Arbuscular mycorrhizal fungi respond to increasing plant diversity

Rhoda L. Burrows and Francis L. Pfleger

Abstract: The effect of plant diversity (1, 2, 8, or 16 species) on arbuscular mycorrhizal fungi (AMF) was assessed at the Cedar Creek Long-Term Ecological Research site at East Bethel, Minnesota, from 1997 to 1999. At each of the five samplings, AMF in 16-species plots produced from 30 to 150% more spores and from 40 to 70% greater spore volumes than AMF in one-species plots. Regressions of spore numbers and volumes with percent plant cover, plant diversity, and soil NO_3 as independent variables suggest that midsummer plot soil NO_3 was the best single predictor of AMF spore production in these plots. Plant diversity influenced spore volume in four samplings and spore numbers in the first three samplings. Plant cover was predictive of spore volume throughout the experiment but of spore number only in the first year. Sporulation by larger-spored AMF species (*Gigaspora* spp. and *Scutellospora* spp.) increased significantly with increasing plant diversity, while sporulation of the smaller-spored species varied in response to host diversity. Spore numbers of several AMF species were consistently negatively correlated and none positively correlated with midseason soil NO_3 concentrations, demonstrating the adaptation of these AMF species to nitrogen-limited conditions.

Key words: mycorrhiza, community, grassland, sporulation, nitrogen, specificity.

Résumé : Les auteurs ont évalué l'effet de la diversité végétale (1, 2, 8, ou 16 espèces) sur les champignons arbusculaires mycorhiziens (AMF) à la station Cedar Creek Long-Term Ecological Research à East Bethel, au Minnesota, de 1997 à 1999. À chacun des cinq échantillonnages, les AMF dans les parcelles portant 16 espèces ont produit 30 à 150% plus de spores, et un volume de spores 40 à 70% plus grand que les AMF dans les parcelles ne portant qu'une seule espèce de plante. Les régressions impliquant les nombres de spores et leurs volumes avec le pourcentage de couverture végétale, la diversité végétale, et le NO_3 du sol comme variables indépendantes suggèrent que le NO_3 du sol au milieu de l'été est le meilleur indicateur pris isolément de la production en spores de ces parcelles. La diversité végétale a influencé le volume de spores dans quatre échantillons, et le volume des spores au cours des trois premiers échantillonnages. La couverture végétale a permis de prédire le volume de spores tout au long de l'expérience, mais le nombre de spores seulement la première année. La sporulation d'espèces AMF à grosses spores (*Gigaspora* spp. et *Scutellospora* spp.) augmente significativement avec l'augmentation de la diversité végétale, alors que la sporulation des espèces à petites spores varie selon la diversité des hôtes. Les nombres de spores de plusieurs espèces AMF sont négativement corrélés et de façon congrue, et aucun ne montre de corrélation positive avec la teneur du sol en NO_3 en mi-saison, ce qui démontre l'adaptation de ces champignons AMF aux conditions limitantes en azote.

Mots clés : mycorhizes, communauté, prairie, sporulation, azote, spécificité.

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Introduction

Arbuscular mycorrhizal fungi (AMF) form symbioses with the roots of most plant species. In exchange for carbon from plant hosts, these fungi can help increase uptake of nutrients (Bolan 1991; George et al. 1995), enhance resistance to disease (Newsham et al. 1995), and increase drought tolerance (Davies et al. 1993). Although AMF are non-host-specific in their ability to infect a wide range of hosts, the degree of benefit to each partner in any given AMF – host plant interaction can depend on the particular species involved. Both host and AMF community structures may be influenced by such differential effects between individual AMF–host partners. The composition of the AMF community may be strongly influenced by the host species through differential effects on hyphal growth and sporulation (Bever et al. 1996; Daniels Hetrick and Bloom 1986; Eom et al. 2000; Johnson et al. 1992; Sanders and Fitter 1992). In return, the plant community structure may be strongly influenced by the specific composition of the associated AMF and the effectiveness of each of the fungal species in promoting growth of each host (Grime et al. 1987; Hartnett et al. 1994; Streitwolf-Engel et al. 1997; van der Heijden et al. 1998*a*, 1998*b*).

Despite their importance, the effects of these mutual feedback mechanisms on natural communities are only now beginning to be studied. Part of the difficulty in studying these symbioses in natural systems is the complexity of interactions between plant and fungal communities. Typically, field surveys find from five to 30 AMF species at a given site

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(Douds and Millner 1999), and as many as eight AMF species have been found colonizing a single 5-cm root segment (Tommerup 1988). A single "individual" may simultaneously colonize the roots of several plants of different ages and (or) species that, in turn, have unique influences on each of the fungi. Field studies must take into account these complex interactions of plant and fungal assemblages.

The differential effects of single host plant species on individual fungi or fungal assemblages have been well documented, but we have not found other studies that directly examine the effects of varying plant species diversity on the associated AMF community. An increase in plant diversity might increase AMF species diversity and (or) spore production in several ways. As the host community becomes more diverse, the likelihood increases that it will contain plant species differing in their ability to support particular AMF species, thus leading to an increase in overall AMF species richness. For example, an array of host species may furnish a broader selection of rhizosphere microenvironments. Root exudates of different species may differ, influencing the germination and growth of specific AMF species (Douds et al. 1996; Tsai and Phillips 1991). More diverse plant communities would also likely result in a widened range of phenology of root growth or of peak photosynthate production or transport to the roots (e.g., C_3 versus C_4 plants), thus supporting AMF growth for longer periods throughout the growing season (Allen et al. 1984). This could lead to an increased number of AMF species that are active at different times throughout a season (Allen et al. 1995) as well as increased total AMF spore production. Increases in plant diversity are frequently accompanied by increases in plant biomass (Hector et al. 1999; Tilman et al. 1996), so that more diverse plant communities may have increased fixed carbon and more root surface area available to support AMF growth and sporulation. Increased plant diversity in grassland ecosystems is linked with reduced soil inorganic nitrogen (NO_3 and NH_4) levels (Hooper and Vitousek 1997; Tilman et al. 1996), which may influence AMF species composition through differential responses in spore production among AMF species (Egerton-Warburton and Allen 2000; Johnson 1993). These factors, singly or in combination, could cause associated AMF communities to respond to increased plant diversity with increased AMF species richness and spore production.

Spore production can be characterized by both spore number and total spore volume; each provides unique information. Spore numbers are more frequently used and can serve as rough indicators of the reproductive capability of the AMF species present. However, the information that spore numbers provide can be skewed by differences in size and germinability among the spores. For example, certain AMF species produce profuse numbers of small spores (<100 μm in diameter) that can germinate only once and tend to lose viability relatively quickly. Other AMF species produce large spores (up to 450 μm in diameter) that can germinate numerous times and may be more effective than small spores in initiating colonization (Daniels et al. 1981; Koske 1987). Calculating total spore volumes helps to account for these differences while also providing an estimate of the carbon resources directed to spore production (Koske 1987).

The objectives of this research were to determine, in a field setting, the effects of plant species diversity on spore production and species composition of the associated AMF. Our hypotheses were that increased plant diversity will result in (*i*) increased AMF spore numbers and total spore volumes, (*ii*) increased AMF species richness, and (*iii*) shifts in AMF species composition. We also investigated possible indirect mechanisms for observed treatment effects, including the role of plant biomass and soil NO_3 changes associated with increased plant diversity in AMF community changes, and evidence for specificity between individual plant and AMF species.

Materials and methods

Field plot design

We tested these hypotheses at the Cedar Creek Long-Term Ecological Research (LTER) site near East Bethel, Minn. (45°35′N, 93°10′W), in field plots in which the number of plant species was experimentally controlled. These plots were established in 1994 to test the effects of increasing plant biodiversity on ecosystem functioning (Tilman et al. 1997; a complete description of the experimental plot setup is available at http://www.lter.umn.edu/research/exper/e120/e120.html [last accessed 28 January 2002]).

The 342, 11 m × 11 m plots were established on a former bromegrass pasture with soil type Nymore series sand, pH 5.9–6.5. The surface 5–10 cm of soil was removed and the remaining soil disked and seeded with native tallgrass prairie species. The plots were irrigated in 1995 and 1996 to help insure seedling establishment and were weeded throughout the experiment to exclude invading plant species. A 2.5 cm × 20 cm deep soil core was collected from a point 2.8 m inward from each of the four corners of each plot in July 1997, 1998, and 1999, and cores from the same plot were pooled for determination of KCl-extractable NO_3 and NH_4 concentrations using a Technicon II autoanalyzer (Wedin and Tilman 1990).

In order to characterize the AMF associated with various plant diversity levels in this experiment, we randomly selected a subset of 24 plots from each of four (1, 2, 8, and 16 species) plant diversity levels for a total of 96 plots. The particular plant species planted in each plot were determined by an independent random draw of the appropriate number of species (1, 2, 8, or 16) from a common pool of 20 species (Table 1). This assignment of species by random draw theoretically minimizes the confounding effects of individual plant species. A 2 m × 0.5 m subplot was established within each selected plot for AMF sampling. Within each subplot, early-season soil samples were taken in mid-June 1997 and mid-May 1998, coinciding with vigorous growth of C_3 grasses each year; late-season samples were taken in late August 1997 and 1998 and in early September 1999, when C_4 grasses were mature. At each sampling time, six 1.25 cm × 15 cm deep soil cores were taken from random locations within each subplot. These were pooled and then thoroughly mixed with a food processor and stored at 5°C until determinations of spore counts and establishment of trap cultures. Plant biomass of each species was estimated by visual assessment of percent cover of each species in each subplot in midsummer of every year, a time selected because both early-season and late-season plant species were present. A 0.5 m × 0.4 m grid was placed over each one-fifth section of each subplot, and the

Table 1. Plant species present in the Cedar Creek LTER biodiversity plots sampled during 1997–1999, East Bethel, Minn.

Latin name	Common name
Achillea millefolium L.	Yarrow
Agropyron smithii Rydb.	Western wheatgrass
Amorpha canescens Pursh.	Leadplant
Andropogon gerardii Vit.	Big bluestem
Asclepias tuberosa L.	Butterfly-weed
Bouteloua gracilis (HBK.) Lagasca	Blue grama grass
Dalea purpureum Venten.	Purple prairie-clover
Dalea candida Michx.	White prairie-clover
Dalea villosum Nutt.	Silky prairie-clover
Elymus canadensis L.	Canada wild rye
Koeleria cristata (Lam.) P. Beauv.	Junegrass
Lespedeza capitata Michx.	Bushclover
Liatris aspera Michx.	Blazingstar
Lupinus perennis L.	Lupine
Monarda fistulosa L.	Wild bergamot
Panicum virgatum L.	Switchgrass
Poa pratensis L.	Kentucky bluegrass
Schizachyrium scoparium (Michx.) Nash	Little bluestem
Solidago nemoralis Aiton. Gray.	Goldenrod
Sorghastrum nutans [L.] Nash	Indiangrass

percentage of ground covered by each plant species in each grid was recorded separately and then averaged to obtain both total plant cover and cover by species.

Spore data

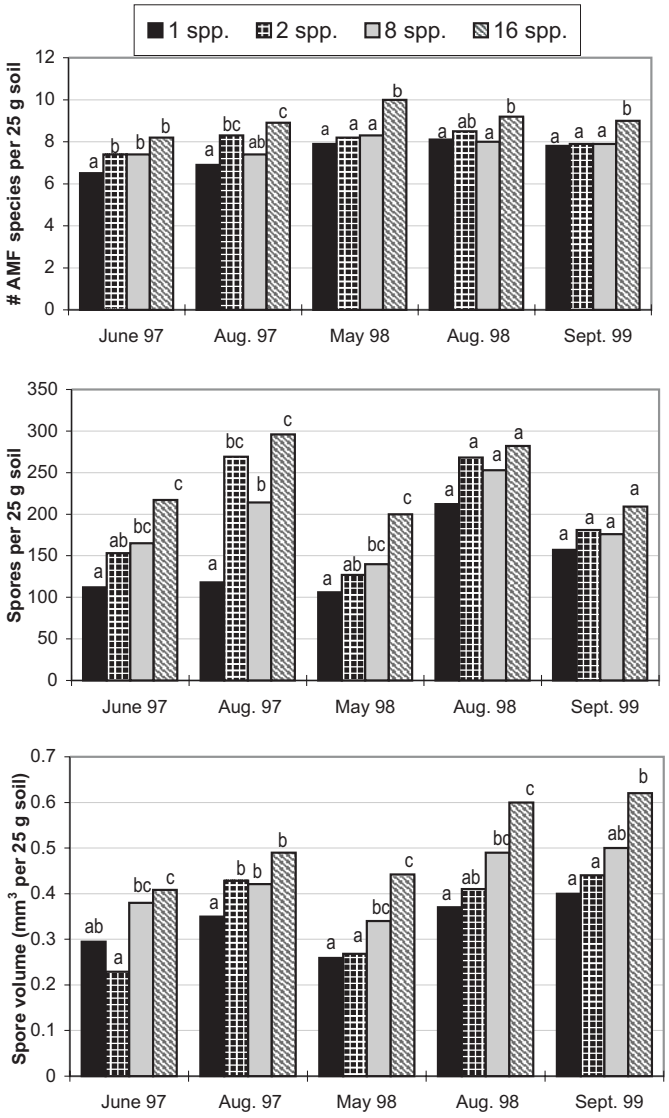
AMF spore density and species numbers

At each sampling, a 25-g dried soil subsample from each plot was wet-sieved through nested sieves (sizes 250, 90, and 25 μm) and separated by sucrose centrifugation (McKenney and Lindsey 1987). Spores were subsequently identified under magnification with species designation based on color, size, and wall structure (Schenck and Perez 1990; online taxonomic descriptions availiable at http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm [last accessed 28 January 2002]), and those of each distinct species or morphotype were counted. Because of difficulty in identification of field specimens, all *Gigaspora* spores were counted as one group, as were all small hyaline spores (*Paraglomus* spp.).

Spore volume

To calculate spore volume in each plot, the average spore size of each AMF species was multiplied by the spore count of that species within each 25-g sample (Koske 1987). Diameters of representative spores were determined for each species when it was first identified, and spore volume was calculated assuming a spherical shape for each spore. In subsequent random measurements, we found that spore sizes within each species did not vary appreciably among plots or sampling times and were in good agreement with published values (online taxonomic description availiable at http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm).

Fig. 1. Plant diversity effects on mean number of AMF species, spore density, and total spore volume of Cedar Creek LTER biodiversity plots. Letters indicate significant differences within sampling time at $p = 0.05$ by Fisher's protected LSD.



Trap cultures

To furnish fresh spores for positive identification of AMF species present and to encourage sporulation of species present only as hyphae in field samples (Stutz and Morton 1996), trap cultures were established for every plot from the pooled soil cores taken at each field sampling in 1997 and 1998. For each trap culture, 125 mL of soil inoculum containing spores and hyphae (stored moist for less than 7 days at 5°C) was mixed with Cedar Creek soil that had been steam-pasteurized twice over a 24-h period. This mix was placed into 15-cm plastic pots, and twelve 2-week-old *Andropogon gerardii* seedlings were transplanted from their germination media of vermiculite into each pot. The soil surface of each pot was covered with sand to a depth of 2 cm to decrease contamination. The pots were maintained in the

Table 2. Regression coefficients for association between sporulation and soil NO_3 or percent plant cover within each plot of the Cedar Creek LTER biodiversity plots.

		No. of spores			Volume of spores		
Independent variable	Sampling	β_1	p	R^2	β_1	p	R^2
Diversity[a]	June 1997		0.004	0.118		0.001	0.155
	Aug. 1997		0.002	0.137		0.174	0.025
	May 1998		0.012	0.088		0.003	0.121
	Aug. 1998		0.470	0.000		0.003	0.123
	Sept. 1999		0.454	0.000		0.019	0.085
Plant cover	June 1997	0.013	0.000	0.132	0.007	0.079	0.036
	Aug. 1997	0.014	0.000	0.142	0.004	0.273	0.014
	May 1998	0.000	0.861	0.000	0.000	0.793	0.001
	Aug. 1998	0.001	0.821	0.001	0.001	0.789	0.001
	Sept. 1999	0.002	0.384	0.010	0.003	0.919	0.001
Soil NO_3	June 1997	–1.23	0.000	0.137	–1.45	0.000	0.173
	Aug. 1997	–1.28	0.001	0.124	–1.06	0.004	0.099
	May 1998	–1.49	0.000	0.131	–1.68	0.000	0.223
	Aug. 1998	–1.22	0.004	0.096	–1.83	0.000	0.272
	Sept. 1999	–0.85	0.009	0.083	–1.63	0.000	0.289
Diversity + soil NO_3	June 1997		0.000	0.189		0.000	0.250
	Aug. 1997		0.000	0.211		0.015	0.102
	May 1998		0.000	0.180		0.000	0.241
	Aug. 1998		0.049	0.066		0.000	0.341
	Sept. 1999		0.122	0.042		0.000	0.275

Note: Numbers of spores and volume of spores were ln transformed to normalize data prior to regressions; regression coefficients are adjusted for the number of factors in the models.

[a]Plant diversity levels (1, 2, 8, and 16) were entered into the regression models as separate independent variables represented by indicator variables; results here summarize overall regression.

greenhouse at approximately 25°C with 14 h of daylight under high-intensity-discharge lights. Pots were watered from the top when the top 1 cm of the soil surface became dry. Every 6 weeks, 5 g of 14:14:14 slow-release fertilizer (Osmocote; The Scotts Co., Marysville, Ohio) was added to each pot. After 3 months, the plants were cut back to the crowns, and four 1.5 cm × 10 cm cores were taken from each pot for spore counts as described above and were reseeded with *Andropogon gerardii* (1997) or with *Sorghum bicolor* (1998) within 2 days. The pots were allowed to grow for an additional 3 months, at which time they were resampled for determination of AMF species.

Analyses

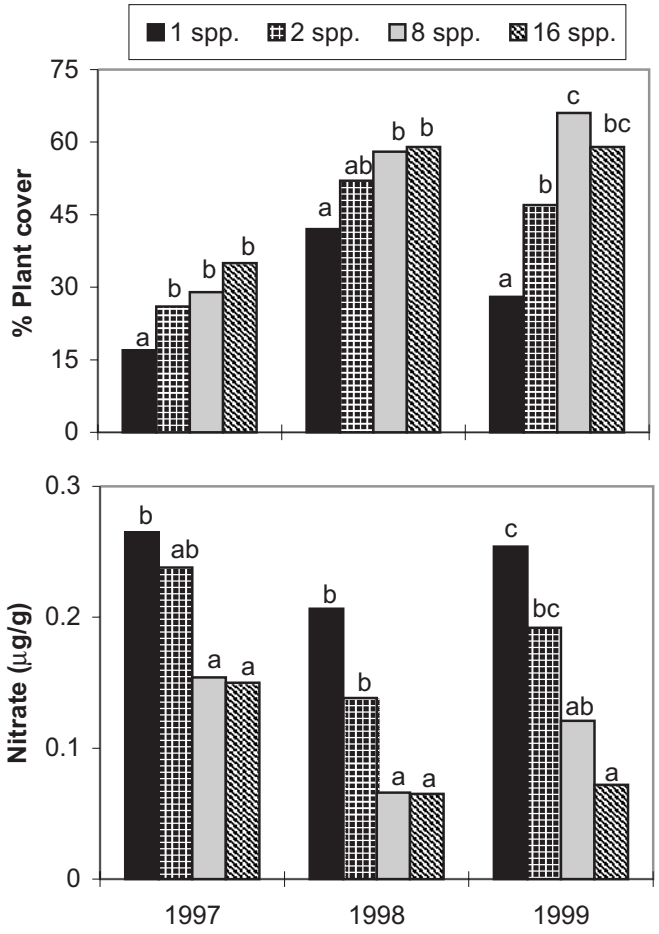
For each plot at each of the five samplings, we thus determined spore numbers and volume by species. Plot data collected in midsummer of each year include percent plant cover by species in each subplot and, from the larger plot, NO_3 and NH_4. Additionally, AMF species counts were obtained from the trap cultures established in 1997 and 1998.

MSUSTAT Statistical Analysis Package version 5.20 (R. Lund, Montana State University, Bozeman, Mont.) was used for all analyses. To normalize data, all spore counts and volumes were log transformed, and soil NO_3 levels were square root transformed, prior to statistical analyses. Bartlett's test for homogeneity was used to confirm equality of variances across treatment levels (Snedecor and Cochran 1980). Analysis of variance was used to test for main effects of plant diversity on AMF species richness, total spore numbers and spore volumes, percent plant cover, and soil NO_3 level. Indicator variable regressions (Snedecor and Cochran 1980) were used to assess diversity treatment effects on spore numbers and volumes and on soil NO_3 and percent plant cover. By entry of each diversity treatment (1, 2, 8, or 16) into the model as an independent indicator variable, linearity of diversity effects need not be assumed. Univariate regression analyses were used to test the influence of soil NO_3 and percent plant cover on spore numbers and volumes. To determine whether there might be some threshold level of plant cover beyond which further effects on sporulation would not be observed, we successively eliminated plots with the lowest amounts of cover from the regressions until the regression was no longer significant. To verify that a loss of significance was not due simply to the decrease in the number of observations, we then ran the regressions with equal numbers of observations randomly selected from the data.

Two measures of AMF species diversity were calculated: the Shannon–Weaver diversity index (Shannon and Weaver 1949), calculated as $H' = -\Sigma (x_i/x_o)\ln(x_i/x_o)$ where x_i is the spore numbers of each individual species and x_o is the total spore populations, and Simpson's index (Simpson 1949), calculated as $D = 1 - \Sigma(x_i/x_o)^2$.

Spearman rank correlation analyses were used to test correlations among spore numbers of all possible pairs of AMF species to determine if there were consistent similarities in their productivity among plots. Probability values of correlations were adjusted for multiple comparisons within sampling times according to Bonferroni's correction (Curtin and Schulz 1998). Rank correlation analyses were also used to assess relation-

Fig. 2. Plant diversity effects on percent plant cover and soil NO_3 of Cedar Creek LTER biodiversity plots. Letters indicate significant differences within sampling time at $p = 0.05$ by Fisher's protected LSD.



ships between percent plant cover of all individual plant species, soil nitrogen (NO_3 and NH_4), and spore production by individual AMF species in each plot. Pearson correlations were used to assess relationships among soil nitrogen, percent plant cover, and plant diversity level in each plot.

Results

Spore production

Plant diversity was positively correlated with total spore number in the first three samplings and with total spore volume throughout the experiment (Fig. 1; Table 2). Spore numbers, spore volumes, and species richness of AMF were consistently lowest in monocultures and highest in 16-species plots, although differences were not always significant. At every sample date, 16-species plots had from 30 to 150% more spores per gram of soil than monocultures. Although the mean number of spores per gram among diversity treatments exhibited the same general trends in all samplings (Fig. 1), the differences among treatments in the last two samplings were smaller than in the earlier samplings and not significantly different. Prior to transformations, within-treatment errors tended to increase with increased spore number, so that monocultures had the smallest errors and 16-species plots the largest, although these differences were significant only in one sampling (August 1997). Spore volumes showed patterns similar to those of spore numbers, with 40–70% greater spore volumes in 16-species plots compared with one-species plots. These differences were maintained throughout the experiment, so that plant diversity had significant effects on spore volume at every sampling. Seasonal effects were apparent: spore numbers and volumes at each diversity level were greater in late summer (August 1997 and 1998) than in early summer (June 1997 and May 1998). Late-summer spore volumes, but not spore numbers, were lowest in 1997 (Fig. 1). Contrary to the within-treatment error pattern seen with spore number, in the first four samplings, monocultures had the greatest within-treatment error of spore volume; again, these differences were significant only in one sampling (June 1997).

Interaction of plant cover and soil NO_3 with plant diversity and spore production

Mean percent plant cover increased with increased plant diversity in all three years (Fig. 2). However, regression analyses show that plant cover was significantly associated with total spore numbers only in the first year (1997) and was not significantly associated with spore volume (Table 2). When plots with less than 15–20% cover were eliminated from the 1997 data sets, the relationships between spore number and plant cover were no longer significant (data not shown).

NO_3 levels ranged from 0 to 1.58 μg/g dry soil and decreased with increased plant diversity in all three years (Fig. 2). Soil NO_3 was correlated with plant cover only in the 1999 sampling ($r = -0.27$, $p = 0.015$) but with plant diversity all three years ($r = -0.27$, $p = 0.012$; $r = -0.37$, $p = 0.000$; $r = -0.28$, $p = 0.010$ in 1997, 1998, and 1999, respectively). Regression analyses showed significant inverse relationships between soil NO_3 and spore production (number and volume) at every sampling (Table 2). Regression models containing only plant diversity treatment or soil NO_3 as the independent variable were compared with models containing both. Models containing both diversity and NO_3 reduced the variation in fit significantly more than models with NO_3 alone in the June 1997 and August 1997 (spore numbers) and June 1997 (spore volume) samplings but not in subsequent samplings. Conversely, at every sampling, models containing NO_3 in addition to diversity explained significantly more of the variation in sporulation than models containing only diversity as the independent variable. Soil NH_4 levels did not differ significantly among plant diversity treatments and showed no significant relationships with spore production (data not shown).

AMF species composition

Of the 15 AMF species detected (Table 3), *Gigaspora* sp., *Paraglomus* sp., *Glomus intraradices*, and *Scutellospora calospora* were the most common; each was found in at least 90% of the plots at every sampling. *Acaulospora morrowiae*, *Acaulospora scrobiculata*, and *Scutellospora pellucida* were also very common, present in 65–85% of the plots at every sampling.

Although trap cultures provided fresh healthy spores for identification, they did not contain any AMF species that were not also present in field soil samples of the same plot in at least one of the five sampling times. Most AMF species identified in field samples also sporulated in the trap cultures that were started with fresh soil inoculum from those samples,

Table 3. Percentage of the 96 Cedar Creek LTER biodiversity plots sampled containing individual AMF species.

	Sampling				
AMF species or species group	June 1997	Aug. 1997	May 1998	Aug. 1998	Sept. 1999
Acaulospora koskei Blaszkowski	6	7	5	3	9
Acaulospora morrowiae Spain & Schenck	84	80	78	85	75
Acaulospora scrobiculata Trappe	71	72	65	69	67
Acaulospora spinosa Walker & Trappe	11	14	20	20	8
Archaeospora leptoticha (Schenck & Smith) Morton & Redecker, comb.nov.[a]	12	32	27	39	51
Entrophospora infrequens (Hall) Ames Schneider	0	1	2	2	2
Gigaspora spp.	98	100	100	99	99
Glomus intraradices Smith & Schenck	95	96	90	99	93
Glomus geosporum (Nicol. & Gerd.) Walker	25	20	33	27	22
Glomus "MN1" (100 μm cream colored)[b]	3	12	10	32	9
Paraglomus spp. (Walker) Morton & Redecker, gen.nov.[b]	98	98	92	97	96
Scutellospora calospora (Nicol. & Gerd.) Walker & Sanders	96	96	95	99	98
Scutellospora pellucida (Nicol. & Schenck) Walker & Sanders	77	85	68	90	71
Scutellospora verrucosa (Koske & Walker) Walker & Sanders	29	28	39	27	26
Scutellospora "MN1" (250 μm red ornamented)[c]	13	12	16	18	16

[a]Syn: *Glomus leptotichum* Schenck & Smith and *Archaeospora gerdmanii* Schenck & Nicolson (Morton and Redecker 2001).
[b]Includes *Paraglomus occultum* (Walker) Morton & Redecker, comb.nov., and *Paraglomus brasilianum* (Spain & Miranda) Morton & Redecker, comb.nov., syn: *Glomus occultum* and *Glomus brasilianum*.
[c]Undescribed species.

indicating that the spores and (or) hyphae were able to initiate new infections in pot cultures. The relative proportion of spores of small-spored species (*Acaulospora morrowiae*, *Paraglomus* spp., and *Glomus intraradices*) compared with that of other species was much greater in trap cultures than in field samples.

There were no consistent significant differences in AMF species diversity indices due to plant diversity treatment (data not shown). However, there were differences in AMF spore populations among treatments and over time. Field spore numbers of the small-spored *Glomus intraradices* and *Paraglomus* spp. were considerably higher in late-summer than in early-summer samples (Fig. 3). Although monocultures contained fewer spores of these species than higher plant diversity plots, in only one case (August 1997) was this difference significant. By contrast, the larger-spored *Gigaspora* and *Scutellospora* showed somewhat less seasonal variation and showed consistent significant responses to plant diversity (Fig. 3), with one-species plots always having the fewest and 16-species plots the most spores of these species.

Specificity of individual AMF species

Shifts in AMF species composition with increasing plant species diversity may be explained in part by differential responses of individual AMF species to individual host plant species. Correlations of spore numbers of many of the AMF species with cover of individual plant species were either not significant or widely variable among sampling times. However, there were significant positive relationships, consistent over several sampling periods, between the percent plant cover of nine plant species and spore numbers of five AMF species (Table 4). Percent cover of the legume *Amorpha canescens* was positively correlated with the most number of AMF species, including *Gigaspora* and *Scutellospora calospora* as well as the smaller-spored *Acaulospora morrowiae*, *Glomus intraradices*, and *Paraglomus* spp. Another legume, *Dalea villosum*, was correlated with spore numbers of the same five AMF in the first year. The correlation between *Dalea villosum* and *Gigaspora* spore numbers was found in all five samplings, while those between this host and the other AMF species varied somewhat in the last three samplings. Although *Lupinus perennis* has been reported to be nonmycorrhizal (Jones 1924), in our plots, percent cover of this legume was not consistently negatively correlated with sporulation of any individual AMF species. Percent cover of the C_4 grasses *Andropogon gerardii*, *Panicum virgatum*, and *Sorghastrum nutans* was consistently correlated with sporulation of the large-spored AMF species *Gigaspora* spp. and *Scutellospora calospora*. The only smaller-spored AMF species consistently associated with a C_4 grass was *Paraglomus*, which was correlated with cover of *Panicum virgatum* at every sampling and with *Andropogon gerardii* at three samplings. The fourth C_4 grass in our study, *Schizachyrium scoparium*, was positively correlated with sporulation only in the first year (June and August 1997). The percent cover of individual nonlegume forbs was rarely correlated with sporulation of individual AMF species. One exception is *Solidago rigida*, which was correlated with spore numbers of *Scutellospora calospora* and, less consistently, with those of *Glomus intraradices*. Sporulation of *Glomus intraradices* was also correlated with percent cover of the forb *Liatris aspera*.

Rank correlation analyses also showed consistent positive associations of spore production among 11 AMF species (Table 5). Significant negative correlations between the spore numbers of individual AMF species were few and inconsistent among the five sampling times (data not shown). Ubiquity of a species did not appear to affect whether its spore numbers were correlated with those of other species. For example, spore numbers of the omnipresent (Table 3) *Gigaspora* were consistently correlated with those of only two species, *Acaulospora morrowiae* and *Scutellospora calospora*, while spore numbers of the equally ubiquitous *Scutellospora calospora* were correlated with those of six

Table 4. Coefficients of Spearman rank correlations between spore counts of selected AMF species and percent plant cover of individual species within individual plots for species with consistently significant correlations present in the Cedar Creek LTER biodiversity plots.

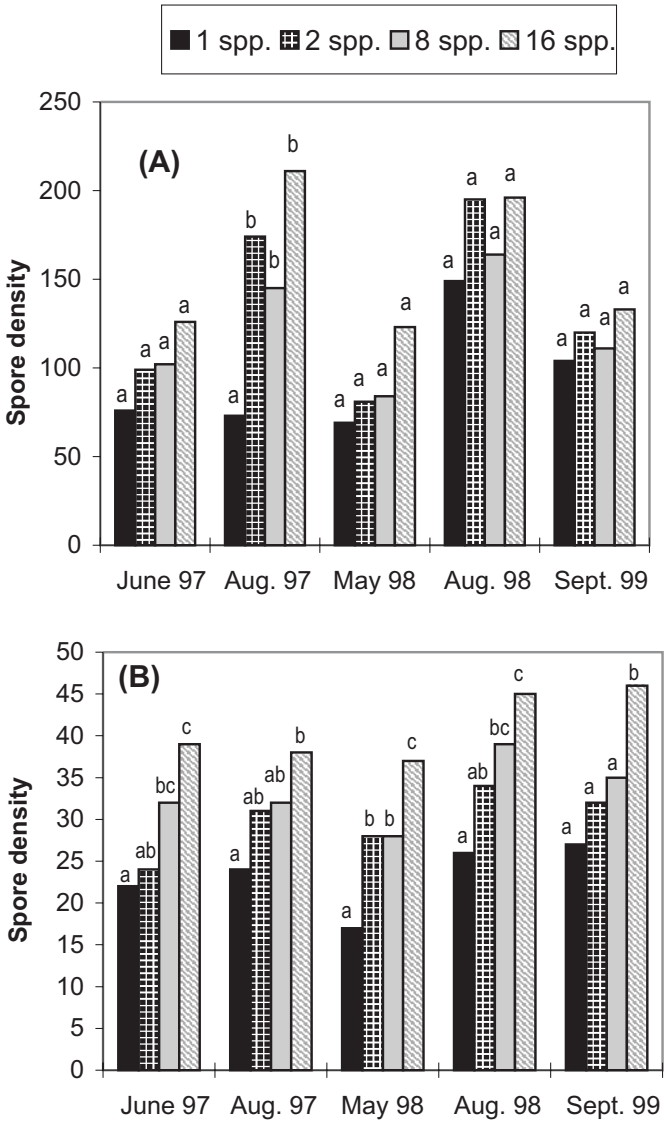
Plant species	Sampling	Amorr	Gigasp	Gintra	Parag	Scalos
Amorpha canescens	June 1997	0.33	0.40***	0.37***	0.45***	0.46***
	Aug. 1997	ns	0.35***	0.37***	0.40***	0.41***
	May 1998	0.35*	0.42***	0.38***	0.36***	0.49***
	Aug. 1998	0.34*	0.46***	0.37***	0.35***	0.47***
	Sept. 1999	ns	0.45***	0.41***	0.46***	0.42***
Andropogon gerardii	June 1997	0.36***	0.38***	ns	0.39***	0.47***
	Aug. 1997	ns	0.34**	ns	0.33**	0.40***
	May 1998	ns	0.39***	ns	ns	0.35***
	Aug. 1998	ns	0.39***	ns	0.30*	0.36***
	Sept. 1999	ns	0.33*	ns	ns	0.37***
Dalea villosum	June 1997	0.32*	0.37***	0.39***	0.41***	0.42***
	Aug. 1997	0.34*	0.35***	0.29*	0.34**	0.36***
	May 1998	ns	0.31*	0.28*	ns	ns
	Aug. 1998	ns	0.30*	ns	0.29*	ns
	Sept. 1999	0.35*	0.33***	0.39***	0.40***	ns
Liatris aspera	June 1997	ns	0.30*	ns	ns	ns
	Aug. 1997	ns	0.42***	0.36***	ns	0.30*
	May 1998	ns	0.37***	0.35***	0.28*	0.28*
	Aug. 1998	ns	ns	0.46***	0.32*	ns
	Sept. 1999	ns	ns	0.36***	ns	ns
Lupinus perennis	June 1997	ns	ns	ns	0.32*	0.32**
	Aug. 1997	ns	ns	ns	0.30*	0.35***
	May 1998	ns	ns	ns	ns	ns
	Aug. 1998	ns	ns	ns	ns	ns
	Sept. 1999	ns	ns	ns	ns	ns
Panicum virgatum	June 1997	ns	0.31*	ns	0.39***	0.38***
	Aug. 1997	ns	0.36***	0.32*	0.35***	0.38***
	May 1998	0.38***	0.39***	ns	0.39***	0.47***
	Aug. 1998	0.42***	0.36***	0.36***	0.40***	0.45***
	Sept. 1999	ns	ns	ns	0.36***	0.50***
Schizachyrium scoparium	June 1997	0.37***	ns	ns	0.36***	0.44***
	Aug. 1997	0.30*	ns	ns	0.44***	0.38***
	May 1998	ns	ns	ns	ns	ns
	Aug. 1998	ns	0.28*	ns	ns	ns
	Sept. 1999	ns	ns	ns	ns	ns
Solidago rigida	June 1997	ns	0.37***	0.38***	0.38***	0.36***
	Aug. 1997	ns	ns	0.39***	0.34**	0.30*
	May 1998	ns	ns	ns	ns	0.31*
	Aug. 1998	ns	ns	0.33*	ns	0.37***
	Sept. 1999	ns	ns	ns	ns	0.31*
Sorghastrum nutans	June 1997	ns	0.42***	ns	ns	0.34***
	Aug. 1997	ns	0.36***	ns	ns	0.48***
	May 1998	ns	0.39***	ns	ns	0.37***
	Aug. 1998	ns	0.42***	ns	ns	0.32*
	Sept. 1999	ns	0.35**	ns	ns	0.47***

Note: Amorr, *Acaulospora morrowiae*; Gigasp, *Gigaspora* spp.; Gintra, *Glomus intraradices*; Parag, *Paraglomus* spp.; Scalos, *Scutellospora calospora*. *p = 0.05; **p = 0.01; ***p = 0.001; ns, not significant.

other species. Sporulation of the less common *Acaulospora spinosa* was correlated with that of six other AMF in at least four sampling times. There was no consistency of correlation strength among species of the same genus or family.

Spore numbers of three AMF species were negatively correlated with soil NO_3 levels in at least four of the five samplings (June 1997, August 1997, May 1998, August 1998, and September 1999, respectively): *Gigaspora* spp. (–0.316 ($p \leq 0.01$), not significant, –0.38 ($p \leq 0.0001$), –0.61 ($p \leq 0.0001$), –0.52 ($p \leq 0.0001$)), *Acaulospora morrowiae* (–0.30 ($p \leq 0.05$), –0.30 ($p \leq 0.05$), –0.45 ($p \leq 0.0001$), –0.38 ($p \leq 0.0001$), –0.41 ($p \leq 0.0001$)), and *Scutellospora calospora* (–0.282 ($p \leq 0.05$), –0.42 ($p \leq 0.0001$), –0.52 ($p \leq 0.0001$), –0.46 ($p \leq 0.0001$), –0.66 ($p \leq 0.0001$)). No AMF species had spore numbers positively correlated with soil NO_3. There were no significant correlations between spore numbers of individual AMF species and soil NH_4 levels.

Fig. 3. Plant diversity effects on spore density (spore number per 25 g of soil) of the (A) small-spored AMF species *Paraglomus* spp. and *Glomus intraradices* and (B) large-spored *Gigaspora* spp. and *Scutellospora* spp. Letters indicate significant differences within sampling time at $p = 0.05$ by Fisher's protected LSD.



Discussion

Increasing plant species richness was correlated with increases in AMF sporulation and species numbers as well as changes in AMF community composition. These increases may be due to the direct effects of increased numbers of plant species on the AMF community or they may be mediated through factors such as plant biomass (estimated here by plant cover) or soil NO_3. Because percent plant cover increased with plant diversity in these plots, spore production may have been elevated due to increases in density of plant roots available for colonization or to increased carbon availability per plot due to greater plant biomass rather than to any direct effect of host diversity. However, regressions of plant cover with sporulation showed that only in the first year was there a significant relationship between overall plant cover and total spore numbers across plots, and cover was not significantly related to spore volume in any sampling. Since total plant cover in 1998 and 1999 was substantially higher than in 1997, it may have reached a threshold value beyond which further increases had minimal influence on total spore number. This possibility is also suggested by the loss of significance between spore numbers and percent plant cover when plots with less than 15–20% plant cover were eliminated from the 1997 data set (data not shown).

The effect of plant diversity on spore volume, but not spore number, in the last two samplings may be due in part to shifts in AMF species composition to include more large-spored species in higher-diversity plots or to proportionally increased sporulation by large-spored species in higher-diversity plots. Comparisons of plant diversity effects on spore numbers of Gigasporaceae, which produce large spores, with those of *Glomus intraradices* and *Paraglomus* sp. (Fig. 3) confirm that the larger-spored species show more consistent significant increases with plant diversity. Although spore volume was significantly associated with plant diversity at each sampling, it was not associated with plant cover (Table 2), suggesting that plant biomass in itself did not lead to increased spore biomass.

Another factor that interacts with plant diversity, but only weakly with plant cover, in these plots is soil NO_3. Midsummer soil NO_3 concentrations were consistently higher in low-diversity plots and were better predictors of plot spore numbers than plant diversity itself. Because plant cover, soil NO_3, and spore volume and number were not controlled treatment variables, we cannot determine causality among them. For example, an inverse relationship between spore numbers and soil NO_3 levels could be due to a direct adverse impact of NO_3 on sporulation, to direct or indirect AMF enhancement of overall NO_3 uptake by host plants (thereby lowering soil levels), or to some correlated factor not measured, such as activity of other soil microorganisms. However, others have reported evidence for adverse impacts of increasing soil NO_3 levels on the sporulation of certain AMF species. Egerton-Warburton and Allen (2000) reported that nitrogen enrichment "was associated with the displacement of the larger-spored species of *Scutellospora* and *Gigaspora*...with a concomitant proliferation of small-spored *Glomus* spp." Johnson (1993) also found spore numbers of *Gigaspora gigantea*, *Scutellospora calospora*, and *Glomus occultum* (= *Paraglomus occultum*) reduced by nitrogen and phosphorus fertilization at a nearby Cedar Creek research site, although at nitrogen levels much higher (a low of 8.6 mg/kg) than the highest concentration (1.6 mg/kg) in this study. Her finding that sporulation of *Glomus intraradices* was actually higher in fertilized plots is consistent with our finding that sporulation of this species was not negatively associated with increased NO_3 levels across the range of NO_3 in our plots. In contrast, even at the relatively narrow range of soil NO_3 levels present in our plots, spore production of *Gigaspora* spp., *Acaulospora morrowiae*, and *Scutellospora calospora* was consistently negatively correlated with increasing NO_3. Although these relationships were consistent across the three years and with data from other investigations, it should be noted that the nitrogen samples were midseason only and that sampling at other points during the growing season may have yielded different results, as nitrogen availability fluctuates seasonally.

Table 5. Coefficients of Spearman rank correlations between spore densities of AMF species with consistently significant interactions present in the Cedar Creek LTER biodiversity plots.

	Date	Gigasp	Amorr	Parag	Gintra	Ggeos	Sverr	Spell	Scalos	Aspin	Arlept
Amorr	June 1997	0.35***									
	Aug. 1997	0.31*									
	May 1998	0.41***									
	Aug. 1998	0.43***									
	Sept. 1999	ns									
Parag	June 1997	0.29*	0.50***								
	Aug. 1997	ns	0.42***								
	May 1998	0.46***	0.46***								
	Aug. 1998	0.30***	0.44***								
	Sept. 1999	ns	ns								
Gintra	June 1997	ns	ns	ns							
	Aug. 1997	ns	0.35***	ns							
	May 1998	ns	0.31*	0.29*							
	Aug. 1998	ns	0.33*	0.43***							
	Sept. 1999	ns	ns	ns							
Ggeos	June 1997	ns	ns	ns	0.38***						
	Aug. 1997	0.43***	0.44***	ns	ns						
	May 1998	ns	0.35***	ns	ns						
	Aug. 1998	0.37***	0.30*	ns	ns						
	Sept. 1999	0.31*	0.43***	ns	ns						
Sverr	June 1997	0.38***	ns	ns	ns	0.36***					
	Aug. 1997	ns	0.36***	ns	ns	0.47***					
	May 1998	0.30*	ns	ns	ns	ns					
	Aug. 1998	0.42***	0.39***	0.30*	ns	0.48***					
	Sept. 1999	ns	ns	ns	ns	0.37***					
Spell	June 1997	0.41***	0.31*	0.33***	ns	ns	ns				
	Aug. 1997	ns	ns	ns	ns	ns	ns				
	May 1998	ns	0.30*	ns	ns	ns	ns				
	Aug. 1998	ns	ns	ns	ns	ns	0.36***				
	Sept. 1999	ns	ns	ns		ns	ns	ns			
Scalos	June 1997	0.34***	0.58***	0.51***	ns	0.29*	ns	0.42***			
	Aug. 1997	0.29*	0.54***	0.45***	ns	0.32*	ns	0.43***			
	May 1998	0.36***	0.47***	0.42***	ns	0.33*	ns	0.38***			
	Aug. 1998	0.42***	0.51***	0.43***	ns	0.44***	0.36***	0.32*			
	Sept. 1999	ns	0.43***	ns	ns	0.45***	ns	ns			
Aspin	June 1997	0.35***	0.39***	0.46***	0.47***	0.60***	0.53***	0.41***	0.42***		
	Aug. 1997	ns	0.51***	0.46***	0.36***	0.68***	0.57***	ns	0.42***		
	May 1998	0.29*	0.49***	0.44***	0.40***	0.56***	0.38***	0.36***	0.47***		
	Aug. 1998	ns	0.32*	ns	0.31*	0.45***	0.37***	ns	0.40***		
	Sept. 1999	0.43***	0.49***	0.39***	0.40***	0.63***	0.63***	0.44***	0.41***		
Arlept	June 1997	0.34***	0.51***	0.42***	0.34***	0.63***	0.55***	0.41***	0.46***	0.74***	
	Aug. 1997	ns	0.45***	0.48***	ns	0.52***	0.35***	ns	0.41***	0.65***	
	May 1998	ns	ns	ns	ns	0.37***	ns	0.31*	0.36***	0.58***	
	Aug. 1998	0.36***	0.33*	0.32*	ns	ns	ns	ns	ns	0.30*	
	Sept. 1999	ns	ns	ns	ns	0.36***	ns	ns	ns	0.41***	
Glmn	June 1997[a]										
	Aug. 1997	0.41***	ns	ns	0.42***	0.55***	0.55***	0.44***	0.32*	0.65***	0.42***
	May 1998	0.50***	0.47***	0.46***	0.45***	0.47***	0.60***	0.54***	0.39***	0.62***	0.57***
	Aug. 1998	ns	ns	0.35***	0.35***	ns	0.32*	0.37***	ns	0.43***	0.44***
	Sept. 1999	0.40***	0.39***	0.40***	0.48***	0.60***	0.54***	0.37***	0.46***	0.73***	0.53***

Note: Gigasp, *Gigaspora* spp.; Amorr, *Acaulospora morrowiae*; Parag, *Paraglomus* spp.; Gintra, *Glomus intraradices*; Ggeos, *Glomus geosporum*; Sverr, *Scutellospora verrucosa*; Spell, *Scutellospora pellucida*; Scalos, *Scutellospora calospora*; Aspin, *Acaulospora spinosa*; Arlept, *Archaeospora leptoticha*; Glmn, *Glomus* "MN1".*p = 0.05; ***p = 0.001; ns, not significant.

[a]Insufficient data.

The majority of the significant correlations that we found between specific plant hosts and spore numbers of individual AMF species were with legumes and C_4 grasses. In a parallel plant diversity experiment located adjacent to these plots, Tilman et al. (1997) found that the presence of legumes and C_4 grasses, but not forbs or C_3 grasses, significantly influenced both plant productivity and soil nitrogen concentration. The fact that no AMF species shows positive correlations with soil NO_3 suggests that the species-specific increases in spore numbers observed in the presence of the legumes *Lupinus perennis*, *Dalea villosum*, and *Amorpha canescens* cannot be explained simply by increased soil NO_3 availability in their plots. Rather, the correlations between the various fungal and host plant species reported in Table 4 may be evidence that plant–fungal specificity plays an important role in determining the structure of AMF fungal communities or at least of the sporulation of such communities. Increased plant diversity increases the number of possible specific host–fungal pairings, which could contribute to the increased AMF diversity and sporulation observed in this study. Our findings suggest that even within plant functional groups, diversity has important impacts on spore production of the associated mycorrhizal communities in each plot. Tilman et al. (1997) also found that increasing species diversity within functional groups (i.e., forbs, legumes, and C_4 and C_3 grasses) caused significant changes in plant productivity, although less significantly than changes in numbers of functional groups.

Correlations among spore numbers of the common AMF species (Table 5) show that a number of them sporulate under similar conditions. The lack of negative correlations among spore numbers of the various AMF species suggest that the species may occupy unique niches rather than competing directly with each other. Smith et al. (2000) recently reported that hyphae of *Glomus caledonium* and *Scutellospora calospora* colonizing the same host plant absorb phosphorus from different spatial regions of the soil. They suggested that such specialization in hyphal growth and phosphorus transport efficiency may help explain why plants inoculated with multiple AMF species can exhibit increased growth compared with those inoculated with single species.

In some cases, AMF species whose spore numbers are positively correlated with one another share similar responses to the presence of specific plant hosts, and their correlation with each other may be due simply to common host preferences. For example, *Scutellospora calospora* and *Gigaspora* spp. both show unique positive responses to the presence of *Sorghastrum nutans* (Table 4). In other cases, although spore numbers of two species tend to parallel each other, they do not share apparent host preferences, and possible explanations are more complex. Although spore production of *Paraglomus* is fairly consistently correlated with that of *Scutellospora calospora*, for example (Table 5), it exhibits a positive response to *Dalea villosum* and *Liatris aspera*, but *Scutellospora calospora* does not (Table 4). Perhaps the presence of *Scutellospora calospora*, which is more prolific, decreases predation or colonization of *Paraglomus* spores by nematodes or parasites. Alternatively, spore production (or degradation) in the two species may be cued to physical environment parameters, such as temperature or soil moisture.

Because AMF data from this experiment was restricted to spore counts, caution must be exercised in interpreting the results. Some species of AMF fungi sporulate only rarely if at all (e.g., Clapp et al. 1995) or only under specific conditions and may therefore be overlooked or their importance underestimated when considering only spore data. Thus, the presence or intensity of colonization by AMF species may be only partially gauged by use of spore data. One method to encourage sporulation of recalcitrant species is the use of repeated trap culture cycles (e.g., Stutz and Morton 1996); however, in our experiment, no new AMF species were found in cultures that were not present in field samples. Species identified from each of our plots were relatively consistent over the five samplings, which included both early and late seasons as well as moist and dry cycles.

In summary, we have presented evidence for positive effects of plant diversity on AMF sporulation and community species composition. At least some of these effects may be mediated through direct or indirect factors associated with midseason soil NO_3 levels, and at low plant density, on percent plant cover. We also found evidence for existence of specificity between host plants and AMF species that may contribute to the positive impact of increasing plant diversity on AMF diversity and sporulation observed in this study. While these data are limited to effects on sporulation, they still contribute an important segment of information to our understanding of the complex relationships between plant hosts and the mycorrhizal community.

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