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Research

Relationships among arbuscular mycorrhizal fungi, vascular plants and environmental conditions in oak savannas

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Summary

• We studied the relationships of plant and AMF (arbuscular mycorrhizal fungi) species richness and community composition to each other and gradients in soil texture, nutrient content, and light availability in three oak savannas in southern Wisconsin, USA.

• Sixty-three samples were analysed for plant and AMF composition along sun–shade and sand–loam gradients. Samples consisted of plant community composition at quadrat and point scales, point-scale AMF community composition, canopy transmittance, soil Kjeldahl nitrogen (N) content, available phosphorus (P), and texture.

• Numbers of AMF and plant species at the point scale were positively correlated with each other and increased with soil texture/N content. The compositions of plant and AMF communities were also significantly correlated with each other and the soils gradient. The paradoxical increase in AMF richness on the most fertile soils may reflect their small soil pores or low P : N ratios.

• Plant and AMF communities appear to respond to underlying environmental gradients in similar ways, perhaps reflecting similar responses to soil conditions by both groups, effects of one group on the other or both.

Key words: arbuscular mycorrhizas (AMF), community composition, environmental gradients, glomalean fungi, oak savannas, species richness.

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Introduction

Arbuscular mycorrhizas (AM) are likely a primitive state for all vascular plants, and the glomalean fungi that produce them (AMF, phylum Glomeromycota) are among the most abundant fungi on the planet (Smith & Read, 1997; Brundrett, 2002). Glomalean fungi have been shown to affect plant species richness in greenhouse microcosms and natural communities. Plant species richness often increases with the number of AMF species added in greenhouse experiments (Grime *et al.*, 1987; van der Heijden *et al.*, 1998, 2002, 2004) but can decline with AMF species richness in natural grasslands, based on comparisons after vs before fungicide treatment (Newsham *et al.*, 1995; Hartnett & Wilson, 1999). These studies have led to models predicting that plant diversity should increase with AMF diversity (van der Heijden, 2002), or that AMF effects on plant diversity should depend on the mycorrhizal status of competitively dominant and subordinate plant species (Hartnett & Wilson, 2002; Allen *et al.*, 2003; Urcelay & Diaz, 2003). According to these models, plant diversity should increase with AMF diversity, unless all plants are independent of mycorrhizae, or unless dominant plant species are strongly dependent on AMF while subordinate ones are weakly dependent. In the field, different plant species, life-stages, or carbon cycling strategies are often associated with different AMF species (Clapp *et al.*, 1995; Eom *et al.*, 1999; Cornelissen *et al.*, 2001; Husband *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002), and experiments show that plants affect AMF community composition (Johnson *et al.*, 1992; Johnson *et al.*, 2004, 2004). Together, these studies suggest that plant and AMF

communities influence each other profoundly; in addition, there are strong empirical and theoretical reasons to expect that external ecological conditions (e.g. light, moisture, phosphorus (P) and nitrogen (N) supplies) would also affect plant and AMF communities, both directly and indirectly (Givnish, 1986; Tilman, 1988; Johnson (1992); Smith & Read, 1997; Clark & Zeto, 2000; Corkidi *et al.*, 2002; Facelli & Facelli, 2002; Miller *et al.*, 2002; Bauer *et al.*, 2003). To date, however, no study has simultaneously examined associations (and potential interactions) among plants, AMF, and ecological conditions, especially in diverse communities.

Here, we examine the relationships among plant and AMF communities and environmental conditions in species-rich, environmentally patchy oak savannas of the US Midwest. To simplify the task, we focus on species richness and community composition of vascular plants and AMF. Our goal is to determine how plant and AMF species richness and composition are related to each other and/or environmental gradients within and among sites.

Midwestern oak savannas are among the most floristically diverse plant communities in the central USA. One survey found 417 plant species in 722 m^2 across 12 remnant Wisconsin savannas (Leach & Givnish, 1999). This represents about 22% of the state's vascular flora, and *c.* 90% of these species are herbs or shrubs. Studies of Wisconsin oak savannas have shown that the understory composition correlates with gradients in soil texture/fertility and light availability. The soil gradient involves collinear increases in per cent clay and silt, Kjeldahl N content, exchangeable calcium (Ca), magnesium (Mg) and per cent organic matter, and explains roughly twice as much variation in plant species composition as light availability, which varies from full sun in openings to deep shade near tree boles (Leach, 1996; Leach & Givnish, 1999; Meisel *et al.*, 2002). The AMF response to such gradients and/or vascular plant composition is, however, almost unknown, with just one study relating AMF composition to the single factor of light availability across a natural prairie–savanna–forest gradient in Illinois (Benjamin *et al.*, 1989).

To elucidate the relationships among plant communities, AMF communities, and the physical environment, we surveyed three remnant oak savannas in southern Wisconsin that vary internally in light regime and collectively span the range of a previously investigated soil-texture gradient (Leach & Givnish, 1999). We used the resulting data to determine whether plant and AMF species richness and composition are correlated with each other and/or light availability and soil texture and fertility. We sampled the biotic communities at two different spatial scales to determine whether associations depend on sampling scale. Our hypotheses are that: (1) AMF and plant species richness should be positively correlated with each other (van der Heijden *et al.*, 1998, 2002); (2) AMF and plant species richness should decrease with increasing soil fertility, as a result of increased selection for AM on nutrient-poor soils; and (3) plant and AMF community composition should be correlated with each other, and with light availability and (especially) soil fertility and texture. Given that AMF and plants might respond directly to light and soil gradients, or indirectly to effects of the latter on partners or enemies of a given fungus or plant, there is the potential for complex patterns of association among AMF, plants, and environment. Since oak savannas are among the most endangered communities in the upper Midwest (Curtis, 1959; Nuzzo, 1986; Leach & Givnish, 1999), the results of this study may have fundamental implications for the role of mycorrhizal diversity and composition in their conservation and restoration.

Morphological vs molecular approaches

We used spores to quantify AMF community composition and species richness. This is a natural approach, given that AMF species are described based on spore morphology. Nevertheless, spore records are subject to bias, as with the pollen records used to investigate past communities of higher plants. Sporulation by different AMF species probably depends on biotic and abiotic conditions, and presence of species in the 'spore community' may thus not always reflect their abundance as mycelia or physiological importance as vascular plant symbionts. However, sporulation itself is an important aspect of AMF life cycles, so the ecological distributions of spores produced by different species are important in their own right, in addition to their use as proxies for species distributions. Spores have a limited lifetime (a few months or years) in the soil, at least as entities identifiable to species level, so spore samples reflect a constantly updated record of recent AMF reproduction.

Molecular approaches have the potential to revolutionize studies of AMF communities by bypassing the need to study spores, but currently present several problems. First, rDNA sequences are the basis for most current identification systems at present, but AMF species are notorious for having polymorphic rDNA sequences (Sanders, 2002; Redecker *et al.*, 2003). This has led some to claim that spores contain ribosomal DNA from several different morphospecies (Clapp *et al.*, 2001). The extent to which such variation reflects simple polymorphism among multiple copies of rDNA in a single genome or multigenomic mycelia is still controversial (Sanders, 2002; Sanders *et al.*, 2003; Pawlowska & Taylor, 2004). Individual spores can contain a thousand or more nuclei, and it is normal to recover multiple sequences by polymerase chain reaction (PCR) amplifications from a single spore (Lanfranco *et al.*, 1999; Antoniolli *et al.*, 2000; Pawlowska & Taylor, 2004). Second, the diversity of sequences detected depends on the details of the molecular screening approach taken, and many (if not all) approaches currently in use are inherently unable to detect all rDNA sequences present (Redecker, 2000; Vandenkoornhuyse *et al.*, 2002; Redecker *et al.*, 2003; Schussler *et al.*, 2003). Competitive interactions during PCR can overwhelm uncommon sequences, so accessions

must be intensively cloned and sequenced before rDNA sequence diversity can be reliably established. Given the large number of species potentially present in a sample, and the large number of genomes present in individual mycelia, it might be necessary to sequence 100 or more clones from a given sample to ensure adequate representation of the AMF species present. For ecological studies of AMF community variation along gradients such as that reported here, involving scores or hundreds of samples, the cost of cloning and direct sequencing could be prohibitive (> US$ 250 000). DNA-based microarrays may soon reduce costs dramatically but are not yet developed. Finally, there are currently no individual rDNA primers that permit identification of all major glomalean lineages, let alone species, and no agreed-upon set of primers to use for such purposes. Given that the identities of rDNA sequences are traced to spore morphology, and the substantial issues regarding methodology and cost of molecular approaches, and inherent interest in conducting an initial study of relationships among plant communities, AMF (or at least AMF spore) communities, and environmental conditions in natural communities, we have based the present study on spore morphology. We incorporate molecular approaches in other facets of our systematic and ecological research and look forward to the development of powerful and cost-effective molecular techniques for projects to complement the results of the one presented here.

Materials and Methods

Site selection

We conducted studies in three remnant oak savannas in southern Wisconsin, USA, that span the range of soil textures found in the 12 such savannas investigated by Leach & Givnish (1999). Marshall is a privately owned, remnant wet savanna/sedge meadow on loamy soils in the floodplain of the Maunesha River (43°10′ N, 89°10′ W). Upper Tarr Creek and Lower Tarr Creek (Tarr 1 and Tarr 2 in Leach & Givnish, 1999) are both located at Fort McCoy in west-central Wisconsin (44°0′ N, 90°39′ W). The Lower Tarr Creek site (TCL) lies on sandy loam soils in the floodplain of Tarr Creek. Upper Tarr Creek (TCU) sits on the sandy uplands south of TCL. The sites at Tarr Creek differ strikingly in most soil and vegetation characteristics and, following Leach & Givnish (1999), we treat them as separate sites. The savannas had comparable overall numbers of plant species: 129 species in TCU, 111 in TCL, and 132 in Marshall (Leach & Givnish, 1999).

Sampling design

We sampled each savanna along six transects located using three criteria: (1) each transect ran north–south (except for one transect that deviated by 15° to avoid a tree); (2) each transect was anchored by a living oak tree at the north end of the transect and by a clearing at the south end of the transect to ensure a marked, light gradient; and (3) each transect was surrounded by oaks to ensure a relatively homogenous soil litter and soil chemistry. The last criterion was critical in Tarr Creek, where the overstory is largely a mix of four *Quercus* species and *Pinus banksiana*. In all three savannas, transects were located in most of the available locations that matched these criteria, not randomly across the landscape.

Each transect was 1.5 times the length of the canopy radius of the oak at its terminus, and then divided into three segments of equal length, with the first two under the oak canopy and the last in the gap. These segments provided a full range of light environments, from deep shade to full sun. We randomly placed two nonoverlapping, 1-m^2 quadrats within each segment of each transect. Within each quadrat, we located four points randomly, where soil samples were collected (see below). Environmental and plant data were collected for each quadrat between 22 August and 7 September, 2001.

Refrigerated AMF samples have a roughly 1-yr shelf life (S. Bentivenga, pers. comm.). This constraint, coupled with a mean sample processing time of about 20 person-hours, limited the analysis to 63 AMF samples, one per quadrat per segment per transect (54 samples = 3 sites × 6 transects × 3 segments), plus three extra quadrats per savanna, representing the additional quadrats along one transect in each (9 samples = 3 sites × 1 transect × 3 segments), for a total of 21 samples per site and seven samples per segment (light microsite) per site. Only data from the 63 samples were fully analysed for all four data sets, and data were not separately analysed at the transect level to avoid issues of pseudoreplication.

Data collection

Light regimes in each quadrat were estimated by computer analysis of hemispherical photographs, using SOLARCALC 5.4.1 (Chazdon & Field, 1987; Pearcy, 1988; Chazdon *et al.*, 1996; Valladares *et al.*, 1997). Photographs were taken over the centre of the quadrat at *c.* 1.5 m above the ground. The film plane was leveled and the top of the camera oriented to true north. We estimated the potential direct photon flux density (PFD, mol m^{-2} d^{-1}) at three points during the growing season: the summer solstice (21 June), August 4 and the autumn equinox (September 22).

Soil samples were collected at four random points within each quadrat, using a clean trowel with a 5 × 20 cm blade. Soil was collected from a cone-shaped hole 25 cm^2 in area and 15–20 cm deep, with the trowel cleaned between samples. Samples were stored in zip-lock bags at 4°C, and duff was discarded prior to processing. Two samples were combined and analysed for Kjeldahl N and 0.002 M H_2SO_4-extractable P (available P) at the University of Wisconsin Soil and Plant Analysis Laboratory, Madison, WI, USA. Since Kjeldahl N

had been previously shown to be significantly positively correlated with exchangeable Ca, Mg and per cent organic matter at these sites (Leach & Givnish, 1999), we did not analyse the samples for these constituents. A third sample was processed to determine soil texture using the hydrometer method (Bouyoucos, 1962; Day, 1965).

For each quadrat, we sampled the plant community at two scales. In each quadrat, we tallied the numbers and identities of plant species and the per cent of ground covered by each taxon. At a point in each quadrat above the fourth soil sample, we also noted the plant species whose shoots contacted a 1 cm diameter stake held vertically over the sample point. Plant species nomenclature followed Gleason & Cronquist (1991).

We extracted AMF spores from a 50-g subsample of the fourth soil sample from each quadrat, corresponding to the point sample of plant composition. We used wet sieving (Daniels & Skipper, 1982; Brundrett *et al.*, 1996) with sieves of 500 μm and 38 μm mesh, and centrifugation using a 60% sucrose gradient. Spores (alive and dead) were extracted from the supernatant by pipette under a Meiji EMZ dissecting microscope (Meiji Techno Co. Ltd, Saitama, Japan), mounted in polyvinyl alcohol-lacto-glycerol (PVLG) without staining, and identified and described using an Olympus BX51 compound microscope with Nomarski optics (Olympus America Inc., Melville, NY, USA). Live spores (identified as such by crushing) were identified as unique types with unique identification codes, measured with an optical micrometer, described, and imaged with a scope-mounted Olympus DP11 digital camera (Olympus America Inc.). Both images and fixed slides were stored as reference collections (access available upon request). Identification of species focused on spore wall anatomy, and followed published descriptions (Schenck & Perez, 1990) and the website of the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) (http://invam.caf.wvu.edu/).

Three important decisions were made in utilizing the AMF data. First, we used only AMF species presence/absence data because previous work has shown that spore abundance data are an unreliable estimate of AMF abundance and activity on plant roots (Clapp *et al.*, 1995). Second, we named AMF species parsimoniously. Several pairs of species have a nearly an identical appearance, especially when spores are damaged and diagnostic features are missing. Morphologically similar taxa were therefore assigned to the one species that was unambiguously identified in each sample, unless both species were clearly present. Third, only 93% of spores could be unambiguously identified, and these alone were used in the analyses reported here. Other species were undoubtedly present, but their spores were too damaged or immature to identify definitively. The AMF species richness values reported here should thus be read as systematic underestimates, particularly in the species-rich samples from TCL and Marshall, where most of the unidentifiable spores were found.

Data analysis

The preceding techniques produced four data sets: quadrat data, with per cent cover for each plant species in each quadrat; point data, with presence/absence for each plant species per point sample within each quadrat; spore data, with presence/absence for AMF species in each point sample; and environmental data, with all environmental variables and the numbers of plant and AMF species per sample for quadrat, point and spore data sets. Environmental variables and units are shown in Table 1.

We calculated frequencies (number of occurrences/number of samples) for plant and AMF species based on the quadrat and spore data sets. Plant species in each savanna were also ranked by per cent cover. We subjected the environmental data set to simple exploratory analysis, calculating means ± SD as well as ranges (minimum to maximum values). Tukey's HSD tests were used to assess the significance of differences in subset means at $P < 0.001$, following the Bonferroni correction.

To examine the direct relationships among plant species richness, AMF species richness, and various environmental measures, we calculated correlations among all pairs of variables in the environmental data set. Assuming a significance level of $P = 0.05$, the overall probability that these correlations contained both Type I and Type II errors (false positives and

Table 1 Reference listing of the codes used for each variable in the analyses (units are included as necessary)

Variable	Code
Number of plant species per quadrat	#PlantSpQuad
Number of plant species per point	#PlantSpPt
Number of glomalean species per point	#SporeSp
Kjeldahl N (mg l^{-1})	N
Available P (mg l^{-1})	P
Per cent sand	Sand
Per cent silt	Silt
Per cent clay	Clay
Estimated daily photon flux density, fall equinox (mol m^{-2} d^{-1})	PFDPEqui
Estimated daily photon flux density, May 1 and August 1 (mol m^{-2} d^{-1})	PFDSumM
Estimated daily photon flux density, summer solstice (mol m^{-2} d^{-1})	PFDSum

false negatives) was greater than 99%. Given that there were more statistical tests than samples, controlling for Type II errors (false negatives) was unrealistic. Therefore, we used a Bonferroni correction of $P = 0.001$, leading to an overall probability of Type I error of $P = 0.10$ for all analyses. This increased the confidence in our positive findings, at the cost of discarding weaker correlations. Correlations, regression coefficients, and other statistical tests were calculated using S-PLUS 6 (2001, Insightful Corp., Seattle, WA, USA).

To test for indirect correlations of plant species richness per point and spore species richness with variables in the environmental data set, we constructed a simple structural equation model (SEM: Joreskog & Sorbom, 1996; Shipley, 2000; McCune & Grace, 2002) using LISREL 8.53 (Scientific Software International, Lincolnwood, IL, USA). Because SEMs are sensitive to non-normal data and large differences in scale among variables, data were square-root transformed. This SEM was functionally similar to a path analysis, and was used to test for the presence of indirect or partial correlations among variables. Major differences between standard correlation coefficients among variables and SEM partial correlations would be evidence for the operation of indirect effects (e.g. an effect of plant species richness on AMF richness once the effects of individual soil variables were taken into account).

We used Mantel tests and ordinations to evaluate the relationships among plant community composition, fungal community composition, and environmental factors. We calculated the standardized Mantel statistic using Sørenson distance matrices to determine whether there were significant correlations between dissimilarities among samples in one variable and dissimilarities among samples in another. Tests were applied to the plant, point, and spore data sets using a Monte Carlo approach with 1000 replicates, and implemented using PC-ORD version 4 (1999, MjM Software, Gleneden Beach, OR, USA).

To evaluate relationships among all four data sets, we first used nonmetric multidimensional scaling (NMS) to ordinate the quadrat, point and spore data sets (Kruskal, 1964a,b; Mather, 1976; McCune & Grace, 2002). Nonmetric multidimensional scaling attempts to minimize the mismatch between the rank order of dissimilarities between pairs of samples and the rank order of distances between the points in abstract space used to represent them, increasing the number of dimensions of that space until stress (a measure of the mismatch) declines at a substantially slower rate (Minchin, 1987). This approach is considered one of the most powerful ordination techniques now available (McCune & Grace, 2002) and reduces the inherently multidimensional variation in each data set to variation in two or three significant dimensions, whose relationship to similar axis scores for other data sets can then be explored using standard regression analysis. All NMS ordinations were conducted with PC-ORD version 4, in the 'slow and thorough' autopilot mode, using a Sørenson distance matrix. For the presence/absence spore data, the final stress calculations on autopilot consistently returned a result that became mildly unstable after about 85 iterations. In this case, we ran NMS for 80 iterations, rather than 100, as used by the autopilot. Linear regressions between each environmental variable and ordination axis score per sample were then calculated. Only regressions with $r^2 > 0.20$ were retained. This cut-off resulted in significant regression coefficients of $P < 10^{-4}$. Significant correlations were overlaid in joint plots, with vector components corresponding to the correlation coefficients between each NMS axis and the variable in question. This approach allowed us to determine the associations not only among plant, AMF, and environmental data, but also the relationships among the quadrat, point, and spore ordinations, and thereby to test for similarities in response to underlying environmental conditions.

Results

We found 137 vascular plant species in 63 quadrats distributed across the three savannas studied. Of these, 62 species were found at Marshall, 62 in TCL and 43 in TCU. At Marshall, the dominant understory species was *Carex lacustris*, at TCU, *Carex pensylvanica*, and at TCL, *Rubus hispidus* (Table 2). Of these dominants, both *Carex* species are considered nonmycorrhizal and *Rubus* is considered facultatively AM (Trappe, 1987; Miller *et al.*, 1999). Plant species showed substantial heterogeneity in their distribution across savannas; only four species are common to all three savannas (Table 2). Marshall shared six and 13 species with TCU and TCL, respectively, and TCU and TCL shared 17 species.

We documented a total of 18 named AMF species, with 14 at Marshall, 17 at TCL, and six at TCU (Table 3). The AMF species are, on average, more widely distributed across savannas than the plant species, with only five AMF taxa restricted to single savannas, and five found in all three savannas. *Glomus constrictum* was most frequently found in Marshall, *Acaulospora scrobiculata* in TCL, and *Glomus intraradices* and *Scutellospora dipapillosa* were equally most frequent in TCU (Table 3).

The mean number of plant species was 11.4 ± 3.7 (SD) per quadrat, and 7.0 ± 2.9 per point (Table 4). TCU had significantly fewer plant species per sample than did the other two savannas at both scales ($P < 0.001$). Numbers of AMF species per sample showed a similar, significant pattern of differentiation, with TCU again having the fewest species per sample ($P < 0.001$). Across savannas, an average of 3.7 ± 2.9 species were found per sample, with the mean value ranging from 1.4 ± 1.4 at TCU to 6.0 ± 2.0 at Marshall (Table 5). As expected, the savannas differed significantly in Kjeldahl N, available P, and soil texture (Table 4). Silt, clay, and N content increased in moving from TCU to TCL to Marshall; available P increased from TCU to Marshall to TCL. The savannas did not differ significantly in estimates of understory PFD at any

Table 2 Forty most common plant species of 137 found in this study

Species	Frequency and cover ranking		
	Marshall	TCL	TCU
Carex pensylvanica	–	0.71 (12)	1.00 (1)
Quercus (subg. *Erythrobalanus*)	–	0.43 (17)	0.71 (4)
Prunus serotina	–	0.43 (10)	0.62 (2)
Solidago canadensis	1.00 (6)	–	–
Carex lacustris	0.81 (1)	–	–
Rubus idaeus var. *strigosus*	0.33 (4)	0.14 (13)	0.33 (8)
Helianthus strumosus	0.67 (5)	–	–
Asclepias syriaca	–	0.05 (30)	0.62 (3)
Cornus racemosus	0.33 (10)	0.33 (25)	–
Potentilla simplex	–	0.67 (15)	–
Athyrium filix-femina	–	0.62 (2)	–
Phalaris arundinacea[1]	0.14 (23)	0.48 (5)	–
Fragaria virginiana	0.48 (20)	0.10 (49)	0.05 (30)
Silphium terebinthaceum	0.57 (3)	–	–
Rubus hispidus	–	0.57 (1)	–
Acer rubrum	0.05 (58)	0.43 (20)	0.10 (37)
Elymus riparius	–	0.29 (19)	0.24 (11)
Zizia aurea	0.52 (14)	–	–
Urtica procera	–	0.19 (24)	0.33 (15)
Aster sagittifolius	0.48 (17)	0.05 (54)	–
Oxalis sp. (*stricta*)	–	0.48 (29)	–
Carpinus caroliniana	–	0.43 (4)	–
Vaccinium angustifolium	–	0.19 (38)	0.24 (6)
Helianthus grosseserratus	0.43 (12)	–	–
Rumex acetosella[1]	–	–	0.43 (7)
Geranium maculatum	0.19 (28)	0.24 (26)	–
Impatiens capensis	0.29 (18)	0.10 (8)	–
Ilex verticillata	–	0.38 (9)	–
Thalictrum dasycarpum	0.38 (15)	–	–
Polygonum sagittatum	–	0.38 (23)	–
Glechoma hederacea	0.14 (41)	0.24 (32)	–
Pinus strobus	–	0.05 (55)	0.33 (18)
Viola sp. (*sororia*)	–	0.33 (33)	0.05 (42)
Spartina pectinata	0.33 (2)	–	–
Apios americana	0.33 (7)	–	–
Carex sp. (*tenera*)	–	0.33 (11)	–
Monarda fistulosa	0.05 (57)	0.14 (31)	0.14 (14)
Aster lateriflorus	–	0.24 (18)	0.10 (36)
Lysimachia lanceolata	–	–	0.33 (16)
Amphicarpaea bracteata	0.05 (59)	0.29 (35)	–

TCL, Lower Tarr Creek; TCU, Upper Tarr Creek. The numbers on the left are frequency of occurrence per savanna. The numbers in parentheses are ranks based on total per cent cover per savanna. Nonflowering species with tentative identifications are in parentheses.
[1]Non-native species.

Table 3 Species of glomalean fungi identified from spores in this study

Species	Frequency		
	Marshall	TCL	TCU
Glomus intraradices	0.86	0.48	0.33
Glomus etunicatum	0.71	0.38	0.24
Acaulospora scrobiculata	0.57	0.76	–
Glomus constrictum	0.95	0.10	–
Glomus mosseae	0.71	0.10	0.19
Glomus claroideum	0.57	0.14	0.14
Glomus clarum	0.43	0.24	–
Scutellospora calospora	0.10	0.38	0.14
Glomus geosporum	0.52	0.05	–
Acaulospora spinosa	0.48	0.05	–
Scutellospora dipapillosa	–	–	0.33
Glomus fasciculatum	0.14	0.14	–
Glomus rubriformis	0.14	0.10	–
Acaulospora morrowae	0.05	0.14	–
Acaulospora colossica	–	0.19	–
Archeospora leptotricha	–	0.19	–
Entrophospora infrequens	0.10	–	–
Paraglomus sp. (*brasilianum*?)	–	0.05	–

TCL, Lower Tarr Creek; TCU, Upper Tarr Creek. The species are arranged in decreasing frequency. Species with tentative identifications are in parentheses.

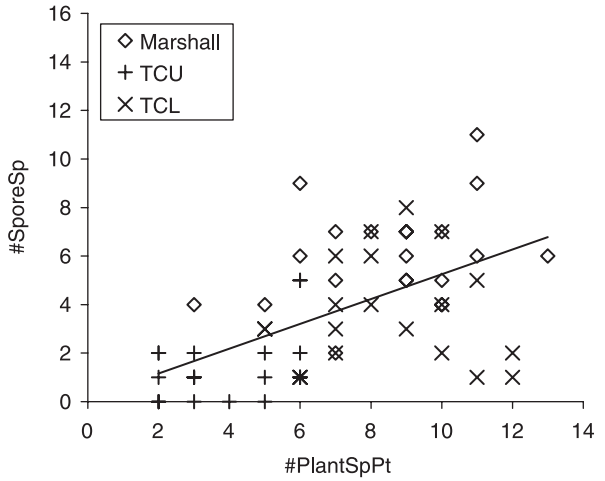


Fig. 1 Number of plant species vs number of arbuscular mycorrhizal fungal (AMF) species, all points (n = 63). For the linear regression shown, r^2 = 0.31, P < 0.00001. TCU, Upper Tarr Creek; TCL, Lower Tarr Creek.

of the times studied, although Marshall was, in general, somewhat shadier than the other savannas.

Species richness

Plant and AMF species richness were strongly and positively correlated when measured at the point scale (r^2 = 0.31, $P < 10^{-5}$) (Fig. 1), but not when plants were surveyed at the quadrat scale (r^2 = 0.03, P > 0.24) (not shown). The number of plant species per point increased significantly with Kjeldahl N, clay and silt contents (r = 0.54–0.59, $P < 10^{-4}$) and decreased significantly with per cent sand (r = –0.59, $P < 10^{-4}$) (Table 5). Numbers of AMF species also increased significantly with Kjeldahl N, per cent silt and per cent clay (r = 0.60–0.70, $P < 10^{-4}$), and decreased with per cent sand (r = –0.70, $P < 10^{-4}$). The number of plant species per

Table 4 Environmental data summary

	#PlantSpQuad	#PlantSpPt	#SporeSp	N (μg g^{-1})	P (μg g^{-1})	% Sand	% Silt	% Clay	PFDEqui (mol m^{-2} d^{-1})	PFDSumM (mol m^{-2} d^{-1})	PFDSum (mol m^{-2} d^{-1})
All sites											
Mean (SD)	18.5 (12.2)	11.35 (3.73)	7.03 (2.92)	3.73 (2.70)	4083.7 (3426.9)	28.9 (19.0)	61.4 (27.4)	17.4 (14.4)	21.2 (15.2)	8.7 (4.7)	14.5 (8.1)
Range	5–21	2–13	0–11	498–13155	11–95	16–95	0–44	4–50	0.73–17.89	0.58–31.63	1.08–43.17
Mean (SD) per savanna											
Mar	11.4 (2.9) ab	8.6 (2.3) a	6.0 (2.0) a	8105.0 (2313.7) a	16.6 (4.5) a	30.7 (10.8) a	29.4 (11.5) a	39.9 (5.8) a	5.96 (4.69) a	10.02 (7.79) a	11.46 (10.33) a
TCU	8.4 (2.2) a	4.1 (1.7) b	1.4 (1.4) b	1035.1 (466.3) b	23.3 (8.1) a	91.3 (2.1) b	1.5 (1.5) b	7.2 (1.5) b	10.33 (3.25) a	16.73 (6.71) a	22.26 (10.22) a
TCL	14.4 (3.5) b	8.4 (2.2) a	3.7 (2.2) c	2861.3 (1267.1) b	48.5 (21.6) b	63.8 (15.2) c	20.8 (8.4) a	15.4 (7.2) c	9.84 (4.95) a	16.93 (8.01) a	22.28 (13.15) a

Mar, Marshall; TCU, Upper Tarr Creek; TCL, Lower Tarr Creek. Variable names and units follow Table 1. Mean (SD) shows mean ± 1 SD on for either all sites or individual savannas. Range values show minimum and maximum. Letters show differences among subsets using a Tukey's HSD test ($P < 0.001$).

quadrat only showed a significant correlation under the Bonferroni correction with available P ($r = 0.43$, $P < 0.001$) (Table 5). As expected, there were highly significant correlations ($P < 10^{-4}$) among Kjeldahl N and all soil texture variables, with correlation coefficients ranging from 0.66 for N–silt, to −0.92 and −0.93 for sand–silt and silt–clay, respectively. Available P showed a significant correlation with clay content ($r = -0.44$, $P < 0.001$). No measure of species richness correlated significantly with any of the light variables, but the latter showed a strong positive correlation with each other ($r = 0.79-0.92$, $P < 10^{-4}$) and a negative correlation to per cent clay ($r = -0.42$ to -0.49, $P < 0.001$).

Generally, there was less than a 0.02 difference between SEM partial correlation coefficients and the linear correlation coefficients in Table 5 (data not shown). The four exceptions, in which the differences between partial and linear coefficients ranged from 0.03 to 0.07, disappeared when we calculated linear correlations on the square-root transformed data used in the SEM. Thus, there appear to be no meaningful indirect correlations, and standard linear correlations (Table 5) seem to represent relationships among plant species richness, AMF species richness and the environmental variables measured as accurately as does a simple structural equation model.

Community composition

Mantel tests showed a significant positive correlation between spore composition and quadrat plant composition across savannas ($r = 0.24$, $P < 0.001$). Spore composition and point plant composition showed an almost identical pattern of correlation ($r = 0.24$, $P < 0.001$).

In addition, all three NMS ordinations showed significant correlations (r^2 from 0.31 to 0.62) among the samples' axis scores on all three ordinations (Table 6). The NMS returned a two-dimensional ordination for the quadrat data (total $r^2 = 0.51$, final stress = 16.98, instability = 10^{-4}) and three-dimensional ordinations for the point data (total $r^2 = 0.69$, final stress = 19.51, instability = 10^{-5}) and spore data (total $r^2 = 0.79$, final stress = 17.49, instability = 0.003) (Fig. 2 and Table 6).

Joint plots of the quadrat, point, and spore compositional data showed the same correlations with environmental variables: strong positive correlations with N, clay and silt and numbers of plant and AMF species per point, and a strongly negative correlation with per cent sand (Fig. 2). That is, AMF spore communities and plant communities at the quadrat and point scales responded in quite similar ways to ecological conditions. The major axes aligned with the N and percent clay gradient explained 25% of the variance in the spore data, 29% of that in the point data, and 34% of that in the quadrat data (Table 6). Scores on the first axes of the quadrat and point ordinations showed much higher correlations with Kjeldahl N (quadrat $r^2 = 0.82$, point $r^2 = 0.55$) and soil texture variables (quadrat $r^2 = 0.53-0.90$, point $r^2 = 0.59-0.74$) than did the corresponding scores for the spore data ($r^2 = 0.33$ for N, and

Table 5 Linear correlations among variables in the environmental data set

	#PlantSpQuad	#PlantSpPt	#SporeSp	N	P	Sand	Silt	Clay	PFDEqui	PFDSumM
#PlantSpPt	0.59***									
#SporeSp	0.18	0.57***								
N	0.12	0.54***	0.63***							
P	0.43**	0.09	−0.23	−0.38*						
Sand	−0.27*	−0.59***	−0.70***	−0.83***	0.31*					
Silt	0.37	0.55***	0.60***	0.66***	−0.12	−0.92***				
Clay	0.14	0.54***	0.70***	0.88**	−0.44**	−0.93***	0.72***			
PFDEqui	0.07	−0.16	−0.18	−0.49***	0.12	0.29*	−0.14	−0.39*		
PFDSumM	0.17	−0.17	−0.18	−0.45***	0.10	0.28*	−0.16	−0.36*	0.85***	
PFDSum	0.18	−0.09	−0.17	−0.42***	0.12	0.28*	−0.16	−0.35*	0.79***	0.92***

*, **, ***, $P < 0.05$, $P < 0.001$ and $P < 0.0001$, respectively.

Table 6 Nonmetric multidimensional scaling (NMS) ordination statistics

											Plot axis		Point axis			Spore axis		
Data Set	Axis	r^2	#Plant SpQuad	#Plant SpPt	#Spore Sp	N	P	Sand	Silt	Clay	1	2	1	2	3	1	2	3
Plot	1	0.34	–	0.32	0.50	0.82	–	−0.82	0.53	0.90	N/A	N/A	−0.32	0.62	–	–	–	0.39
	2	0.17	–	–	–	–	–	–	–	–	N/A	N/A	–	–	0.59	−0.31	–	–
Point	1	0.21	–	–	–	0.22	–	0.22	–	0.27	–	0.32	N/A	N/A	N/A	–	–	–
	2	0.29	–	0.40	0.41	0.55	–	−0.72	0.59	0.64	–	0.62	N/A	N/A	N/A	–	–	−0.20
	3	0.19	0.18	–	–	–	0.2	–	–	–	–0.59	–	N/A	N/A	N/A	–0.39	–	–
Spore	1	0.25	0.13	0.42	0.23	0.33	–	–0.39	0.29	0.37	–	0.43	0.22	0.23	–	N/A	N/A	N/A
	2	0.22	–	–	–	–	–	–	–	–	–	–	–	–	–	N/A	N/A	N/A
	3	0.33	0.16	–	–	–	0.2	–	–	–	0.31	–	–	–	0.40	N/A	N/A	N/A

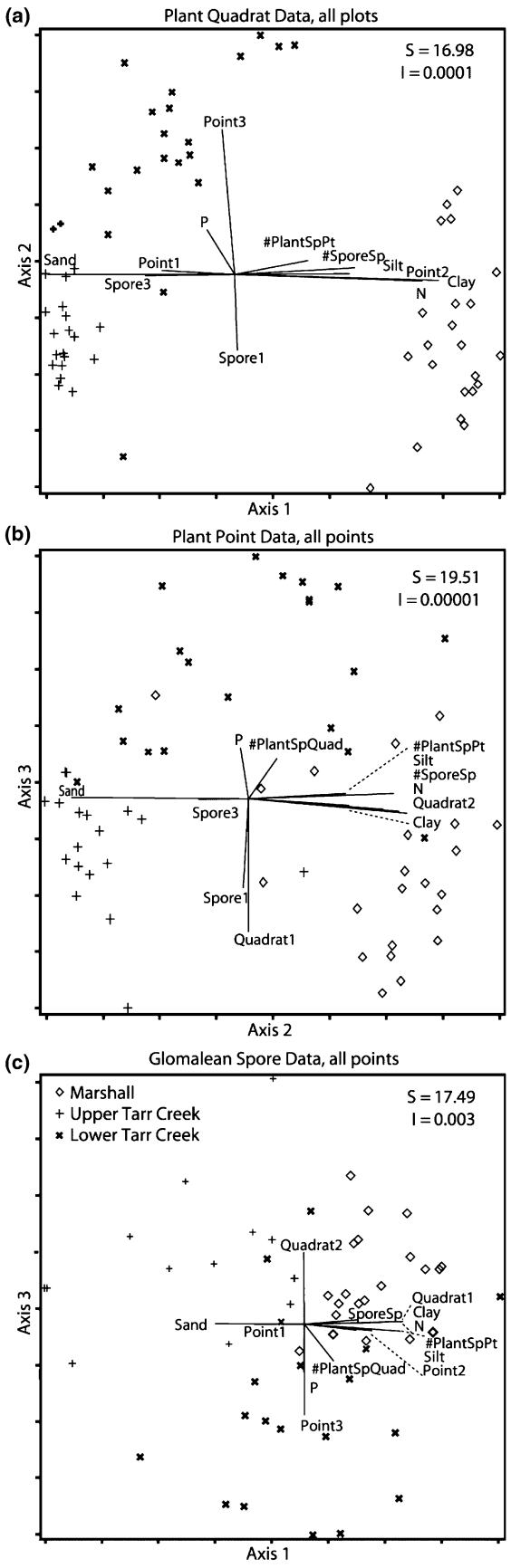
Data sets are the ordinations in Fig. 2, and r^2 values show the proportion of variance explained by each axis. All environmental and axis correlates are r^2 values, with the minus sign showing the direction of the correlation. Environmental correlates are as listed in Table 1, The Plot, Point, and Spore correlates show correlations among the NMS axis scores for every sample. Only correlates with $P < 0.0001$ (linear regression against axis) are shown.

r^2 = 0.29–0.39 for other soil variables) (Table 6). Intriguingly, plant and AMF diversity also showed similar responses to this major environmental gradient: numbers of plant species per point had r^2 values of 0.32, 0.40 and 0.42 for the first axes of the quadrat, point, and spore ordinations, respectively, while numbers of AMF species had a r^2 values of 0.50, 0.41, and 0.23 for same axes of these ordinations (Table 6). Available P (quadrats and point ordinations r^2 = 0.20) and numbers of plant species per quadrat (spores and point ordinations r^2 = 0.20) correlated more strongly with other axes (Table 6).

Discussion

We found support for our first hypothesis: the numbers of plant and AMF species per sample were indeed positively correlated across savannas and microsites within savannas (Fig. 1). Given that the dominant understory species in each of the savannas studied are nonmycorrhizal (*C. pensylvanica* and *C. lacustris*) or facultative AM (*R. hispidus*), these results are consistent with the models proposed by Hartnett & Wilson (2002), van der Heijden (2002), and Urcelay & Diaz (2003): increased mycorrhizal diversity is likely to benefit competitively subordinate plants and thus increase overall plant diversity. Significant correlations between AMF and plant species richness were found only at the point level and not between AMF species richness and plant species richness at the quadrat level. This result underscores the importance of sampling scale in determining patterns in species richness. Reports of scale dependence of patterns in AMF species richness have surfaced in recent years (Schultz, 1996; R. M. Miller, unpubl. data), but few, if any, detailed accounts have been published. Correlations between plant and AMF diversity tend to be lower within individual savannas than across savannas, but this would be expected simply based on restricting an identical correlation structure across savannas to a narrower range of ordinates within savannas. On average, each sample contained the largest number of plant and AMF species at Marshall, and the fewest plant and AMF species at Upper Tarr Creek (TCU).

Our second hypothesis was contradicted, at least at first glance: the numbers of AMF and plant species increased



significantly with soil fertility (specifically, Kjeldahl N content, per cent clay and per cent silt). Our prediction had been to expect the greatest number of AMF species in the sandiest, least fertile substrates at TCU (Table 4). However, Marshall, with the highest N concentrations, had the greatest numbers of AMF and plant species (Table 4; Leach & Givnish, 1999). Paradoxically, our data might support a rephrased version of our second hypothesis, in which increased AMF incidence is favored by a greater relative limitation of plant growth by phosphorus vis-à-vis nitrogen or other soil nutrients. Most importantly, AMF may provide no added value in harvesting P from sandy soils with large pores that can be penetrated by root hairs. Alternatively, low P : N ratios might favor the dominance of strongly mycorrhizal plants, if P uptake is one of the major benefits of mycorrhizal association. Soil P : N ratio is lowest (0.002) at Marshall and highest (0.225) at TCU, and shows a strong correlation ($r = 0.63$) with AMF species number across all samples. Paralleling our expections, Phoenix *et al.* (2004) recently found that N deposition induced greater root-surface phosphatase activity in graminoids and forbs in calcareous grasslands. Soil P : N ratio might not provide the best estimate of the actual relative supplies of these elements to plants and AMF. However, several studies suggest that foliar P : N ratios are reliable indicators of the degree to which plant growth is limited by P vs N (Koerselman & Meuleman, 1996; Bedford *et al.*, 1999; Lockaby & Conner, 1999; Tessier & Raynal, 2003).

Our data demonstrate some distributional differences among genera of Glomales along the major nitrogen–soil texture gradient. *Scutellospora* was most common in the TCU, the sandiest, low-N savanna, whereas most *Glomus* species were found in Marshall, the high clay, high-N savanna, and *Acaulospora* showed little apparent preference. This distribution pattern of *Scutellospora*, *Glomus* and *Acaulospora* by soil texture has been observed elsewhere (Egerton-Warburton & Allen, 2000; Treseder & Allen, 2002).

We found support for our final hypothesis: plant and AMF community compositions were significantly correlated with each other and showed strikingly similar responses to environmental gradients (Table 6; Fig. 2). Mantel tests showed significant positive correlations between AMF composition and plant composition at the point and quadrat levels. Furthermore, NMS axis scores for all three ordinations (point, quadrat and spore data sets) were correlated with each other, suggesting that there are fundamental similarities in the ways in which plant

Fig. 2 Joint plots of nonmetric multidimensional scaling (NMS) ordinations for Quadrat, Point and Spore datasets. The two strongest axes are shown, and figures are rotated to show the overlays in the same orientation. S, final stress; I, final instability. Overlays show environmental variables as listed in Table 1. In addition, axes from each ordination are overlaid on each other. Quadrat1 and Quadrat2 are scores from the two Quadrat axes, Point1–Point3 are from the Point ordination, and Spore1–Spore3 are from the Spore ordination. Correlation coefficients are given in the Table 6.

and AMF community composition vary along environmental gradients generally, and not merely those we measured directly. These ordinations represent reductions of 18- to 137-dimensional data (one dimension per species) to two or three dimensions, so there is no a priori mathematical reason to expect significant correlations among ordination scores as an artifact.

Both AMF and plant community composition were significantly correlated with the N–soil texture gradient and with species richness per point at all measurement scales. No relationship was found between community composition and the light gradient, however, which seems consistent with the weak effect of the latter on plant communities in the more extensive sample analysed by Leach & Givnish (1999). The remarkable collinearity of shifts in AMF composition and species richness, plant composition and species richness, soil silt and clay content, and N content, observed in this study makes it difficult to infer any possible interaction of the effects of two or more of these variables on the others (e.g. plant species richness and soil texture on AMF species richness) using studies of direct or partial correlations. However, our results are perhaps the first to extend the finding of a positive association between AMF species richness and plant species richness – originally documented in greenhouse microcosm experiments (Grime *et al.*, 1987; van der Heijden *et al.*, 1998, 2002, 2004) – to natural communities.

The rise in AMF species richness with soil N content has a number of potential explanations beyond those based on soil texture or soil P : N ratio. Sporulation might be stimulated by higher soil N content. Leach & Givnish (1999) found that Kjeldahl N content was positively correlated with the levels of Ca, Mg and soil organic matter across the savannas surveyed, so one or more of these nutrients may be driving AMF species richness. Finally, because a substantial fraction of variation in soil N involves variation among the three study sites, it is possible that historical contingencies associated with different savannas (e.g. different burning frequencies or differential invasion by various organisms) might be largely responsible for the differences seen. Although these possibilities are worth pursuing, we cannot test them with current data.

We did not find support for any ecological effect of light availability similar to those reported in previous studies on savanna plant communities (Leach & Givnish, 1999; Meisel *et al.*, 2002). We believe that this reflects the low sample size in our study and the inherently weaker association of plant communities with light vs soil seen in previous studies, rather than being an artifact of Type II errors introduced by the Bonferroni correction (use of $P < 0.001$). The previous studies cited had at least twice as many samples per savanna as ours did, and none of the correlations between AMF or plant communities and light availability in our study would have been significant even if we had used $P < 0.05$ as the cut-off.

As indicated in the Introduction, a key question in result interpretation is whether the sampling protocol for AMF was adequate, given that we studied only spores and thus missed nonsporulating taxa, and could not determine which fungi were active on plants roots at the time (Clapp *et al.*, 1995; Husband *et al.*, 2002). Trap culturing and some molecular techniques could sample nonsporulating fungi, but we chose to use spore morphology after evaluating these methods. We experimented with trap cultures, but cultures from all three savannas generally returned only about 50% of the AMF species we found in the samples that were visually analysed, and the cultured species were all found among spores identified microscopically (F. C. Landis and C. J. Andrew, unpubl. data). Spore identification thus appears to provide a more accurate measure of AMF species composition and richness than trap culturing for this study.

Molecular surveys using techniques such as terminal restriction fragment length polymorphisms (T-RFLPs) might, in principle, provide more accurate estimates of AMF community composition and diversity than spore data. However, the methodological problems associated with current molecular approaches are by no means trivial to resolve (see Introduction). More importantly, a molecular version of the present study would have been far more costly and logistically complex than any AMF study published to date (e.g. Husband *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002; Vandenkoornhuyse *et al.*, 2003; D Johnson *et al.*, 2004), and extrapolating AMF species diversity from sequence diversity would not be straightforward (Clapp *et al.*, 2001; Kuhn *et al.*, 2001). Finally, most objections to the use of spore data appear to come from previous uses of spore *numbers* to estimate AMF abundance and activity (Clapp *et al.*, 1995). In this study, we used spore data only to infer the *presence/absence* of AMF species in different savannas. We look forward to testing the preliminary results reported here by using molecular approaches once they become sufficiently powerful and cost-effective. However, we find it extremely hard to believe that the striking correlations among AMF community composition and species richness, plant community composition and richness, and environmental factors that are demonstrated in this paper by our use of spore morphology could be artefacts.

Our findings indicate that the composition and diversity of understory plant and AMF assemblages in Midwestern oak savannas display similar responses to environmental gradients, including primarily soil N content, per cent silt and per cent clay. AMF and plant composition and species richness are positively correlated with each other across the savannas studied, although the correlations of species richness across plants and fungi are scale-dependent. Surprisingly, AMF species richness was highest on the richest soils, contrary to expectations based on proposed advantages to plants of mycorrhizal nutrient capture, but consistent with the observed trend for plant species richness to increase toward richer soils in savannas (Leach & Givnish, 1999). In Midwestern oak savannas, plant and AMF communities appear to be linked to each other across the mycorrhizal interface, and to the soil they both inhabit. It is remarkable that both groups show such strong and similar responses to soil texture and N content.

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