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Elevated CO_2 and plant species richness impact arbuscular mycorrhizal fungal spore communities

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Summary

• We enumerated arbuscular mycorrhizal (AM) fungal spore communities for 3 yr as part of a long-term CO_2 enrichment experiment at Cedar Creek, Minnesota, USA. Complete factorial combinations of two levels of CO_2 and N, and 16 perennial plant species grown in monoculture and 16-species polyculture were arranged in a split-plot design.

• In 1998–2000, spore communities were quantified under monocultures of eight plant species. In 2000, measurements were expanded to include monocultures and polycultures of all of the plant species.

• Under plant monocultures, only *Glomus clarum* responded significantly to CO_2 elevation out of 11 species present. This response was not detectable under plant polycultures. *Glomus clarum* was also significantly more abundant under plant polycultures. Nitrogen addition had small negative effects on AM fungal spore abundance and species richness in 2000. The interaction of CO_2 and N did not affect arbuscular mycorrhizal fungal spore communities.

• We show that CO_2 enrichment and plant species richness impact arbuscular mycorrhizal fungal community structure. These findings are important because altered symbiotic functioning may result.

Key words: arbuscular mycorrhizal (AM) fungi, elevated CO_2, species richness, N addition, free-air CO_2 enrichment (FACE), multi-response permutation procedure (MRPP), BioCON (Biodiversity, CO_2 and N) experiment, *Glomus clarum*.

Introduction

The species composition of arbuscular mycorrhizal fungal communities is important because mutualistic function varies among pairings of fungal and plant species (Ravnskov & Jakobsen, 1995; Johnson *et al.*, 1997; van der Heijden *et al.*, 1998). Arbuscular mycorrhizal (AM) fungi only acquire C from living plants. Anthropogenic enrichment of atmospheric CO_2 and soil N is expected to impact AM symbioses, because the availability of these elements frequently mediates plant allocation of C to AM fungal symbionts (Jifton *et al.*, 2002; Miller *et al.*, 2002). Enrichment of CO_2 and N is known to have diverse effects on plant communities, but little is known about the responses of AM fungal communities to these changes.

Atmospheric CO_2 is expected to rise by 75–350% over preindustrial levels by the end of the century (IPCC, 2001). This change is expected to increase the growth of AM fungi through increased availability of photosynthate (Fitter *et al.*, 2000). Increases in photosynthetic rates (Lee *et al.*, 2001), fine root production and turnover (Arnone *et al.*, 2000; Norby & Jackson, 2000), plant C : N and C : P ratios (Gifford *et al.*, 2000), and production of phenolic compounds (Castells *et al.*, 2002) have been measured under elevated CO_2, but impacts are not universal and vary among plant taxa (Lüscher *et al.*, 1998; Field, 1999; Bassiriad, 2000). Concomitant to the rise in atmospheric CO_2, annual terrestrial N fixation has doubled over the last century (Vitousek *et al.*, 1997). Nitrogen eutrophication generally reduces plant allocation of C below-ground (Marschner *et al.*, 1996) and reduces

production of AM fungal structures (Bååth & Spokes, 1989; Sylvia & Neal, 1990). In addition, AM fungal community structure can be strongly influenced by N enrichment (Johnson, 1993; Edgerton-Warburton & Allen, 2000; Eom *et al.*, 2000; Edgerton-Warburton *et al.*, 2001), suggesting the possibility of different adaptations to decreased C availability among AM fungal species. The variability in plant and AM fungal responses requires that the effects of enriched CO_2 and N be examined at the AM fungal species level, and under many host plant species.

To better understand ecosystem responses to changing atmospheric CO_2 and soil N, AM fungal responses must be examined within plant and fungal communities (Wolters *et al.*, 2000). No studies to date have quantified AM fungal responses to CO_2 at the species level, despite evidence that AM fungal species have unique functional traits. Another largely unexplored area of AM ecology is the effect of plant diversity on AM fungal communities. The presence of many plant species might be associated with a larger or more diverse AM fungal community, because the variety in colonizable roots under high plant species richness might lead to greater niche differentiation.

To address the issues raised above, we made use of the BioCON experiment (Biodiversity, CO_2 and N). This is a unique long-term field experiment in Minnesota, USA, in which 16 perennial plant species were grown in monoculture and polyculture, under factorial combinations of ambient and enriched CO_2 and N (Reich *et al.*, 2001a,b). Within this experiment we test the following hypotheses: (1) enrichment of atmospheric CO_2 increases total AM fungal spore production and alters community composition; (2) enrichment of soil N decreases AM fungal spore production and alters community composition; (3) AM fungal spore production and species diversity will be greater under plant polycultures than under plant monocultures; and (4) the above factors may have interactive effects on AM fungal communities.

Materials and Methods

Experimental design

We evaluated our hypotheses using experimental plots within the six free-air CO_2 enrichment (FACE) rings of the BioCON experiment at Cedar Creek Natural History Area in east central Minnesota, USA (45° N, 93° W); the experimental site is described by Reich *et al.* (2001a,b). Soil at this site is excessively drained outwash sand (Nymore series, subgroup Typic Upidsamment, suborder Psamments, Order Entisols). The six FACE rings were established within a 300×450 m successional grassland. The rings were located in flat, unshaded areas and were selected to minimize differences in soil parameters and land-use history. In June of 1997, the soil within the rings was tilled and fumigated with methyl bromide, to remove plants and eliminate the seed bank. Although methyl bromide has been demonstrated to reduce AM fungal populations (Menge, 1982), elimination of the seed bank was necessary for the maintenance of target plant species.

Each FACE ring was divided into $61–2 \times 2$ m plots, separated by 0.3-m deep sheet metal isolation barriers and by 0.2-m wide cement walkways. Experimental plots were seeded in June 1997 and again in May of 1998. Plots were weeded several times per growing season to maintain only the desired plant species. Plant litter was not removed from the plots, but the entire experimental area was burned in April 2000.

The experimental design was a randomized split-plot arrangement of treatments, with complete factorial combinations of CO_2 level, N level, and 16 perennial plant species grown in monoculture ($n = 2$) and in 16-species polycultures ($n = 12$). The CO_2 treatments were maintained within six FACE rings at the whole-plot level; ambient (368 μmol mol^{-1}) and elevated (560 μmol mol^{-1}) levels were replicated in three rings each. The CO_2 was enriched during daylight hours from April to October 1998, April to November 1999 and April to October 2000. Nitrogen and plant treatments were randomly assigned to individual plots within the FACE rings. Ambient N plots did not receive any N fertilization; N-enriched plots were fertilized with NH_3NO_4 at a total rate of 4 g N m^{-2} per year; this was delivered over three dates each year (in May, June and July). The 16 perennial plant species are all native or naturalized to Cedar Creek (Reich *et al.*, 2001a,b) and include four C_4 grasses (*Andropogon gerardii* Vitman, *Bouteloua gracilis*, *Schizachyrium scoparium* (Michaux) Nash, *Sorghastrum nutans* (L.) Nash), four C_3 grasses (*Agropyron repens* (L.) Beauv., *Bromus inermis* Leysser, *Koeleria cristata* Pers, *Poa pratensis* L.), four N-fixing legumes (*Amorpha canescens* Pursh, *Lespedeza capitata* Michaux, *Lupinus perennis* L., *Petalostemum villosum* Nutt.), and four herbaceous forbs (*Achillea millefolium* L., *Anemone cylindrica* A. Gray, *Asclepias tuberosa* L., *Solidago rigida* L.). Only half of the monoculture plots were monitored for AM fungal communities during the entire 3 yr of the experiment; these included two species from each of the functional groups (*Bouteloua gracilis* and *Schizachyrium scoparium*; *Agropyron repens* and *Koeleria cristata*; *Lespedeza capitata* and *Lupinus perennis*; *Achillea millefolium* and *Solidago rigida*). In 2000, all 16 plant species were monitored in monoculture and in 16-species polyculture.

Sampling, spore extraction and identification

Soil samples were obtained in mid-September of each year. In each plot, soil cores were taken using 5-cm diameter PVC pipe to a depth of approximately 20 cm. The location of coring was changed on every sampling date to avoid repeated coring of any area. Samples were stored frozen for no longer than 1 yr before they were processed.

Spores were extracted from soil using wet sieving and sucrose density gradient centrifugation (Johnson *et al.*, 1999).

The extracted spores were observed under a compound microscope and identified to species using current taxonomic criteria (Schenck & Perez, 1990; INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi) http://invam.caf.wvu.edu/). Total spore biovolume, in addition to total spore numbers, was used to compare responses of total AM fungal spore production. This is to account for the large variation in spore size and numbers produced by the different AM fungal species and genera. Spore biovolume was calculated as $V = 1/6\pi D_3$ for species with spherical spores, or as $V = 1/6\pi D_1 D_2^2$ (where D_1 is the larger dimension and D_2 is the smaller dimension) for species with elongated spores. The dimensions used for each species were based on the average sizes observed for the spores in these soils and are not always identical to those in published species descriptions.

Statistical analyses

This experiment involved a complex statistical model and multivariate responses. We avoided the use of multiple univariate analyses, because community data does not meet the univariate requirement that response variables be independent. In addition, separate analysis of the responses of each AM fungal species might not capture overall changes in community composition. Therefore, we used multi-response permutation procedure (MRPP) and indicator species analysis (IS) to look at the main effects of treatments on spore community composition. We then used analysis of covariance to further examine main effects and interactions of treatments, within the full statistical model.

Multi-response permutation procedure (MRPP) and indicator species analysis (IS) Spore communities under the different experimental treatments were compared with MRPP (Mielke, 1984) using the PC-ORD statistical package version 4.14 (McCune & Mefford, 1999). This method accommodates the nonparametric and multivariate nature of community response data, and calculates the probability (*P*) that community differences detected are due to random chance alone (Zimmerman *et al.*, 1985). If MRPP detected significant changes in communities as aresult of treatment, we used IS to reveal which particular species, if any, were the primary sources of the change (Dufrene & Legendre, 1997).

MRPP can only test for differences between two or more groups of communities; it cannot examine multiple factors or interactions within a single test. It can, however, account for blocked designs if all blocks are of equal size. Blocked MRPP calculations require Euclidean distance measurements; for consistency we used Euclidian distance calculations for all MRPP and blocked MRPP tests. For blocked MRPPs, median alignment of the blocks was used. Individual FACE rings, which were the split-plot level of CO_2 treatment, cannot be used as blocks, because each ring contained plots receiving only one level of CO_2 treatment. Thus, pairs of ambient and elevated FACE rings were used to block communities under plant polycultures, allowing us to examine effects while minimizing large-scale spatial variation. The sets of 64 or 128 monocultures, which could not be distributed symmetrically among six FACE rings, were instead blocked by plant functional group. Because root biomass varied greatly with plant functional group, but not with CO_2 treatment (data not shown), blocking by plant functional group allows us to examine treatment effects while controlling for the variance caused by innate differences in below-ground allocation among the groups.

For all significant MRPP analyses, the chance-corrected within-group agreement (A) is presented. The value of A represents the degree of within-group homogeneity and is analogous to 'effect size'. When all plots within a treatment group have identical community compositions, A = 1; when within-group heterogeneity equals that expected by random chance (i.e. no 'effect' of treatments), A = 0. Values of A between 0.1 and 0.3 are often meaningful in ecological community data (McCune & Mefford, 1999).

Analysis of covariance (ANCOVA) Because MRPP cannot accommodate multiple treatment effects and interactions, univariate responses of interest (total spore abundance and biovolume, Brillouin's diversity (*HB*) values, and the abundances of significant indicator species) were compared using ANCOVA with the full model of all experimental treatments, to test the effects and two-factor interactions of: CO_2 level (ambient or elevated), the random blocking effect of ring nested within CO_2 level (no interactions tested), N level (ambient or enriched) and plant species richness (monoculture or 16-species, 2000 only), plant functional group (forb, legume, C_3 grasses, or C_4 grasses; monocultures only), or plant species (monocultures only). In the 3-yr data set, the univariate responses of interest were also tested for main effects and interactions of experimental year with all treatments. There were no significant interactions of experimental year with any treatments (not shown). Therefore we present the 3-yr means of all responses, except where indicated.

Because sample sizes and model characteristics differed at different levels of plant species richness (for monocultures, $n = 2$ at each combination of plant species, CO_2 level and N level; in polyculture, $n = 12$ at each combination of CO_2 and N level) the above-listed response variables from 2000 monocultures data were also analysed separately, in a model incorporating host plant functional group or species (for monocultures).

Fine root biomass in each plot, collected in August of each experimental year, was used as the covariate in all ANCOVA tests. We wished to use a plant biomass covariate so that we could (1) account for as much nontreatment variation as possible and (2) uncouple the statistical significance of AM

fungal responses to treatments from plant responses to treatments. Significant increases in a spore parameter cannot be attributed solely to increased host plant growth when the covariate is included in the model. We chose fine root biomass (as opposed to total root or total plant biomass) because it explained the greatest amount of variation in total spore biovolume, in a linear regression model ($r^2 = 0.084$, $P < 0.0001$, not shown). Spore counts of individual AM fungal species were ranked before ANCOVA (Eom *et al.*, 2000) but the actual means are reported here. Brillouin's index of diversity was selected for analysis because it does not assume an equal probability of encounter of species (Eom *et al.*, 2000).

If the effect of a treatment on an indicator species did not retain significance under the full ANCOVA model, the response could not be attributed to the treatment with certainty; these cases are indicated as such. All ANCOVA was performed using the JMP 4.0 statistical package (SAS Institute Inc., Cary, NC, USA).

Results

AM fungal dynamics under eight plant monocultures (1998–2000)

Total spore abundance, species richness and diversity did not change with CO_2 enrichment, but mean total spore biovolume was significantly greater under CO_2 enrichment in each of the three years of the study (Table 1). Spore community composition was significantly affected by CO_2 level when communities were blocked by plant functional group (A = 0.350, $P = 0.032$). Spores from 11 distinguishable morpho-species of AM fungi from five genera were observed, but *G. clarum* was the only significant indicator species of elevated CO_2. Over the three years of the study, mean abundances of this species were 2.5–4.5 times greater under elevated CO_2 than under ambient (Table 2, Fig. 1a). When spore biovolume is summed over all species except *G. clarum*, there is no longer any difference in total spore biovolume at ambient and elevated CO_2 (3-yr means of 0.035 $mm^3\ g^{-1}$ soil at ambient CO_2 and 0.034 $mm^3\ g^{-1}$ soil at elevated CO_2; SE = 0.002 for both). In contrast to the response of *G. clarum*, *G. fasciculatum* was an indicator of ambient CO_2, but this response was not consistent over the three years of the study, and its significance was not retained in the full ANOVA model (Table 2, Fig. 1b). The biological significance of this response cannot be evaluated further with this data.

Neither N treatment nor the $CO_2 \times N$ interaction were significant predictors of any AM fungal responses in this data set. Plant functional group and species were both significant predictors of total spore abundance and diversity (not shown), but neither predicted total spore biovolume. Abundance and diversity of AM fungal spores were significantly greater under both forb species (*A. Millefolium* and *S. rigida*), and significantly lower under both legume species and one C_3 grass (*L. perennis*,

Table 1 Three years of arbuscular mycorrhizal fungal (AMF) spore community responses to CO_2, N and experimental year, under plant monocultures ($n = 64$ per year). There were no significant interactive effects

	CO_2 (df = 1, error df = 4) Mean (SE)			N (df = 1, error df = 46) Mean (SE)			YEAR (df = 2, error df = 175) Mean (SE)			
Response	Ambient	Elevated	P^1	Ambient	Enriched	P^1	1998	1999	2000	P^1
Total spore abundance (spores g^{-1} soil)	116 (10.4)	137 (13.4)	0.97	122.2 (12.3)	130.5 (11.7)	0.42	**112 (5.7)**	**215 (19.5)**	**52 (4.8)**	**0.000**
Total spore biovolume ($mm^3\ g^{-1}$ soil)	**0.041 (0.003)**	**0.055 (0.008)**	0.023	0.049 (0.008)	0.047 (0.004)	0.72	**0.040 (0.002)**	**0.081 (0.012)**	**0.024 (0.003)**	**0.000**
AMF species richness (species $plot^{-1}$)	7.18 (0.19)	6.90 (0.20)	0.58	6.98 (0.215)	7.09 (0.174)	0.71	**6.75 (0.22)**	**7.64 (0.24)**	**6.72 (0.25)**	**0.039**
Brillouin's diversity (*HB*) ($plot^{-1}$)	0.579 (0.033)	0.597 (0.035)	0.61	0.572 (0.034)	0.604 (0.033)	0.41	**0.382 (0.027)**	**0.601 (0.036)**	**0.780 (0.044)**	**0.000**

^1P = level of significance using the full ANCOVA model of effects. Significant *P*-values and associated group means and standard errors are in bold type.

Table 2 Mean arbuscular mycorrhizal fungal spore community composition under plant monocultures (n = 64 per year)

	CO_2 Abundance (SE)			Year Abundance (SE)			
Morpho-species	Ambient	Elevated	P[1]	1998	1999	2000	P[1]
Acaulospora scrobiculata	0.170 (0.034)	0.291 (0.088)	0.85	0.267 (0.065)	0.337 (0.121)	0.086 (0.032)	0.50
Acaulospora spinosa	0.042 (0.017)	0.053 (0.026)	0.93	0.071 (0.023)	0.068 (0.040)	0.004 (0.004)	0.16
Archeospora trappei	4.86 (2.64)	3.19 (1.12)	0.75	3.13 (1.55)	6.08 (3.86)	2.86 (1.11)	0.86
Gigasporaceae spp.	0.558 (0.087)	0.460 (0.088)	0.40	**0.527 (0.081)**	**0.716 (0.152)**	**0.284 (0.060)**	**0.050**
Glomus aggregatum/intraradices	90.5 (7.64)	99.1 (8.15)	0.44	**100.0 (5.0)**	**152.1 (11.7)**	**32.3 (2.5)**	**0.001**
Glomus clarum	**6.1 (1.5)**	**22.7 (8.3)**	**0.013**	**1.94 (0.812)**	**31.0 (12.2)**	**10.2 (2.48)**	**0.001**
Glomus constrictum	0.406 (0.078)	0.718 (0.0121)	0.35	1.07 (0.161)	0.518 (0.115)	0.093 (0.034)	0.001
Glomus fasciculatum	**2.06 (0.66)**	**1.10 (0.51)**	**0.047**[2]	0.926 (0.421)	1.51 (0.732)	2.30 (0.919)	0.16
Paraglomus occultum	9.20 (3.04)	8.20 (2.40)	0.72	**2.26 (0.557)**	**20.8 (5.4)**	**3.04 (0.79)**	**0.001**
Scutellospora calospora/pellucida	0.723 (0.106)	0.871 (0.160)	0.67	0.651 (0.088)	1.02 (0.240)	0.718 (0.132)	0.60
Scutellospora erythropa	0.883 (0.196)	0.519 (0.085)	0.06	**0.717 (0.085)**	**1.23 (0.297)**	**0.154 (0.023)**	**0.002**

[1]P, level of significance from indicator species analysis, performed for all factors with significant effects in multi-response permutation procedure. Significant indicator species' P-values and group means are in bold font.[2]This response was not detectable using ANOVA with the full model of treatment effects.

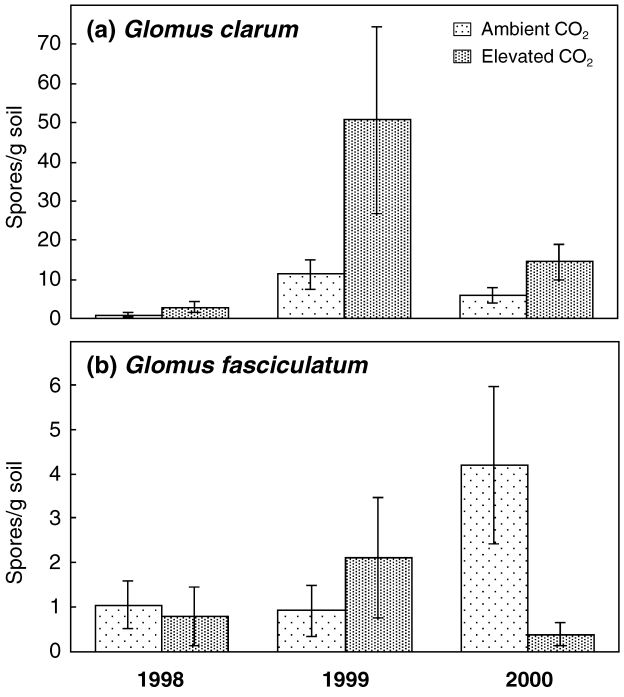

Fig. 1 Mean spore abundance (± SE, n = 64) for arbuscular mycorrhizal (AM) fungal species found to be significant by Indicator Species analysis of CO_2 level, under plant monocultures. (a) *Glomus clarum* and (b) *Glomus fasciculatum* are shown under ambient and elevated CO_2, in each experimental year. Light columns, ambient CO_2; dark columns, elevated CO_2.

L. capitata and *K. cristata*), than under the other groups and species. While plant functional group was a significant predictor of these AM fungal responses, there was variation in spore abundance and diversity under the different plant species within the functional groups (not shown). The four plant functional groups and eight plant species were associated with significantly different spore communities (A = 0.061, $P < 0.001$, for both factors tested separately with MRPP). However, the effect sizes (A) of these factors are small and no individual AM fungal species indicated particular plant functional groups or species.

There were yearly and spatial variations in AM fungal spore populations (Tables 1 and 2, Fig. 1). Overall means of total spore biovolume and species richness increased from 1998 to 1999 and decreased from 1999 to 2000. In contrast, Brillouin's diversity index (*HB*) rose over all three years, indicating that evenness of AM fungal spore communities continued to increase in 2000. By the second year of the study, spore communities in these soils were similar in abundance to those documented by Johnson *et al.* (1991) in adjacent undisturbed soils at the site, but were slightly lower in species richness. The neighboring soils, undisturbed for 10–30 yr before measurements, contained between 200 spores g^{-1} soil and 316 spores g^{-1} soil and 12–22 species per site; we measured a mean of 215 spores g^{-1} soil in 1999, and a total of 11 AM fungal species. Community analysis of the 3-yr data set revealed significant differences in spore communities among all three experimental years (A = 0.10, $P < 0.0001$). Five AM fungal species significantly indicate collection year, four of which demonstrate large increases in abundance from 1998 to 1999 and large decreases from 1999 to 2000 (Table 2). *Glomus aggregatum/intraradices* was the most abundant species and was the only species that occurred in 100% of the experimental plots. The large drop in spore biovolume and the continuing increase of HB in 2000 are due primarily to the decreasing dominance of this species. The FACE rings were also significant predictors of total spore abundance and diversity, and of the abundances of five AM fungal species (means not shown). These species reached peak abundances in different

rings, and there was no indication of an overriding spatial gradient across the experimental area for any response.

AM fungal dynamics in 16 plant species monocultures and polycultures (2000)

When all plots were analyzed together, only plant species richness and N had significant impacts on AM fungal responses. Mean spore abundance, biovolume, species richness and diversity were not significantly affected by plant species richness, although all four means were greater under polycultures (Table 3). Plant species richness level was associated with significantly different AM fungal communities, although the effect size was small (A = 0.006, $P = 0.008$). *G. clarum* was the only indicator species for plant species richness, with significantly greater abundance under polycultures ($P = 0.006$, means of 8.6 spores g^{-1} soil under monocultures and 15.7 spores g^{-1} soil under polycultures). In 2000, N enrichment was always associated with decreased spore abundance, biovolume, species richness and diversity, with significant or near-significant reductions in all four responses (Table 3). Spore community composition was not detectably altered by N addition. There were no interactive effects of CO_2 and N on any of the fungal variables that were measured.

Monocultures and polycultures were also analyzed separately. This was so that (1) blocked MRPPs could be run on the two sets, and (2) the effects of plant functional group and plant species could be included in monoculture analyses. Under plant monocultures, the AM fungal spore communities differed between ambient and elevated CO_2 (A = 0.089, $P = 0.048$). This was not the case under plant polycultures ($P = 0.84$). *Glomus clarum*, in addition to indicating high plant species richness, indicated elevated CO_2 under monocultures (Table 4, Fig. 2a). *Glomus fasciculatum* indicated ambient CO_2 under monocultures, but as in the 3-yr data set, the statistical significance of this response was not retained within the full ANCOVA model of effects (Table 4, Fig. 2b). We also used MRPP to compare spore communities under the four groups created by CO_2 and plant species richness treatments (Fig. 3). Comparisons among all four groups yield significant differences, but the effect size is small (A = 0.013, $P = 0.009$). *Glomus clarum* and *G. fasciculatum* were significant indicator species for the interaction of plant species richness with CO_2, but neither retained a significant response to the interaction in the full model of effects. Pairwise comparisons of all groups demonstrate that communities under monocultures in ambient CO_2 are different from communities under the remaining groups. Although this finding must be interpreted with caution because of sample size differences and the lack of significance in the full ANCOVA model, it does depict an interactive effect of plant species richness and CO_2 on spore community composition.

Within the monoculture plots in 2000, total spore abundance and biovolume were significantly lower under the C_3

Table 3 One-year (2000) arbuscular mycorrhizal fungal (AMF) spore community responses, in all plots ($n = 176$)[1]. There were no significant interactions of any factors

Response	CO_2 (df = 1, error df = 4) mean (SE)			N (df = 1), error df = 110) Group means			Plant species richness (df = 1, error df = 110) Group means		
	Ambient	Elevated	P[1]	Ambient	Enriched	P[1]	1 sp.	16 spp.	P[1]
Total spore abundance (spores g^{-1} soil)	49.4 (4.38)	59.2 (4.33)	0.38	58.2 (4.90)	50.4 (3.75)	0.10	50.0 (3.30)	65.8 (7.05)	0.33
Total spore biovolume (mm^3 g^{-1} soil)	0.023 (0.002)	0.028 (0.003)	0.39	**0.028 (0.003)**	**0.024 (0.002)**	**0.040**	0.023 (0.002)	0.032 (0.003)	0.76
AMF species richness (species $plot^{-1}$)	6.38 (0.20)	6.45 (0.18)	0.97	**6.47 (0.19)**	**6.15 (0.19)**	**0.015**	6.31 (0.16)	6.68 (0.24)	0.55
Brillouin's diversity (*HB*) ($plot^{-1}$)	0.082 (0.039)	0.856 (0.034)	0.88	0.864 (0.039)	0.810 (0.035)	0.06	0.821 (0.032)	0.882 (0.046)	0.36

[1]*P*, level of significance using full ANCOVA model. Significant *P*-values and associated group means are in bold type.

Table 4 One-year means (2000) of arbuscular mycorrhizal fungal spore abundances under 16 plant species in monoculture (n = 128) and 16-species polyculture (n = 48)

	Monocultures (spores g^{-1} soil) Mean (SE)			16 spp. Polycultures Mean (SE)		
Morpho-species:	Ambient CO_2	Elevated CO_2	P[1]	Ambient CO_2	Elevated CO_2	P[2]
Acaulospora scrobiculata	0.039 (0.015)	0.063 (0.030)	0.92	0.080 (0.045)	0.069 (0.030)	–
Acaulospora spinosa	0.117 (0.100)	0.182 (0.111)	0.85	0.021 (0.019)	0.310 (0.166)	–
Archeospora trappei	0.866 (0.288)	2.25 (1.08)	0.20	1.23 (0.52)	0.425 (0.200)	–
Gigasporaceae spp.	0.228 (0.056)	0.210 (0.051)	0.89	0.243 (0.140)	0.262 (0.158)	–
Glomus aggregatum/intraradices	28.6 (2.4)	34.9 (2.7)	0.10	41.8 (10.1)	39.3 (4.0)	–
Glomus clarum	**5.3 (1.3)**	**11.9 (2.6)**	**0.012**	15.2 (4.2)	16.2 (3.4)	–
Glomus constrictum	0.124 (0.043)	0.072 (0.023)	0.64	0.073 (0.033)	0.114 (0.049)	–
Glomus fasciculatum	**2.65 (0.95)**	**0.60 (0.21)**	**0.016**[3]	1.13 (0.67)	0.63 (0.42)	–
Glomus occultum	3.57 (0.65)	6.47 (1.84)	0.07	7.23 (3.89)	4.89 (1.71)	–
Scutellospora calospora/pellucida	0.739 (0.158)	0.90 (0.201)	0.56	0.987 (0.232)	0.896 (.0275)	–
Scutellospora erythropa	0.225 (0.074)	0.220 (0.088)	0.74	0.193 (0.054)	0.240 (0.082)	–

[1]P = level of significance from indicator species analysis. Significant indicator species' P-values and group means are in bold type. [2]Indicator species analysis was not run for CO_2 in plant polycultures, because multi-response permutation procedure did not show a significant effect of CO_2 on spore community composition in this subset. [3]This response was not detectable using ANOVA with the full model of treatment effects.

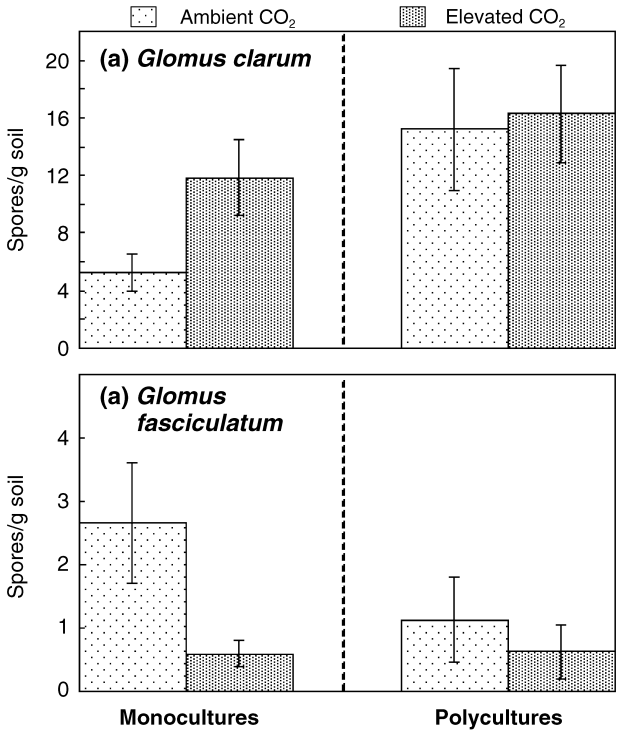

Fig. 2 Mean spore abundance (± SE, n = 128 monocultures and 48 polycultures) of the arbuscular mycorrhizal (AM) fungal species found to be significant by indicator species analysis of CO_2 and plant species richness in the year 2000. (a) *Glomus clarum* and (b) *Glomus fasciculatum* abundances are shown under plant monocultures and polycultures. Light columns, ambient CO_2; dark columns, elevated CO_2.

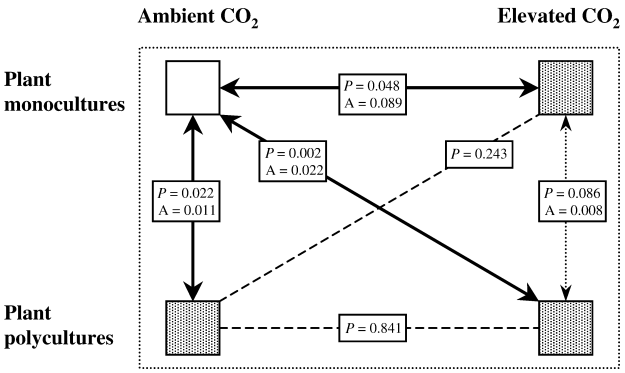

Fig. 3 Pair-wise comparisons of the four groups of AM fungal spore communities created by CO_2 and plant species richness treatments, as analysed by multi-response permutation procedure. Boxes represent the groups of communities. Dark arrows connect pairs that were found to be significantly different, light arrows connect pairs that are marginally different, and dashed lines link groups that did not differ significantly. The P-value (and effect size A, for significant differences) is given over the connector for each comparison. Communities under monocultures at ambient CO_2 were different from the other three treatment groups; no other treatment groups differed in this way.

grasses than the other functional groups, after the model accounted for root biomass covariation (not shown). However, the four plant functional groups were not associated with significantly different spore communities or with different species richness or diversity. Plant species did not significantly affect any AM fungal variables in 2000.

Discussion

We expected that enrichment of atmospheric CO_2 would increase total AM fungal spore production and alter community composition. Only the second of these predictions was supported by our study, and only when plants were grown in monoculture. While total AM fungal spore

biovolume was larger under plant monocultures grown in elevated CO_2, this increase was due to increased sporulation of only one AM fungal species in the genus *Glomus*. This result is consistent with the only previous work on AM fungal community responses to elevated CO_2; communities were measured to the genus level only, and only spores within the genus *Glomus* increased under elevated CO_2 (Klironomos *et al.*, 1998). We also expected that plant species richness would affect AM fungal spore abundance and diversity, but again this was not the general case; again, only one AM fungal species responded to plant species richness. Like most FACE experiments, this study has low statistical power to examine the effects of CO_2. There might be further effects of CO_2 on AM fungal sporulation that we cannot detect statistically. As always, further years of study are needed. Despite the statistical shortcomings of this experiment, it is clear that even within one year of data (2000), the response of *G. clarum* to CO_2 elevation was more pronounced under plant monocultures.

On average, polyculture plots contained twice as much fine root biomass as monoculture plots (607 vs 289 g m^{-2} based on two harvests in 2000; P. B. Reich, unpubl. data) but were not affected by CO_2 level (617 g m^{-2} and 581 g m^{-2} under polycultures in ambient and elevated CO_2, respectively; 303 g m^{-2} and 304 g m^{-2}, respectively, under monocultures in ambient and elevated CO_2). Thus, CO_2-induced community differences may be dampened through the 'saturating effect' of polyculture rhizospheres. In addition, in polycultures, the roots of many plant species are present. Although we did not detect it in this study, AM fungal species do appear to have 'preferred' host plant species under which their sporulation is maximized (Bentivenga & Hetrick, 1991; Dhillion, 1992; Johnson *et al.*, 1992; Sanders & Fitter, 1992; Bever *et al.*, 1996; Eom *et al.*, 2000). Therefore, the quantity and/or variety of roots available may be sufficient to explain the effect of high plant species richness on AM fungal communities and their responses to CO_2.

We observed a negative response of *G. fasciculatum* to CO_2 enrichment in 2000, but cannot be sure that this is a real response without more years of measurements. Even so, the response of *G. clarum* to CO_2 was unique. It is possible that other species responded, but were too rare to be detected statistically. However, this is clearly not the case for *G. aggregatum*/*intraradices*, which was present in very large numbers and in 100% of plots within the experiment, but did not respond to CO_2. Hence, within a single genus, AM fungal species are individualistic in their responses to elevated CO_2. Such individualism may result from species differences in: (1) competitive abilities to obtain photosynthate from plants; (2) C allocation patterns (i.e. between spores and other structures); and/or (3) different affinities for altered forms of photosynthate under elevated CO_2 (e.g. increased phenolic concentrations).

We found spatial and year-to-year variation in the overall abundance and community composition of AM fungal spores. Annual variability within AM fungal spore communities is well documented (Bentivenga & Hetrick, 1991; Edgerton-Warburton & Allen, 2000). However, the variation in our study may also reflect postdisturbance succession (following the tillage and methyl bromide application) in addition to interannual differences. Continuing AM fungal succession is indicated by the fact that spore diversity continued to increase in the third year of the study, although total spore production and species richness peaked in the second year. Regardless of the cause, the temporal variation observed here indicates that repeated measurements over time are important for discerning responses of AM fungi from background variation. The consistent positive response of *G. clarum* to elevated CO_2 indicates that this species' preference for conditions associated with CO_2 enrichment supersedes temporal, successional and spatial community variation. However, other AM fungal community responses may be obscured by this inevitable background variation.

We did not find strong effects of N enrichment or of host plant species on AM fungal communities, in contrast to the findings of other researchers at this site (Johnson *et al.*, 1992; Johnson, 1993). However, the present study has had a much shorter history of N and host plant treatment than the previous studies (1–3 yr in this study, compared with eight or more years of N addition and 4-yr-old host plants in previous work). We expect that stronger AM responses to N enrichment will emerge in future years of the study. We also expect that differentiation of AM fungal communities under the different plant species in monoculture will be manifested in the future.

What is the ecological significance of the spore community changes that we observed? Spore communities do not necessarily reflect the community composition of active root- and soil-colonizing AM fungi (Clapp *et al.*, 1995). However, when looking for ecological change in studies with replicated treatments, spore analysis can still be a useful indicator even though it may not capture the entire cohort or the absolute community composition of AM fungal symbionts. In the analysis of pollen from sediment, the communities enumerated are interpreted as reflections of the vegetation at the time, even though they do not describe the entire plant community. Therefore, detectable changes in pollen assemblages over time indicate significant changes in the plant community (Traverse, 1999). Enumeration of AM fungal spore communities may be interpreted similarly; if we detect changes in what we can measure (spores), we may assume that the unmeasurable source of these changes (the collective thalli of the entire AM fungal community) is also undergoing change, possibly even greater change than what was detected in the subset of AM fungi that have sporulated.

The CO_2 enrichment experiments using plant monocultures and, more extremely, using individual plants in pots, are precise and easy to model. While these types of experiments are the starting point for answering many research questions,

our findings indicate that AM fungal responses to elevated CO_2 may vary with different levels of plant species richness. Therefore future CO_2 experiments should incorporate growth conditions similar to those in the communities and ecosystems of interest. Changes in AM fungal community structure may have complex feedbacks on C cycling, soil quality, and soil responses to rising atmospheric CO_2. The importance of plant species richness in buffering such changes in AM fungal communities should be investigated further.

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