL, 75 samples in total), identified to morphospecies, and counted.

Soil analysis

Soils were analyzed for pH, P, K, Mg, Ca, organic matter (OM), total N, NH_4–N, and NO_3–N by the Analytical Services Lab at Pennsylvania State University. Specific methods are: Mehlich 3 (ICP) for plant-available P, K, Mg, and Ca; loss on ignition for OM; combustion for total N;

specific ion electrode for NH_4–N and NO_3–N. Bioavailable contents of soil Ni, Cr, and Fe were analyzed with ICP-AES according to EPA Method 6010C by the Energy Technology Laboratory, US Department of Energy (Pittsburgh, PA, USA).

AM fungal spore community examination

We used soil-borne spore numbers to quantify AM fungal community composition, recognizing that spores are discrete units identifiable to species and represent allocation to propagules. Spores are often used as inoculum in ecological studies (Klironomos 2002).

AM fungal spores were extracted from soil using the modified wet sieve method (McKenney and Lindsey 1987; Castelli and Casper 2003). Representative spores were mounted in a 1:1 mixture of polyvinyl lactoglycerol (PVLG) and Melzer's reagent, and identified to species under a compound microscope based on their color, size, surface ornamentation, hyphal attachment, Melzer's reaction, and wall structure (Schenck and Pérez 1990).

For field soils, spore abundance per morphospecies was determined by counting the number of apparently viable spores in each soil extract. The relative abundance (proportion) of each AM fungal species in an extraction was calculated as n_i/N_i, where n_i = number of spores from the ith species and N_i = total number of spores counted in that extraction (Castelli and Casper 2003). Relative abundance is more commonly used than absolute abundance because absolute numbers are highly sensitive to the overall sporulation level of fungi. The following community descriptors were calculated for each soil sample: number of species per sample S = the number of fungal species present; Brillouin's index of species diversity $H = (1/N)\ln(N!/(n_1!n_2!n_3!\ldots))$, and evenness $E = H/H_{max}$ where H_{max} = maximum value of H (Eom et al. 2000).

Trap cultures were primarily employed to confirm AM fungal species identifications for species recovered from field soil, and they were used for scoring the presence/absence of species only. For each culture, a subsample of field-collected soil was mixed with sterile sand in a 1:5 ratio (soil:sand), placed in a 15 cm plastic pot, and planted with 80–100 surface-sterilized (0.5% sodium hypochlorite for 15 min) seeds of a sorghum-sudangrass hybrid (*Sorghum bicolor* × *sudanense* var. "Super Su 22"). Trap cultures were maintained in greenhouses (Department of Biology and Microbiology, University of Wisconsin Oshkosh), watered daily, supplemented with artificial light on cloudy and winter days, and fertilized regularly. Six months later, the AM fungal spores were extracted from a 100 ml soil sample collected from the center of each trap culture pot and all species were identified. A second generation of trap cultures (reseeding and growing for an additional six months) produced no additional AM fungal species.

Statistical analyses

We first asked whether soil chemical properties and field-collected AM fungal spore communities varied among the different plant species within each of the four sites. Separate ANOVAs were performed for each of the following dependent variables: each individual soil chemical property, total spore number, number of species per sample (S), Brillouin's diversity index (H), and evenness (E) or relative abundance of each AM fungal species, as a function of host plant species and collection point (random factor). In order to meet assumptions of normality, all data were log transformed, except that an arcsine square-root transformation was applied to the relative abundance of individual fungal species. Then, for each site separately, canonical correlation analysis (CCorA) was used to examine potential relationships between all measured soil chemical properties and the AM fungal spore communities, as described by the relative abundance of each species (McGarigal et al. 2000).

We also compared individual soil chemical properties and several variables describing fungal communities among the four sites. Fungal spore communities were described by total spore number, the number of fungal species (S), Brillouin's diversity index (H), evenness (E), and the relative abundance of spores of each AM fungal species. Each was examined using a separate ANOVA. Field site and collection point (random factor nested within field site) served as the two independent variables. Since only a few soil and AM variables showed significant differences among plant species within sites (based on previous ANOVAs), plant species was not treated as an independent factor in these ANOVAs. Post hoc analyses were conducted using Tukey's HSD tests in order to identify significant differences among sites ($P < 0.05$).

To visually compare AM fungal spore communities both among host plants and among sites, we performed nonmetric multidimensional scaling (NMDS) using the Sørensen (Bray–Curtis) distance measure on untransformed spore abundance data (McCune and Grace 2002). NMDS scores for spore communities associated with the different plant species were plotted in two dimensions for each site separately; NMDS scores were also plotted to compare spore communities among the four sites, combining results for all host plants.

We followed NMDS ordination with a multi-response permutation procedure (MRPP), using the Sørensen (Bray–Curtis) distance measure, to test for differences in spore communities among sites and among host plants within sites (McCune and Grace 2002). The MRPP result provides a T statistic and its associated P value, which describes the degree of dissimilarity among groups—with more negative values indicating stronger dissimilarity. Output also includes an A value, which represents within-group agree-

ment; its possible values range from -1 to 1, where 1 indicates that all samples are identical within groups (McCune and Grace 2002). When differences were detected ($P < 0.01$), we performed pair-wise comparisons to detect the groups that differed from each other. An indicator species analysis (ISA) was used to further detect which species discriminated the groups that showed significant differences in MRPP analysis (two serpentine sites vs. two prairie sites, in our case). The significance of the discriminating power of each fungal species was determined by the Monte Carlo permutation test (4,999 permutations).

Finally, canonical correlation analysis (CCorA) was used to examine potential relationships between all measured soil chemical properties and the AM fungal spore communities, as described by the relative abundance of each species (McGarigal et al. 2000), for all sites simultaneously. Only the 75 samples (S-NOT = 20, S-SOL = 15, P-AND = 20, and P-HAY = 20) for which both soil chemical properties and fungal data were available were included in the analysis. The first canonical variables of soil chemical properties and AM fungi were paired to construct the CCorA plot.

ANOVAs and post hoc tests were performed in JMP IN version 5.1 (SAS Institute Inc., Cary, NC, USA). NMDS, MRPP and ISA were conducted using the program PC-ORD, version 5 (McCune and Mefford 2006). CCorA was performed in SAS version 9.1.3 (SAS Institute Inc.).

Results

Soil chemical properties

Edaphic characteristics of prairie soils varied more among plant species than did those of serpentine soils (Table 2). For both prairie sites (P-AND and P-HAY), K, NH_4–N and NO_3–N varied among plant species, often with soils under *Sorghastrum* among the lowest values and those under *Andropogon* among the highest. No single soil chemical property differed among plant species at S-NOT; at S-SOL, soils under *Schizachyrium* had lower levels of NO_3-N than did soils under other species.

Every soil chemical property except pH differed among sites (Table 3). Although differences of greater magnitude occurred between serpentine and prairie, a number of variables differed statistically between the two serpentine sites (P, K, Mg, OM, NH_4–N, Ni, Cr), and a few differed between the two prairie sites (P, Ca/Mg, NH_4–N). In comparison to prairie, serpentine sites were lower in P, K, Ca, Ca/Mg, but higher in NO_3–N. The low Ca/Mg ratio is thought to be one of the major plant stressors created by serpentine (Proctor 1971; Brooks 1987). Serpentine soils also differed predictably from prairie soils in having higher

Table 2 ANOVA results (F statistics) and mean values of soil properties differing significantly among host plant species within a field site

Field site	Soil property	F statistics	Host plant species			
			Andropogon	*Schizachyrium*	*Sorghastrum*	*Sporobolus*
S-SOL	NO₃N (ppm)	$F_{3,18} = 7.26^{**}$	10.7[a]	7.7[b]	12.1[a]	
P-AND	pH	$F_{3,27} = 4.82^{**}$	6.7[a]	6.8[a]	6.7[a]	6.3[b]
	K (ppm)	$F_{3,27} = 5.34^{**}$	242.4[ab]	219.9[ab]	170.6[b]	288.3[a]
	NH₄–N (ppm)	$F_{3,27} = 13.8^{***}$	10.3[a]	8.5[b]	7.6[b]	7.9[b]
	NO₃–N (ppm)	$F_{3,27} = 4.29^{*}$	4.4[a]	3.9[ab]	3.6[ab]	2.5[b]
P-HAY	K (ppm)	$F_{3,27} = 5.93^{**}$	135.2[ab]	116.8[b]	109.5[b]	159.8[a]
	Mg (ppm)	$F_{3,27} = 5.86^{**}$	560.9[a]	530.3[ab]	508.5[b]	573.3[a]
	OM (%)	$F_{3,27} = 5.68^{**}$	9.3[a]	8.7[ab]	8.3[b]	9.7[a]
	NH₄–N (ppm)	$F_{3,27} = 12.8^{***}$	11.2[b]	11.0[b]	9.1[b]	14.6[a]
	NO₃–N (ppm)	$F_{3,27} = 3.93^{*}$	4.6[a]	4.5[a]	3.3[b]	3.2[b]

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Different superscript letters in a row indicate significant differences at $P < 0.05$ based on post hoc Tukey's HSD tests

Table 3 ANOVA results (F statistics) and mean values (±SE) for pH, soil nutrients (plant-available P, K, Mg, Ca, OM, total N, NH₄–N, and NO₃–N), Ca/Mg ratios and bioavailable metal contents (Ni, Cr, and Fe) at different field sites S-NOT ($n = 40$), S-SOL ($n = 30$), P-AND ($n = 40$), and P-HAY ($n = 40$)

Soil variables	F statistics ($df = 3,36$)	S-NOT	S-SOL	P-AND	P-HAY
pH	1.78	6.42 ± 0.07	6.66 ± 0.03	6.63 ± 0.07	6.37 ± 0.07
P (ppm)	162.20***	4.9 ± 0.4[c]	2.7 ± 0.1[d]	9.3 ± 0.5[b]	21.6 ± 0.8[a]
K (ppm)	91.67***	74.5 ± 4.0[c]	45.6 ± 1.9[d]	230.3 ± 14.1[a]	130.3 ± 6.1[b]
Mg (ppm)	201.68***	2471.6 ± 105.1[a]	1655.1 ± 52.5[b]	647.1 ± 16.4[c]	543.2 ± 12.0[c]
Ca (ppm)	296.68***	570.5 ± 26.8[a]	430.8 ± 15.5[a]	3520.7 ± 178.7[b]	3330.1 ± 84.7[b]
Ca/Mg	770.23***	0.24 ± 0.02[c]	0.26 ± 0.01[c]	5.41 ± 0.19[b]	6.14 ± 0.10[a]
OM (%)	5.12**	11.3 ± 0.9[a]	7.0 ± 0.2[b]	7.3 ± 0.3[b]	9.0 ± 0.2[ab]
Total-N (%)	4.26*	0.39 ± 0.03[ab]	0.34 ± 0.01[b]	0.44 ± 0.02[ab]	0.49 ± 0.01[a]
NH₄–N (ppm)	11.68***	14.7 ± 0.9[a]	9.7 ± 0.5[bc]	8.6 ± 0.3[c]	11.4 ± 0.5[ab]
NO₃–N (ppm)	46.66***	11.4 ± 0.7[a]	10.2 ± 0.7[a]	3.6 ± 0.3[b]	3.9 ± 0.3[b]
Ni (ppm)	482.39***	163.6 ± 12.3[b]	219.7 ± 8.1[a]	3.4 ± 0.2[c]	2.9 ± 0.1[c]
Cr (ppm)	18.12***	0.25 ± 0.01[b]	0.38 ± 0.03[a]	0.14 ± 0.01[c]	0.15 ± 0.01[c]
Fe (ppm)	11.74***	44.0 ± 8.7[a]	21.0 ± 2.3[ab]	7.8 ± 0.9[c]	14.0 ± 1.0[bc]

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Different superscript letters in a row indicate significant differences at $P < 0.05$ based on post hoc Tukey's HSD tests

levels of Ni and Cr. There was no clear separation between serpentine and prairie for OM, total N, NH₄–N, and Fe,

AM fungal spore communities

A total of 13 AM fungal species were identified from field soils and trap cultures across all four sites: *Glomus aggregatum*, *G. claroideum*, *G. constrictum*, *G. etunicatum*, *G. geosporum*, *G. mosseae*, *G. rubiforme*, *G. tortuosum*, *Acaulospora spinosa*, *Archaeospora leptoticha*, *Entrophospora infrequens*, *Gigaspora gigantea*, and *Scutellospora calospora*. Among these species, *G. geosporum*, *G. tortuosum* and *A. spinosa* were found only in trap cultures, and they were generally present at low frequency (<23% of the cultures per site). As mentioned previously, a second generation of trap cultures did not yield additional AM fungal species.

The relative abundance of each AM fungal species per sample was calculated for all ten AM fungal species making up the standing spore community in field samples. Except for *Gi. gigantea*, which was absent from the prairie sites, all ten were found at all four sites. Differences in the relative abundance of the individual AM fungal species and spore community descriptors among host plants within sites were negligible. The relative abundance of only one species at only one site (*G. aggregatum* at P-AND) varied among
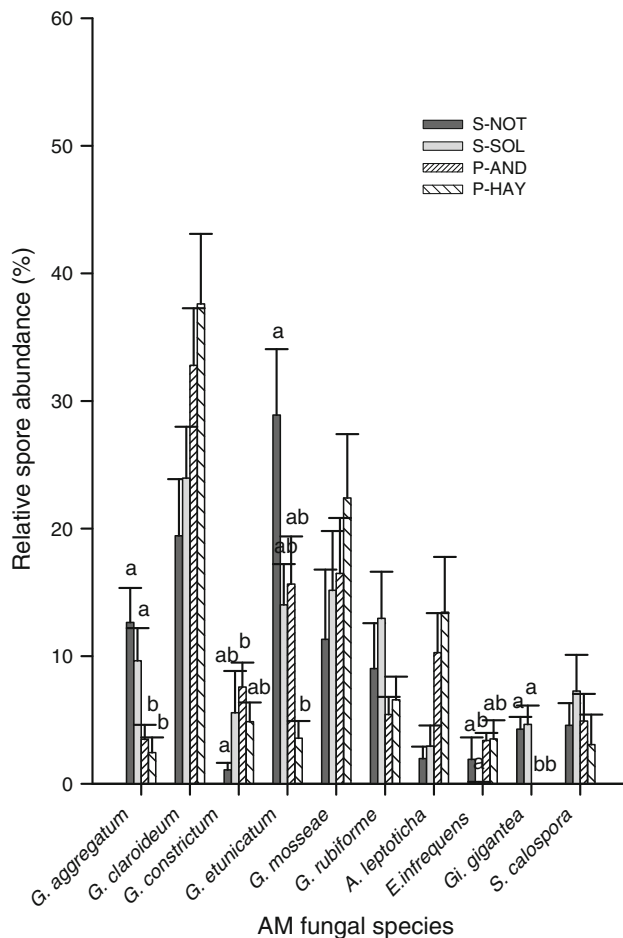
Fig. 1 Relative abundances of spores (mean ± SE) at each field site (S-NOT, $n = 20$; S-SOL, $n = 15$; P-AND, $n = 20$; and P-HAY, $n = 20$) for the ten AM fungal species found in field samples. Differences among sites for a particular fungal species are indicated by *different letters at the tops of bars* (ANOVA, post hoc Tukey HSD tests; $P < 0.05$)

host plant species ($F_{3,12} = 12.30$, $P < 0.001$), with its relative abundance being greater on *Andropogon* and *Sorghastrum* and lower on *Schizachyrium* and *Sporobolus*. Among the community descriptors (total spore number, number species per extraction, Brillouin's diversity index, and

evenness), total spore number varied among host plants only at S-NOT ($F_{3,12} = 3.80$, $P < 0.05$), and Brillouin's diversity index varied among host plants only at P-HAY ($F_{3,12} = 4.43$, $P < 0.05$).

Some fungal species showed considerable variation among sites in relative abundance; ANOVA revealed significant differences among sites for five species (Fig. 1): *G. aggregatum* ($F_{3,15} = 15.82$, $P < 0.001$), *G. constrictum* ($F_{3,16} = 6.09$, $P < 0.01$), *G. etunicatum* ($F_{3,16} = 9.28$, $P < 0.001$), *E. infrequens* ($F_{3,16} = 5.56$, $P < 0.01$), and *Gi. gigantea* ($F_{3,16} = 20.50$, $P < 0.001$). Among these, *G. aggregatum*, *G. etunicatum*, and *Gi. gigantea* were more abundant in at least one serpentine site, while *G. constrictum* and *E. infrequens* were more abundant in at least one prairie site. For AM fungal spore community descriptors, Brillouin's diversity index and evenness did not vary among sites, while total spore number and the number of species per extraction did (Table 4). S-SOL had the highest spore abundance with 506.2 spores, on average, in 50 ml soil—roughly twice that of S-NOT and P-HAY. Both S-SOL and P-AND averaged about six species per sample, while S-NOT and P-HAY averaged less than five species per sample.

Within each of the four sites, MRPP did not reveal any significant differences in spore communities among host plants, but the difference among sites was highly significant (Table 5). Pair-wise comparisons between either of the serpentine sites and either of the prairie sites were always significant, while neither the two prairie sites nor the two serpentine sites differed from each other (Table 5). NMDS ordination echoed MRPP results. There was no significant separation among host plants within any site (Fig. 2a–d), but there were patterns in community composition among sites; the two serpentine sites (S-NOT and S-SOL) clustered together, and the two prairie sites (P-HAY and P-AND) formed a separate cluster (Fig. 2e).

ISA identified eight of the ten AM fungal species as being significant indicators of serpentine versus prairie sites (Table 6). Serpentine sites had three indicator species: *Gi. gigantea*, *G. etunicatum*, and *G. aggregatum*, with *Gi. gigantea* having the highest indicator value

Table 4 ANOVA results (F statistics) and mean values (±SE) for the total spore numbers in 50 ml of soil, number species per sample (S), Brillouin's diversity index (H), and evenness (E) compared among the four field sites S-NOT ($n = 20$), S-SOL ($n = 15$), P-AND ($n = 20$), and P-HAY ($n = 20$)

Community descriptor	F statistics ($df = 3,16$)	S-NOT	S-SOL	P-AND	P-HAY
Total spore number	5.59**	224.7 ± 23.8^b	506.2 ± 70.0^a	334.2 ± 32.2^{ab}	288.4 ± 41.2^b
Number species per sample	6.01**	4.90 ± 0.24^{ab}	6.20 ± 0.30^a	5.95 ± 0.36^a	4.45 ± 0.25^b
Brillouin's index	2.88	0.37 ± 0.03	0.45 ± 0.03	0.45 ± 0.03	0.36 ± 0.03
Evenness	0.46	0.56 ± 0.03	0.58 ± 0.04	0.62 ± 0.04	0.60 ± 0.05

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Different superscript letters in a row indicate significant differences at $P < 0.05$ based on post hoc Tukey's HSD tests

Table 5 Results of the multi-response permutation procedure (MR-PP) analyses for AM fungal spore communities among host plants within each site (S-NOT, S-SOL, P-AND, and P-HAY) and for spore communities among the four sites

Groups	T statistic	A value	P value
Hosts within NOT	−0.42	0.02	0.30
Hosts within SOL	0.75	−0.03	0.76
Hosts within AND	−0.24	0.01	0.38
Hosts within HAY	−0.47	0.02	0.29
Sites	−8.20	0.07	<0.001
NOT versus SOL	−1.27	0.01	0.108
NOT versus AND	−6.48	0.06	<0.001
NOT versus HAY	−10.17	0.10	<0.001
SOL versus AND	−2.70	0.03	0.019
SOL versus HAY	−4.80	0.05	0.001
AND versus HAY	-1.42	0.01	0.091

Pair-wise comparisons were made among sites only. P values refer to the T-statistics and differences among groups

(80.0). The prairie sites had five indicator species: *G. claroideum, G. constrictum, G. mosseae, A. leptoticha*, and *E infrequens*.

Relating soil properties and AM fungal spore communities

CCorA analyses detected no relationship between fungal spore communities and soil chemical properties within any of the four sites. CCorA results revealed a strong correlation between fungal communities and soil chemistry when all sites were examined together (correlation = 0.85, Wilks' lambda = 0.03, $F_{120, 426} = 1.98$, $P < 0.001$). The first soil canonical variable and the first AM fungal community canonical variable accounted for about 50% of the variability in each data set. The CCorA plot showed a clear division between serpentine and prairie samples (Fig. 3). As shown along the *x*-axis of the CCorA plot, Ca (correlation value = 0.85), P (0.83) and K (0.60) drove the first soil canonical variable towards the prairie samples, with Ni (−0.81), Mg (−0.79), NO_3-N (−0.72), and Cr (−0.62) driving the serpentine samples. Along the *y*-axis, *A. leptoticha* (0.50), *G. claroideum* (0.48), *E. infrequens* (0.28), and *G. constrictum* (0.23) drove the first fungal canonical variable towards the prairie samples, with *Gi. gigantea* (−0.58), *G. aggregatum* (−0.53), *G. etunicatum* (−0.41) and *G. rubiforme* (−0.36) driving the serpentine samples.

Discussion

We found variation in the AM fungal communities only at the largest spatial scale—between Eastern serpentine grass-lands and Iowa prairies. Environmental factors, rather than dispersal limitations, likely shape these differences, since all AM fungal species but one—*Gi. gigantea*—were found at all four sites. While we show that AM fungal community composition is strongly correlated with soil properties when all sites are examined simultaneously, climatic differences between the Eastern serpentine sites and the Iowa sites may also play a role. Both temperature (Douds and Schenck 1990) and moisture (Koske 1987) can impact AM fungal community structure.

Previous experimental work has demonstrated functional differences between the Hayden prairie and Nottingham serpentine AM fungal communities (Ji et al. 2010) when applied in the same (greenhouse) environment, supporting the idea that soil variation influences differences in community composition. That work involved a reciprocal transplant experiment with AM fungal spores collected at the sites and native combinations of soils, plants, and other soil microbes. For *Sorghastrum nutans* but not *Schizachyrium scoparium*, both prairie and serpentine plants grew larger with their native AM fungal community. Moreover, the plant–soil environment into which a single inoculum type was placed (either from P-HAY or from S-NOT) influenced the composition of the spore community produced. Similarly, Schechter and Bruns (2008) reported distinct AM fungal assemblages associated with serpentine and nearby nonserpentine ecotypes of a California native plant species, *Collinsia sparsiflora*.

The absence of *Gi. gigantea* from the prairie sites, and its presence at the serpentine sites, may have functional significance. We base this interpretation on three pieces of evidence. (1) *Gi. gigantea* was also absent from the prairie inoculum used in the reciprocal transplant experiment described above (Ji et al. 2010), where individuals of *S. nutans* grew better in their native soil when inoculated with their native AM fungal spore community, whether prairie or serpentine. (2) Yet another study using controlled, mixed spore communities of *Gi. gigantea*, *G. etunicatum*, *G. mosseae, E. infrequens* and *S. calospora*, all collected at the S-NOT, showed that the deletion of *Gi. gigantea* depressed the growth of *S. nutans* and *A. gerardii* in S-NOT soil more than the deletion of any of the other four AM fungal species did (Doherty 2009). (3) While *Gi. gigantea* has been recorded in studies of other prairie sites, its abundance is typically low (Johnson et al. 1992; Eom et al. 2000; Egerton-Warburton et al. 2007). Species of AM fungi with large spores are thought to be more costly to the host plant (Liberta and Anderson 1986; Vogelsang et al. 2006; Cumming and Kelly 2007), so its absence from or low frequency in particular soils may indicate that its costs outweigh its benefits in those soils. We note, however, that the delineation between serpentine and prairie communities in our study is not

Fig. 2 Nonmetric multidimensional scaling (NMDS) ordination of AM fungal spore communities among host plant species within each field site (**a**–**d**) and among the four field sites (**e**). *Points* represent the centroid for all samples, with a group with *bars* indicating ±1SE of the centroid mean along each axis. Field site codes are as in Fig. 1
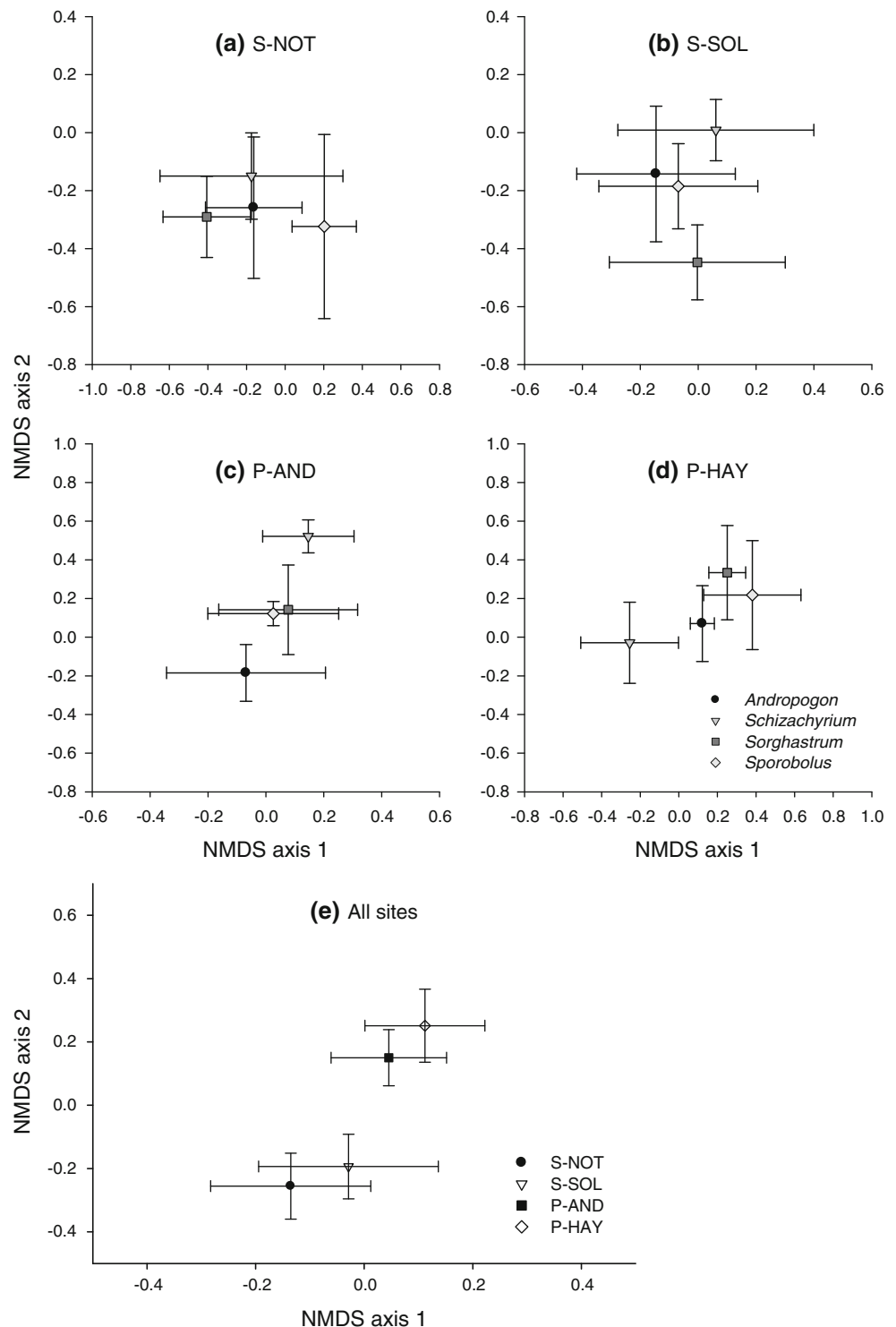
entirely due to *Gi. gigantea*; some small-spored AM fungal species are also more abundant in serpentine, but others are more abundant in prairie (Fig. 1; Table 6).

We were surprised to find no differences in AM fungal communities between the two prairie sites or between the two serpentine sites despite their geographic separation and differences in soil chemistry, which for the serpentine sites

is substantial. The two prairie sites differ in P levels, which is thought to be a primary limiting nutrient driving the formation of mycorrhizae (Smith and Read 1997). The two serpentine sites are in closer proximity, but differ in a suite of soil properties, including P and the heavy metals characteristic of serpentine. Previous studies have reported quite different sets of AM fungal species across prairie sites

Table 6 Results of indicator species analysis (ISA) between two groups, S-NOT and S-SOL are the serpentine group and P-AND and P-HAY are the prairie group

AM fungal species	Group identifier	Observed indicator value	P value
Glomus aggregatum	Serpentine	62.9	<0.001
Glomus claroideum	Prairie	61.0	0.002
Glomus constrictum	Prairie	47.3	0.008
Glomus etunicatum	Serpentine	64.0	<0.001
Glomus mosseae	Prairie	40.6	0.049
Glomus rubiforme	Serpentine	36.6	0.204
Archaeospora leptoticha	Prairie	41.7	0.010
Entrophospora infrequens	Prairie	41.7	0.001
Gigaspora gigantea	Serpentine	80.0	<0.001
Scutellospora calospora	Serpentine	30.3	0.234
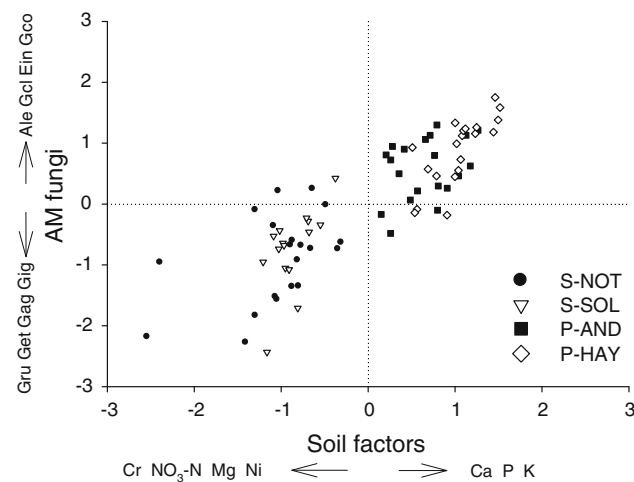
Fig. 3 Canonical correlation analysis (CCorA) plot showing the correlation between the relative abundance of AM fungi and soil variables across all four field sites. The *x*-axis is the first canonical variable of soil factors, and the *y*-axis is the first canonical variable of AM fungi. Each abbreviation of the field site represents an individual sample. AM fungal species or soil variables that made the greatest contribution to their first canonical variable were labeled along their canonical axes. Abbreviations of AM fungal species: *Gag, Glomus aggregatum*; *Gcl, Glomus claroideum*; *Gco, Glomus constrictum*; *Get, Glomus etunicatum*; *Gru, Glomus rubiforme*; *Ale, Archaeospora leptoticha*; *Ein, Entrophospora infrequens*; *Gig, Gigaspora gigantea*

(Bentivenga and Hetrick 1991; Bentivenga and Hetrick 1992; Wilson and Hartnett 1998; Eom et al. 2000), but whether they varied systematically with soil properties was not specifically examined.

Replicated across all four of our sites is the finding that AM fungal community structure did not vary either among host plant species, despite some associated differences in nutrients, or with local variation in soil chemistry, independent of host plant species. Low levels of host specificity have been reported for similar grasslands (Johnson et al.

1991; Sanders 1993; Eom et al. 2000), but the difference may be that the four C$_4$ grasses on which we focus are very similar in phenology, physiology, and root structure. Other studies have shown that in a complex community, plant species composition can influence the AM fungal community (Fitzsimons et al. 2008). The effect of soil edaphic properties on the community of AM fungi may be greater than the influence of plant composition, especially when chemical conditions (such as pH) vary dramatically within a single field (Dumbrell et al. 2010).

Our data provided some support for the classic view that AM fungi proliferate more in low-nutrient soils (Smith and Read 1997). S-SOL had the highest total spore number and more species per sample, which could be related. The community could be responding as much to the lower levels of P, K, and Ca as to the higher levels of Ni and Cr. Plants at S-SOL growing in nutrient-poor soils enriched by heavy metals might allocate more carbon to the formation of mycorrhizae or require a more taxonomically diverse set of AM fungi.

We consciously chose to assess the AM fungal community qualitatively and quantitatively using spore morphology. Other approaches include the use of (1) terminal restriction fragment length polymorphisms (Johnson et al. 2003; Dumbrell et al. 2010), (2) polymerase chain reaction and denaturing gradient gel electrophoresis (Ma et al. 2005), and (3) the cloning and sequencing of extracted DNA (Hempel et al. 2007). Each method has its strengths and drawbacks. Our approach allowed us to identify each morphospecies of the community positively, which is not always achievable with sequence data from field isolates, and make broad geographic comparisons at multiple scales. AM fungi can possess multiple genomes (Kuhn et al. 2001), and even neighboring isolates of a single fungal morphospecies can display a high degree of polymorphism at multiple loci (Croll and Sanders 2009). Because of their clonal nature, it is possible that geographically distant isolates of AM fungal morphospecies would possess unique polymorphisms, making comparisons across scale difficult. Furthermore, trap culturing from comparatively large volumes of soil (relative to DNA-based techniques) allowed us to detect less common AM fungal species. Finally, we used spore communities from S-NOT and P-HAY in the reciprocal transplant study described above (Ji et al. 2010), and such manipulations would be impossible with DNA sequences or polymorphisms. We do recognize that, on the other hand, morphological characteristics are not always sufficient to differentiate similar species. This is especially true for field-collected spores, which are often degraded, and is a main reason that we backed up the field spore collections with trap cultures.

In summary, our systematic examination of AM fungi associated with the same four grasses demonstrates a

remarkable constancy in AM fungal community composition across a range of spatial scales and soil chemistries, except for the most disparate. Differences in AM fungal communities between prairie and serpentine sites cannot be due to dispersal limitation alone, and are likely influenced by environmental factors, including basic chemical and possibly other biotic differences between serpentine and prairie soils.

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