Seven years of carbon dioxide enrichment, nitrogen fertilization and plant diversity influence arbuscular mycorrhizal fungi in a grassland ecosystem

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Received: *16 February 2011*
Accepted: *25 April 2011*

New Phytologist (2011) **192**: 200–214
doi: 10.1111/j.1469-8137.2011.03776.x

Key words: arbuscular mycorrhiza, CO_2 enrichment, community composition, grassland, niche partitioning hypothesis, nitrogen fertilization, plant richness, structural equation model.

Summary

- We tested the prediction that the abundance and diversity of arbuscular mycorrhizal (AM) fungi are influenced by resource availability and plant community composition by examining the joint effects of carbon dioxide (CO_2) enrichment, nitrogen (N) fertilization and plant diversity on AM fungi.
- We quantified AM fungal spores and extramatrical hyphae in 176 plots after 7 yr of treatment with all combinations of ambient or elevated CO_2 (368 or 560 ppm), with or without N fertilization (0 or 4 g N m^{-2}), and one (monoculture) or 16 host plant species (polyculture) in the BioCON field experiment at Cedar Creek Ecosystem Science Reserve, Minnesota, USA.
- Extramatrical hyphal lengths were increased by CO_2 enrichment, whereas AM spore abundance decreased with N fertilization. Spore abundance, morphotype richness and extramatrical hyphal lengths were all greater in monoculture plots. A structural equation model showed AM fungal biovolume was most influenced by CO_2 enrichment, plant community composition and plant richness, whereas spore richness was most influenced by fungal biovolume, plant community composition and plant richness.
- Arbuscular mycorrhizal fungi responded to differences in host community and resource availability, suggesting that mycorrhizal functions, such as carbon sequestration and soil stability, will be affected by global change.

Introduction

Arbuscular mycorrhizal (AM) fungi influence plant nutrition, water relations and pathogen interactions (Linderman, 1988; Newsham *et al.*, 1995; Augé, 2001). These ubiquitous fungi also structure the soil by contributing substantial amounts of organic matter and stabilizing soil aggregates (Miller & Jastrow, 1990; Olsson *et al.*, 1999; Wilson *et al.*, 2009). Plant species, cultivars and ecotypes vary greatly in their dependence on mycorrhizas; consequently, mycorrhizal symbioses differentially affect plant community structure and stability (Van der Heijden *et al.*, 1998; Hartnett & Wilson, 2002). Taxa of AM fungi (Glomeromycota) likewise vary in their symbiotic effects on host plants (Klironomos, 2003), and resource availability influences the degree to which plants benefit from AM trading partnerships (Mosse, 1973; Koide *et al.*, 2000; Johnson, 2010). Thus, predicting the function of AM fungi within communities and ecosystems requires an understanding of the biotic and abiotic factors that control the abundance and diversity of AM fungi in their environment (Johnson *et al.*, 2006).

Environmental changes caused by human activities influence plant communities. Increasing atmospheric carbon dioxide (CO_2) influences plant water relations and often preferentially benefits plant species with a C_3 photosynthetic pathway, thus changing the species composition of plant communities (Koch & Mooney, 1996; Reich *et al.*, 2001a; Reich, 2009). Increasing the availability of mineral nitrogen (N) often increases total plant production but decreases diversity as a few nitrophilic plant species become dominant (Silvertown, 1980; Tilman, 1987). Furthermore, interactions between CO_2 and N can complicate plant community responses. For example, the magnitude of plant productivity responses to simultaneous CO_2 and N enrichment has been

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shown to be greater in plant communities with greater species or functional group richness (Reich *et al.*, 2001b, 2004), and recently it was shown that elevated CO_2 can decrease plant species loss caused by N fertilization (Reich, 2009).

Enrichment of CO_2 and N can impact AM symbioses by changing the availability of essential resources as well as by changing the plant community composition (Fitter *et al.*, 2000; Treseder & Allen, 2000; Staddon *et al.*, 2003). Resource enrichment affects AM symbioses because mycorrhizal trading partnerships are based upon the exchange of fixed carbon (C) for mineral elements. Elevated CO_2 is predicted to reduce plant C limitation relative to below-ground resources, making mycorrhizal delivery of mineral resources more valuable and stimulating plants to allocate more C to their fungal symbionts (Treseder, 2004). The effects of N enrichment on AM fungi have been shown to be mediated by soil phosphorus (P) availability; N enrichment of P-limited soils has been shown to increase the biomass of AM fungi while N enrichment of P-rich soil decreases it (N. Johnson *et al.*, 2003b). Because resource availability affects plant community composition, it is important to consider the effects of interactions among N, CO_2 and plant diversity on AM fungal communities.

The diversity and species composition of plant communities can influence the abundance, diversity and species composition of AM fungal communities (Oehl *et al.*, 2003; Landis *et al.*, 2004; Wu *et al.*, 2007). Net primary productivity is often higher in taxonomically rich plant communities than in more depauperate communities (Tilman, 1997; Grime, 1997; Wardle, 2001; Grace *et al.*, 2007), and since AM fungi gain all of their C from living plant hosts, it is logical to expect higher AM fungal biomass in more diverse and productive plant communities where more host C is available. To a certain extent, AM fungi are generalist biotrophs and will colonize a remarkably wide variety of plant hosts (Allen *et al.*, 1995; Rosendahl, 2008; Helgason & Fitter, 2009); however, many studies indicate a degree of ecological specificity among fungal–plant pairs (Helgason *et al.*, 1998; Klironomos, 2000; Bever, 2002; Vandenkoornhuyse *et al.*, 2003). Although it is intuitive to expect highly diverse plant communities to generate more potential AM fungal niches and support a larger diversity of AM fungal taxa compared with plant communities that are depauperate of species (Urcelay & Diaz, 2003; De Deyn *et al.*, 2010), this expectation is not universally supported (D. Johnson *et al.*, 2003a; Börstler *et al.*, 2006). The lack of a consistent relationship between diversity of AM fungi and their plant hosts suggests that factors other than plant diversity are also important in structuring AM fungal communities.

We studied the AM fungi in a multi-factor long-term field experiment to better understand the relative degree to which communities of AM fungi may be structured by resource availability, host community richness, host community composition, and the interactions of these factors. Our focus was on AM fungi within the soil, measured by extramatrical hyphae (EMH) and spores, because up to 90% of the biomass of AM fungi can be found outside plant roots (Olsson *et al.*, 1999). Furthermore, EMH are critical to soil structure and nutrient cycling (Miller & Jastrow, 2000; Rillig & Mummey, 2006), and changing their abundance may have important consequences for soil C sequestration (Rillig *et al.*, 2002; Wilson *et al.*, 2009). Although recent advances in molecular methods are revolutionizing AM research, there is also still value in quantitative analysis of AM fungal spores across gradients and experimental treatments. For example, quantitative spore analysis has revealed successional patterns in Glomeromycotan communities (Johnson *et al.*, 1991), changes in species occurrences with anthropogenic N deposition (Egerton-Warburton & Allen, 2000), and strong correlations with plant community composition and soil properties (Landis *et al.*, 2004). A major criticism of spore analysis is that Glomeromycotan fungi vary in the degree to which they form spores; and thus, spore communities in the soil may not reflect the community of fungi active within plant roots (Clapp *et al.*, 1995; Börstler *et al.*, 2006). Nevertheless, since Glomeromycota are obligate biotrophs, the presence of high populations of particular spore taxa in an area suggest some degree of activity of that fungus in that area. Furthermore, spore production has been shown to reflect the relative fitness of some AM fungi (Bever, 2002). Thus, although we are likely missing nonspore-forming AM fungi, variation in spore populations of the spore-forming taxa of AM fungi across controlled CO_2, N, and plant diversity treatments provides a meaningful metric for testing our hypotheses. We hypothesized that: (1) N enrichment of the P-rich soil at our study site would reduce AM fungal hyphae and diversity of AM spores in the soil as plants allocate less C below ground and more above ground (Table 1a); (2) enrichment of atmospheric CO_2 would increase AM fungal hyphae and diversity of AM spores in the soil as C becomes relatively less limiting and below-ground resources relatively more limiting to plants; (3) diverse plant communities would support a higher density of hyphae and diversity of AM fungal spores than monocultures; (4) AM fungi would respond differently to different plant functional groups. Specifically, we expected that mycorrhizal responses to CO_2 and N enrichment would vary among plants with C_3 and C_4 photosynthetic pathways because of their differences in mycotrophy (Wilson & Hartnett, 1998; Hoeksema *et al.*, 2010) and their contrasting responses to CO_2 enrichment and N fertilization (N. Johnson *et al.*, 2003c; Antoninka *et al.*, 2009).

Materials and Methods

Study design

We studied the direct and interactive effects of plant diversity and CO_2 and N availability on AM soil fungi in

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Table 1 Our hypothesized (a) and observed (b) impacts on arbuscular mycorrhizal (AM) biovolume in the soil and AM spore morphotype richness (spore richness) from our structural equation model (SEM), including direct, indirect and total effect coefficients (λ) for hypothesized causal relationships

	(b) Observed outcomes (λ)		
(a) Expected outcomes	Direct path	Indirect paths	Total effects
Abiotic resource effects			
H1 – N fertilization will:[a]			
Decrease AM biovolume	−0.05	0.02	−0.03
Decrease spore richness	NA	−0.02	−0.02
H2 – Enrichment of CO_2 will:			
Increase AM biovolume	0.37	0.02	0.39
Increase spore richness	NA	0.09	0.09
Host-mediated effects			
H3 – Higher plant richness will:			
Increase AM biovolume	**−0.27**[b]	0.04	**−0.23**
Increase spore richness	−0.38	−0.01	**−0.37**
H4 – Plant community structure will:[c]			
Affect AM biovolume	−0.10	−0.15	−0.25
Affect spore richness	0.23	0.20	0.43
Weed biomass will			
Affect AM biovolume	**0**	0	**0**
Affect spore richness	−0.20	0	−0.20
Target plant biomass will:			
Increase AM biovolume	0.10	0	0.10
Increase spore richness	**−0.01**	0.09	0.08
AM-mediated effects			
H5 – AM biovolume will:			
Increase spore richness	0.31	NA	0.31

H, hypothesis.
[a]Expected outcomes correspond to arrows in the SEM (Fig. 1).
[b]Bold text highlights instances where the expected effect is different from the observed effect.
[c]Plant community structure is a composite variable derived from the biomass of each plant functional group.

the split-plot factorial biodiversity, CO_2 and nitrogen (BioCON) field experiment at the Cedar Creek Ecosystem Science Reserve, MN, USA (45°N, 93°W; Reich *et al.*, 2001a,b). BioCON's six free-air CO_2 enrichment (FACE) rings were established in 1997, with three 20-m-diameter rings maintained at ambient CO_2 (368 ppm in 2004) and three at elevated CO_2 (560 ppm) alternated spatially on the landscape. Within each FACE ring, 2 m^2 plots were randomly assigned to several plant species richness (0, 1, 4, 9, 16) and N treatments (0 or 4 g N m^{-2} yr^{-1} as NH_4NO_3; Reich *et al.*, 2001b). We examined the plots containing one (monoculture; N = 128) or 16 (polyculture; N = 48) species with all combinations of CO_2 and N in 2004, after 7 yr of treatment. Polyculture plots diverged to some extent in community composition and richness by treatment over time (Reich *et al.*, 2001a,b, 2004; Reich, 2009), and as a result, the actual species richness in these polycultures ranged from five to 13 species (mean ± SD: 9.8 ± 3.4 species) at the time of our sampling,

At BioCON, the mean annual temperature is 5.6°C and mean annual precipitation is 679 mm, distributed relatively evenly throughout the year. Soil at this site is excessively drained outwash sand (Nymore series, subgroup Typic Upidsamment, suborder Psamments, Order Entisols), with low soil organic matter (*c.* 1.4%), high soil phosphorus (*c.* 46.5 μg PO_4-P g^{-1} soil, Bray-1) and low available N (*c.* 10 μg NH_4-N + NO_3-N g^{-1} soil; Johnson, 2010). Soil was treated with methyl bromide 8 yr before this sampling to remove the seed-bank before seeding with target species. Although methyl bromide is known to reduce Glomeromycotan propagules, AM fungi can recolonize quickly following fumigation (Menge, 1982). High densities of AM fungi were observed in the soil within a year of fumigation (Wolf *et al.*, 2003).

Four functional groups composed of four plant species each were seeded into polyculture plots or individually into monoculture plots; the C_3 grasses included *Agropyron repens* (L.) Beauv., *Bromus inermis* Leysser, *Koeleria cristata* Pers and *Poa pratensis* (L.); the C_4 grasses included *Andropogon gerardii* Vitman, *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths, *Schizachyrium scoparium* (Michaux) Nash and *Sorghastrum nutans* (L.) Nash; the herbaceous forbs included *Achillea millefolium* L., *Anemone cylindrica* A. Gray, *Asclepias tuberosa* L. and *Solidago rigida* L.; and the N-fixing legumes included *Amorpha canescens* Pursch, *Lespedeza capitata* Michaux, *Lupinus perennis* L., and *Petalostemum villosum* Nutt. All species form mycorrhizas, even the *Lupinus* (A. Antoninka, unpublished). After planting in 1997, the plots were only reseeded once in 1998 with sparse species, but have been regularly weeded to remove nontarget species. The dominant species in polyculture were legumes and C_3 grasses, particularly *Luinis perennis* and *Poa pratensis.*

Analysis of AM fungi

Three soil cores (5 cm diameter, 20 cm deep) were taken in September 2004 from each plot. Soil was homogenized in the laboratory and stored frozen. Spores were extracted from *c.* 25 g of fresh soil and a subset of soil was oven-dried (105°C) and weighed to calculate spores g^{-1} soil. Spores were extracted using wet sieving and sucrose centrifugation (McKenney & Lindsey, 1987; Johnson *et al.*, 1999), mounted on permanent glass slides with polyvinyl lactoglycerol, examined under 100–1000× magnification with a compound microscope, identified to species using morphological characteristics (Schenck & Perez, 1990; INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi) http://invam.caf.wvu.edu/; Schüβler's Glomeromycota Species List, http://www.lrz.de/~schuessler/amphylo/), and counted. Voucher slides and photographs are maintained at Northern Arizona University. Average diameter was measured for each species and spore biovolume

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was calculated as $V = 4/3\ \pi r^2$ for spherical spores and $V = 1/6\ \pi D_1 D_2^2$, where D_1 is the length and D_2 is the width for elongated spores. We used *c.* 1.25 g of soil to extract EMH from the homogenized soils using the methods of Abbott *et al.* (1984). EMH length was quantified using a compound microscope with a gridded reticule at ×250 magnification. Biovolume of EMH was calculated using the equation for a cylinder ($V = \pi r^2 h$), where r was set to one-half the average hyphal diameter observed (r = 2.5 μm) and h was the calculated hyphal length. We calculated AM fungal biovolume in the soil as the sum of spore and hyphal biovolume g^{-1} soil.

Mean responsiveness of AM spore abundance, richness and EMH to CO_2 and N treatments were calculated by plant functional group to compare responses across groups in all plots. We calculated responses to CO_2 by subtracting each value at ambient CO_2 from each value at elevated CO_2 with and without N fertilization and taking the mean. Responses to N were calculated by subtracting each value without N fertilization from each value with N fertilization at elevated and ambient CO_2 and taking the mean.

Plant measurements

Plant above-ground biomass was clipped to the soil surface in a 10 × 100 cm strip in each plot in August 2004. Plant biomass was separated by species, dried and weighed. Root biomass estimates were obtained by collecting roots from five soil cores (5 cm diameter × 1 m deep) in each plot. Roots were washed, dried and weighed. Root mass fraction was calculated as the root biomass divided by the total biomass.

Some nontarget plants colonized the experimental plots. These 'weeds' were removed on six occasions during the growing season. The sum total biomass of weeds removed from each plot across the entire growing season was included as a predictor of mycorrhizal responses to the experimental treatments. Weed biomass contains both shoots and roots, but root biomass is almost certainly an underestimate.

Statistical analysis

Response variables were checked for normality of distribution and homogeneity of variance. We built two models using a nested ANCOVA design in JMP 4.04 (SAS 2000) to test for treatment and interaction effects where all treatment effects were considered fixed except the random effect of the blocking variable, ring nested within CO_2 concentration. Fine root biomass was used as a covariate to uncouple plant and AM responses to treatment (Wolf *et al.*, 2003). The first model included: ring, two concentrations of N, two concentrations of CO_2, two values of plant richness and the interaction terms of N × CO_2, N × plant richness and CO_2 × plant richness (degrees of freedom (df) denominator = 1 for all terms except ring, df = 4). In the second model we looked only at monocultures to see if there were differences in AM responses to plant functional groups. This model was as described earlier, except that we used functional group instead of plant richness (df denominator = 1 for all terms except ring, df = 4, and all terms including functional group, df = 3). Three-way interactions were not considered in either model because the complexity of interpretation would make it difficult to assess if results were biologically meaningful, particularly because of low replication.

We performed multivariate response permutation procedures (MRPP) in PC-ORD, (PC-ORD, Version 5.0, 2006) to look for AM spore community differences among treatments using Bray–Curtis distance (McCune & Mefford, 1999). The reported test statistic (*A*-value) is a description of the effect size describing within-group homogeneity, where zero indicates that heterogeneity within a sample is equal to that expected by chance. A higher value indicates higher within-sample heterogeneity than expected by chance, and a negative value indicates lower within-sample heterogeneity than expected by chance (McCune & Grace, 2002). We conducted indicator species analyses with Monte Carlo randomization tests in PC-ORD to determine if individual AM spore species were sensitive to treatments (Dufrene & Legendre, 1997). We report the *P*-value and indicator value (IV), which is a product of the relative abundance and frequency of occurrence of a species within a treatment. Dufrene & Legendre (1997) suggest that species with IVs > 25 are strong indicators. Because of differences in sample size, we modeled species accumulation curves based on sample number and also number of AM spores using EstimateS V. 8.2 (Colwell, 2009) to determine if we had adequately sampled both plant richness values (monocultures and 16-species polycultures).

We built an *a priori* structural equation model (SEM) in AMOS (SPSS, 2006), hypothesizing the relationships among biotic and abiotic influences on AM fungal biovolume in soil and AM richness (i.e. CO_2, N, plant richness, plant community structure, plant biomass and weed biomass) in order to partition the variance among possible causal influences (Table 1a, Fig. 1, Supporting Information Table S1). This approach allows analysis of net treatment effects on response variables and also interactions among response variables (Bollen, 1989; Shipley, 2000; McCune & Grace, 2002). Because few treatment interactions were found, we did not include interaction terms in the model. CO_2 and N were treated as bivariate variables with CO_2 represented as 368 or 560 ppm, and N represented as 0 or 4 g N^{-1} g^{-1} soil. Instead of using the experimental plant richness values (i.e. 1 or 16) we used the realized plant richness values (range: one to 13 species) found in 2004 for our plant richness variable. Plant community structure was modeled as a statistical composite variable with four causal response variables: total plant biomass in each plot made up of C_3 grasses, C_4 grasses, forbs or legumes. The composite variable allowed us to account for multivariate responses by

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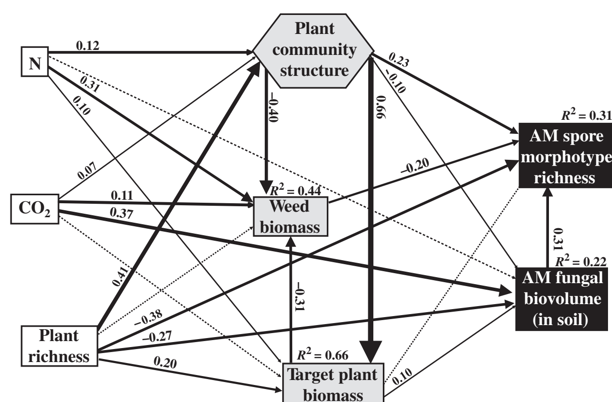

Fig. 1 A structural equation model (SEM) showing the causal influences of treatments (white rectangles), plant response variables (gray rectangles) and a composite variable that represents plant community structure based on biomass by functional groups (gray hexagon) on arbuscular mycorrhizal (AM) fungal biovolume and richness (black rectangles). Width of arrows indicates the strength of the causal influence. Numbers above arrows indicate path coefficients; a measure of the strength of causal relationships that are mathematically equivalent to either partial regression coefficients or correlation coefficients. Nonsignificant pathways are represented with dashed lines ($\lambda \leq 0.05$). R^2 values represent the proportion of variance explained for each endogenous variable.

representing the collective effects of the four plant biomass variables on the downstream response variables of interest. Target plant biomass represents the total biomass of all target plants in a plot. We modeled a pathway from AM biovolume to AM spore richness, hypothesizing that higher AM biovolume would increase the probability of encountering new AM species.

Path coefficients (λ) are mathematically equivalent to partial correlation coefficients. Using path rules we were able to calculate the direct, indirect and total influences of each treatment and response variable on all downstream variables (Grace, 2006). By comparing the covariance structure that is implied by the model with the actual covariance structure of the data, we tested how well the model fit the data (Grace & Pugesek, 1998) using the Bollen & Stine (1992) bootstrap test, the maximum likelihood χ^2 goodness-of-fit (GFI) test, and root mean square error of approximation (RMSEA; Steiger, 1990; df = 5). Low *P*-values indicate lack of fit; therefore, high *P*-values indicate that the data fit the model well. We chose to use multiple measures of model fit as recommended by Grace (2006) because each has different strengths and weaknesses. If all indices show a significant model fit, we can be confident in our model structure. No pathways were removed from our model; hence the final and *a priori* models are the same (Fig. 1).

Supplemental analysis of plant richness findings

Contrary to our hypothesis, higher plant richness led to lower AM spore abundance and richness as well as reduced EMH. We used regression analysis (JMP 4.04), Mantel tests (PC-ORD 5.0) and our structural equation model (Table 1a) to investigate possible reasons for this unexpected result. First, we hypothesized that this relationship may have been driven by a particular plant species or functional group. Secondly, we hypothesized that weed biomass might be higher in monoculture plots because more open space was available to colonize, leading to a richer plant community and a temporary C source for AM fungi. Thirdly, we hypothesized that resource availability and plant community composition and biomass would differentially impact AM biovolume and richness. We used our SEM to partition the variance in AM biovolume and spore richness among plant and resource variables to determine the strongest drivers.

Results

Twenty-five morphotypes of Glomeromycotan spores were found across all of the experimental plots. The mean abundance of spore morphotypes by treatment, relative abundance and frequency across all plots are given in Table 2. Bold values show significant indicator species for a particular treatment. Four species were present in > 90% of plots (*Archeaospora trappei*, *Glomus aggregatum/ intraradices*, *Glomus microaggregatum*, and *Paraglomus occultum*) and three species were present in < 10% of plots (*Acaulospora spinosa*, *Glomus pansihalos* and *Glomus tenue*). The most abundant species were *G. aggregatum/ intraradices* and *G. microaggregatum*. While no species were unique to polyculture, eight were unique to monoculture (Table 2).

Species accumulation curves, based on number of plots sampled and the number of individual spores counted, verify that adequate sampling was conducted at both plant richness levels (Fig. S1). In monoculture, 25 morphotypes were observed compared with the first- and second order jackknife estimates of 27.0 and 29.0, respectively. In polyculture, 18 morphotypes were observed compared with 18.0 and 18.9 for the first- and second-order jackknife estimates, respectively. This indicates that not only do polycultures have fewer spore species per plot, but they accumulate AM spore morphotypes at a slower rate and reached an asymptote with fewer morphotypes than monocultures.

Treatment effects: main and interactive

Despite significant main effects of all three treatments on AM fungi and plants, there were few significant interactions among treatments in the full data set (Table 3) or in the monocultures analyzed separately (Table 4). The blocking variable, ring nested in CO_2 treatment, accounted for significant variability for all variables in the monoculture plots (data not shown), indicating that blocking was necessary, and the substantial variation among rings within CO_2 treatments required large differences between CO_2 levels

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Table 2 Morphospecies abundances based on treatment (mean spores g^{-1} soil (± SE))

Species name	No nitrogen	Nitrogen	Ambient CO_2	Elevated CO_2	Monoculture	Polyculture	Relative abundance[a]	Frequency (%)[b]
Acaulospora delicata	0.23 (0.06)	0.19 (0.06)	0.23 (0.08)	0.19 (0.04)	0.05 (0.01)	**0.69** (**0.14**)[c]	0.25	29.03
Acaulospora laevis	0.10 (0.02)	0.06 (0.01)	0.06 (0.02)	0.09 (0.02)	**0.11** (**0.00**)	0.01 (0.01)	0.11	20.97
Acaulospora morrowiae	0.11 (0.03)	0.07 (0.03)	0.05 (0.02)	0.13 (0.04)	**0.13** (**0.03**)	0.00 (0.00)	0.13	16.67
Acaulospora scrobiculata	0.11 (0.04)	0.11 (0.08)	0.12 (0.08)	0.10 (0.04)	0.16 (0.07)	0.04 (0.02)	0.15	13.98
Acaulospora spinosa	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.03 (0.01)	0.02 (0.01)	0.04 (0.02)	0.06	8.60
Archeaospora trappei	7.99 (0.49)	7.28 (0.48)	7.13 (0.46)	8.14 (0.51)	7.41 (0.40)	8.43 (0.75)	8.24	98.92
Entrophospora infrequens	0.41 (0.18)	0.20 (0.08)	0.10 (0.03)	**0.51** (**0.19**)	0.21 (0.09)	**0.62** (**0.29**)	0.36	21.51
Gigaspora gigantea	0.16 (0.06)	0.13 (0.03)	0.15 (0.05)	0.14 (0.03)	0.08 (0.02)	0.35 (0.10)	0.19	43.01
Gigaspora margarita	0.06 (0.02)	0.20 (0.15)	0.20 (0.15)	0.06 (0.02)	0.14 (0.08)	0.03 (0.01)	0.13	20.97
Glomus aggregatum/intraradices	48.38 (3.17)	42.64 (2.66)	48.67 (3.35)	42.29 (2.41)	48.33 (2.71)	33.77 (2.48)	48.92	100
Glomus clarum	6.51 (0.92)	5.66 (0.81)	5.38 (0.96)	6.78 (0.76)	4.84 (0.60)	**10.58** (**1.57**)	6.57	96.24
Glomus constrictum	2.57 (0.73)	1.80 (0.69)	1.27 (0.38)	**3.09** (**0.92**)	2.62 (0.69)	1.44 (0.71)	2.37	55.38
Glomus etunicatum	0.23 (0.07)	0.78 (0.41)	0.37 (0.19)	0.64 (0.38)	**0.56** (**0.23**)	0.00 (0.00)	0.43	20.43
Glomus fasciculatum	6.32 (1.34)	7.17 (1.47)	6.11 (1.34)	7.39 (1.47)	**8.24** (**1.42**)	4.15 (0.76)	7.29	89.24
Glomus fistulosum	0.41 (0.19)	0.37 (0.14)	0.26 (0.06)	0.51 (0.23)	**0.57** (**0.17**)	0.00 (0.00)	0.45	24.73
Glomus globiferum	0.06 (0.02)	0.03 (0.02)	0.03 (0.01)	0.06 (0.02)	0.05 (0.02)	0.00 (0.00)	0.08	10.21
Glomus microaggregatum	14.99 (2.91)	10.49 (1.36)	9.55 (1.02)	**15.88** (**3.00**)	13.48 (1.71)	12.18 (4.28)	13.70	98.39
Glomus mosseae	0.14 (0.04)	0.11 (0.03)	0.11 (0.03)	0.14 (0.04)	0.15 (0.03)	0.08 (0.03)	0.16	27.42
Glomus tenue	0.13 (0.06)	0.19 (0.17)	0.28 (0.18)	0.03 (0.02)	0.23 (0.13)	0.00 (0.00)	0.20	8.60
Glomus versiforme	4.57 (1.32)	4.77 (1.35)	3.28 (0.81)	6.06 (1.70)	6.86 (1.35)	0.31 (0.19)	5.05	38.17
Paraglomus occultum	4.21 (0.91)	3.31 (0.63)	3.72 (0.72)	3.78 (0.84)	5.06 (0.79)	1.00 (0.14)	4.06	94.09
Scutellospora arborensis	0.03 (0.01)	0.01 (0.01)	0.01 (0.01)	0.02 (0.01)	0.03 (0.01)	0.00 (0.00)	0.05	10.22
Scutellospora calospora	0.54 (0.13)	0.52 (0.15)	0.49 (0.12)	0.56 (0.16)	0.57 (0.11)	0.00 (0.00)	0.45	46.24
Scutellospora erythropa	0.18 (0.05)	0.07 (0.01)	0.12 (0.02)	0.12 (0.05)	0.13 (0.04)	0.11 (0.02)	0.16	43.55
Scutellospora pellucida	0.42 (0.08)	0.31 (0.05)	0.31 (0.06)	0.42 (0.07)	0.27 (0.03)	0.71 (0.15)	0.42	68.28

[a]Relative abundance was calculated as n_i/N where n_i is the abundance of a particular morphotype at a site and N is the total abundance of all spores at a site.
[b]Frequency was calculated as the proportion of the 176 samples in which the morphotype was observed.
[c]Bold font indicates that the morphotype is a significant indicator species for a particular site.

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Table 3 ANCOVA (a; for arbuscular mycorrhizal (AM) fungal responses) and ANOVA (b; for plant responses) results for interaction terms and main effects (F (*P*)) within the full data set (*N* = 176)

Response variable	N × CO_2	N × plant richness	CO_2 × plant richness	N	CO_2	Plant richness
(a) AM fungal responses						
AM spore abundance	0.21 (0.65)	0.95 (0.33)	0.30 (0.59)	**6.35** (**0.01**)	2.56 (0.11)	**16.36** (**< 0.0001**)
AM spore biovolume	0.43 (0.51)	0.21 (0.65)	**3.99** (**0.05**)	0.95 (0.76)	**19.83** (**< 0.0001**)	**11.89** (**0.0007**)
AM spore richness	0.06 (0.81)	0.53 (0.47)	0.16 (0.69)	2.72 (0.10)	3.29 (0.07)	**31.18** (**< 0.0001**)
AM spore evenness	0.99 (0.32)	0.03 (0.87)	0.003 (0.96)	0.22 (0.64)	0.99 (0.32)	**1.20** (**0.28**)
External hyphal density	0.44 (0.51)	0.0001 (0.99)	3.63 (0.06)	0.36 (0.55)	**47.55** (**< 0.0001**)	**20.68** (**< 0.0001**)
(b) Plant responses						
Total biomass	0.25 (0.62)	0.43 (0.51)	2.79 (0.10)	**13.35** (**0.0004**)	5.23 (0.06)	**86.78** (**< 0.0001**)
Shoot biomass	0.00 (0.99)	0.17 (0.68)	2.61 (0.11)	2.88 (0.09)	**8.31** (**0.02**)	**53.36** (**< 0.0001**)
Root biomass	0.35 (0.56)	0.92 (0.34)	1.40 (0.24)	**11.88** (**0.0007**)	1.60 (0.25)	**54.10** (**< 0.0001**)
Root mass fraction	0.60 (0.43)	0.20 (0.66)	0.21 (0.65)	1.83 (0.18)	0.37 (0.56)	2.33 (0.13)
Weed biomass	0.15 (0.70)	2.22 (0.14)	0.27 (0.60)	1.87 (0.17)	0.08 (0.78)	**37.35** (**< 0.0001**)

Bold values indicate a significant difference at alpha level equal to 0.05.

Table 4 ANCOVA (a; for arbuscular mycorrhizal (AM) fungal responses) and ANOVA (b; for plant responses) results for interaction terms and main effects (F (*P*)) within monocultures of different functional groups (*N* = 128)

Response variable	N × CO_2	N × plant functional group	CO_2 × plant functional group	N	CO_2	Plant functional group
(a) AM fungal responses						
AM spore abundance	0.10 (0.76)	2.20 (0.09)	2.15 (0.09)	1.73 (0.19)	0.67 (0.42)	0.822 (0.49)
AM spore biovolume	0.84 (0.36)	0.43 (0.73)	1.69 (0.17)	0.45 (0.50)	**4.85** (**0.03**)	1.10 (0.35)
AM spore richness	0.24 (0.63)	0.33 (0.80)	**2.95** (**0.04**)	**4.93** (**0.03**)	1.87 (0.17)	**8.12** (**< 0.0001**)
AM spore evenness	1.09 (0.30)	0.21 (0.93)	1.96 (0.10)	0.11 (0.75)	1.31 (0.25)	**10.43** (**< 0.0001**)
External hyphal density	2.27 (0.14)	0.33 (0.80)	0.20 (0.89)	0.24 (0.63)	**20.59** (**< 0.0001**)	0.45 (0.71)
(b) Plant responses						
Total biomass	0.65 (0.58)	**4.02** (**0.02**)	2.36 (0.10)	0.001 (0.97)	2.36 (0.13)	0.65 (0.58)
Shoot biomass	0.03 (0.96)	1.72 (0.74)	1.70 (0.17)	0.73 (0.40)	0.26 (0.61)	0.34 (0.79)
Root biomass	2.84 (0.09)	1.36 (0.26)	**3.47** (**0.02**)	0.03 (0.85)	2.36 (0.13)	0.72 (0.54)
Root mass fraction	1.24 (0.27)	0.40 (0.75)	0.46 (0.70)	1.19 (0.28)	0.67 (0.41)	0.39 (0.76)
Weed biomass	0.26 (0.61)	0.17 (0.92)	0.11 (0.95)	3.54 (0.06)	0.18 (0.67)	**9.16** (**< 0.0001**)

for statistical significance. Small differences in soil bulk density and soil water availability correlated with the differences seen in AM fungal variables across the six FACE rings (Antoninka, 2011).

AM fungal responses to N

As a main effect, N fertilization decreased spore abundance but did not affect EMH or spore richness. Spore community composition did not respond to N treatment either (Fig. 2a; $A = -0.001$, $P = 0.74$); however, *S. erythropa* spores were more common without fertilization (IV = 36.0, $P = 0.01$). In monocultures alone, N fertilization modestly decreased richness (mean ± SE) of AM spores (no N, 10.0 ± 0.04; N, 9.7 ± 0.04; Table 4). Across functional groups within monocultures responses to N fertilization generally reduced AM metrics with the exceptions of spore abundance in forb monocultures with ambient CO_2 and EMH in legume and C_4 monocultures with elevated CO_2 (Fig. 3a).

AM fungal responses to CO_2

Elevated CO_2 increased EMH density and spore biovolume (Fig. 2b; Table 3a). Spore community composition did not respond to CO_2 enrichment ($A = -0.20$, $P = 0.84$); however, there were three significant indicator species of elevated CO_2 from two families: *Gl. microaggregatum* (IV = 61.8, $P = 0.05$), *Gl. constrictum* (IV = 43.5, $P = 0.03$), and *E. infrequens* (IV = 25.2, $P = 0.005$), and no indicators of ambient CO_2. Within monocultures alone, spore biovolume was greater with elevated CO_2 (Table 4b; elevated CO_2, $7.3 \times 10^4 \pm 7.7 \times 10^3$; ambient CO_2, $5.3 \times 10^4 \pm 5.3 \times 10^3$). An interaction with CO_2 and functional group occurred where spore richness responses were greater in C_3 grasses grown with elevated CO_2 compared with those grown at ambient CO_2 (Table 4a). We also saw positive responses to elevated CO_2 in polycultures alone and across monocultures in abundance and richness of AM spores and EMH, with the exception of C_4 grasses (Fig. 3b). Although there

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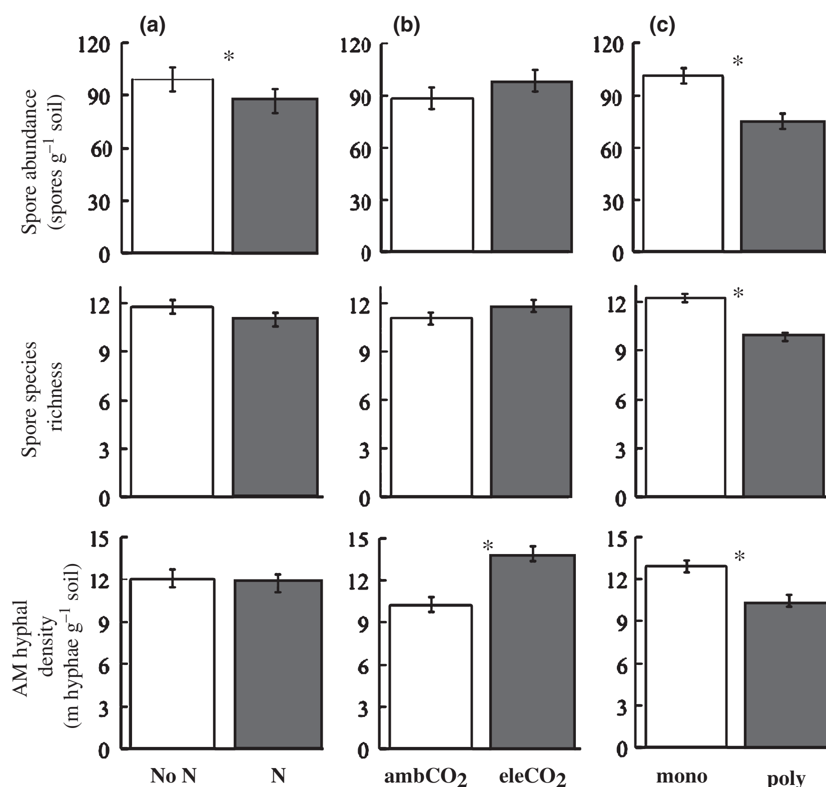

Fig. 2 Mean abundance of arbuscular mycorrhizal (AM) fungal spores, spore richness and density of AM fungal hyphae by: (a) nitrogen (N) treatment; (b) treatment with ambient (amb) and elevated (ele) carbon dioxide (CO_2); (c) plant richness within monocultures (mono) and polycultures (poly). Error bars represent one SE and asterisks above bars indicate significance at the P = 0.05 level. Polyculture plant richness ranged from 5 to 13 total species. m, meters.

were no $CO_2 \times N$ interactions in the full model that pooled all functional groups in the monocultures, there were some interactions in individual functional groups of plants. In several cases, N fertilization changed the magnitude of AM fungal responses to CO_2 enrichment; and reversed the increase in spore abundance caused by CO_2 in forbs (Fig. 3b). EMH increased with elevated CO_2 in all cases except C_4 grasses in monoculture (Fig. 3b). N fertilization increased the CO_2 enhancement of EMH in C_4 grasses and legumes in monoculture, and decreased the response in polyculture (Fig. 3b).

AM fungal responses to plant richness

Spore abundance, biovolume, spore richness and EMH densities were all 25–30% higher, on average, in monoculture than in polyculture (Fig. 2c; Table 3a). The mean spore richness in polycultures was lower than or similar to that found in each of the 16 monocultures (Fig. 4a). Monocultures of C_3 and C_4 grasses had greater spore richness than forbs or legumes (Fig. 4a; Table 4a). AM spore evenness was greatest in C_3 (0.59 ± 0.02) and C_4 grasses (0.63 ± 0.02) and lowest in forbs (0.55 ± 0.02) and legumes (0.48 ± 0.02; Table 4a). Although EMH densities and spore richness were variable across individual species and functional groups, both were generally higher in monoculture plots than in polyculture plots (Fig. 4c; Table 4b).

Plant richness affected Glomeromycotan spore community composition (A = 0.05, P < 0.0001) and there were significant indicator species in both plant richness levels. In monoculture, the following morphospecies were most common: *Gl. fasciculatum* (IV = 52.3, P = 0.04), *S. calospora* (IV = 55.1, P = 0.002), *Paraglomus occultum* (IV = 67.0, P = 0.001), *Gl. etunicatum* (IV = 30.2, P = 0.01), *Acaulospora laevis* (IV = 31.0, P = 0.008), *Acaulospora morrowiae* (IV = 24.6, P = 0.02), *Gl. versiforme* (IV = 51.5, P = 0.002), and *Gl. fistulosum* (IV = 33.5, P = 0.01). The following were most common in polyculture: *Gl. clarum* (IV = 64.0, P = 0.001), *S. pellucida* (IV = 59.2, P = 0.002), *Acaulospora delicata* (IV = 68.2, P = 0.0001) and *E. infrequens* (IV = 26.9, P = 0.03).

Supplemental analysis of the relationship between AM fungi and plant richness

A Mantel test revealed no strong relationships between plant community composition and AM fungal community composition for monocultures or polycultures (monoculture: R^2 = 0.02 P = 0.18; polyculture: R^2 = 0.04, P = 0.64). Likewise, regression analysis comparing AM fungal response variables and plant biomass by individual plant species or weed biomass revealed no patterns in monoculture alone, polyculture alone or with the combined dataset; contrary to

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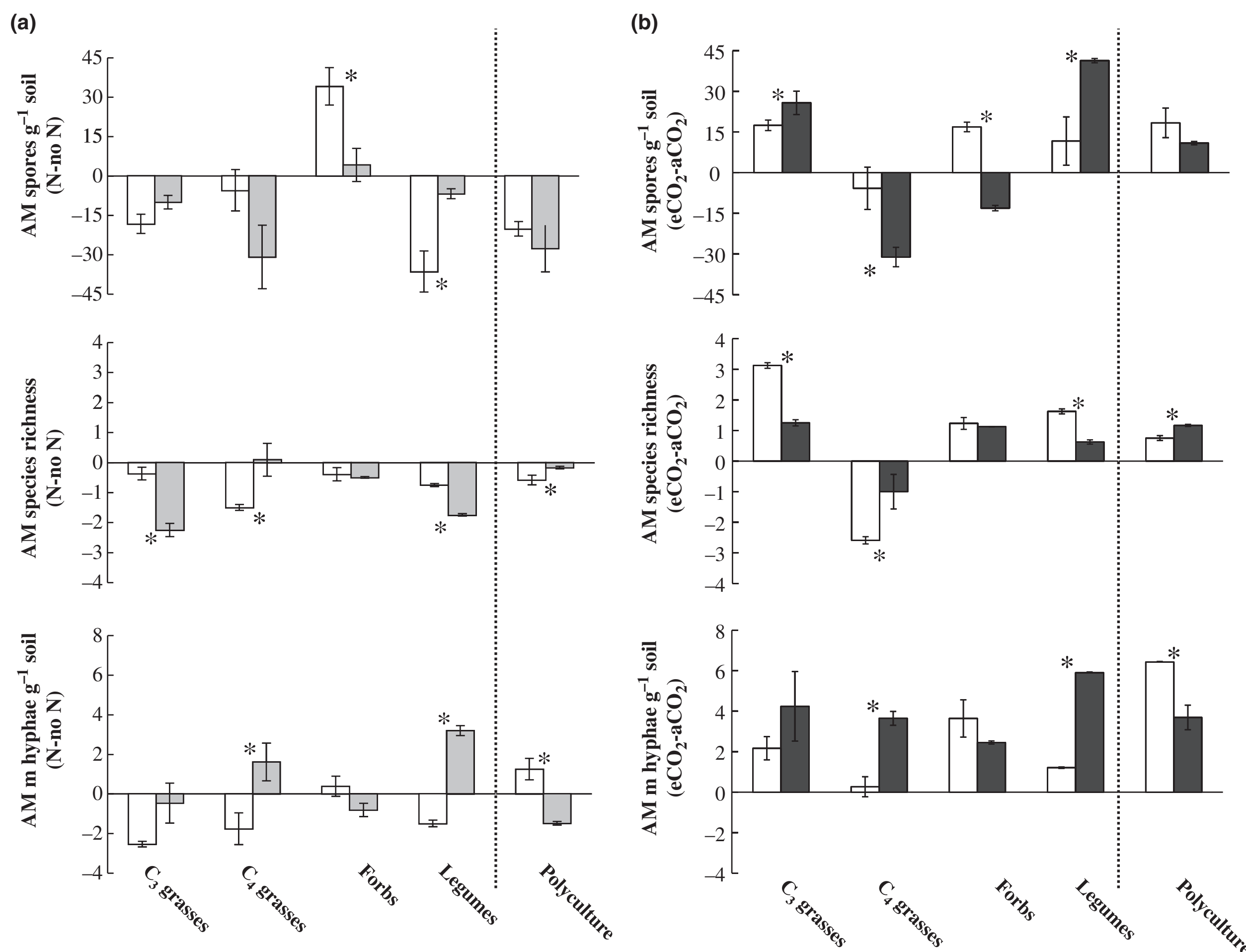

Fig. 3 (a) Mean responsiveness of arbuscular mycorrhizal (AM) spore abundance, richness and hyphal length to N fertilization at ambient CO_2 (aCO_2, open bars) and elevated CO_2 (eCO_2, closed bars) separated by plant functional groups grown in monoculture, as well as overall mean responsiveness of polycultures. (b) Mean responsiveness of AM spore abundance, richness and hyphal length to CO_2 enrichment without N addition (open bars) and with N (closed bars) separated by plant functional groups grown in monoculture, as well as overall mean responsiveness of polycultures. Asterisks indicate significant differences between (a) N fertilization concentrations or (b) CO_2 concentrations within a group. The dashed line separates monocultures (left) from polycultures (right). Polyculture plant richness ranged from five to 13 total species.

our hypothesis, there were no significant relationships (P > 0.05) and all R^2 values were < 0.10.

Plant responses

Total plant biomass (g m^{-2}) was higher with N fertilization (no N, 774.8 ± 88.9; N, 924.9 ± 94.5) and in polyculture (polyculture, 1286.9 ± 114.7; monoculture, 497.7 ± 68.7), but was only marginally affected by CO_2 (ambient CO_2, 842.7 ± 88.8; elevated CO_2, 991.7 ± 94.6; Table 3b). Shoot biomass was higher with CO_2 and in polyculture, but was not significantly affected by N (Fig. 5; Table 3b). Root biomass was not affected by CO_2 but was greater with N and in polyculture (Fig. 5; Table 3b). There were no significant differences in root mass fraction for any treatments (Fig. 5; Table 3b).

The biomass of weeds (g m^{-2}) summed over the growing season was only affected by plant richness (polyculture, 4.6 ± 1.0; monoculture, 162.4 ± 16.3; Table 3b). In monoculture alone, weed biomass only responded to functional group, with forbs (233.2 ± 43.3) and legumes (251.5 ± 32.3) having greater weed biomass (g m^{-2}) than C_3 (72.2 ± 17.2) and C_4 grasses (92.8 ± 16.7); Table 4b). No other plant variables responded to main treatment effects. However, root biomass (g m^{-2}) was affected by the interaction between CO_2 and plant functional group where C_3 grasses with elevated CO_2 had greater root biomass than C_3 grasses in ambient CO_2 (ambient CO_2, 1018.9 ± 210.2; elevated CO_2, 1674.0 ± 218.2). Total biomass (g m^{-2}) in monoculture was affected by an interaction between N and plant functional group where C_3 grasses grown with N fertilization had greater total biomass than C_3 grasses grown

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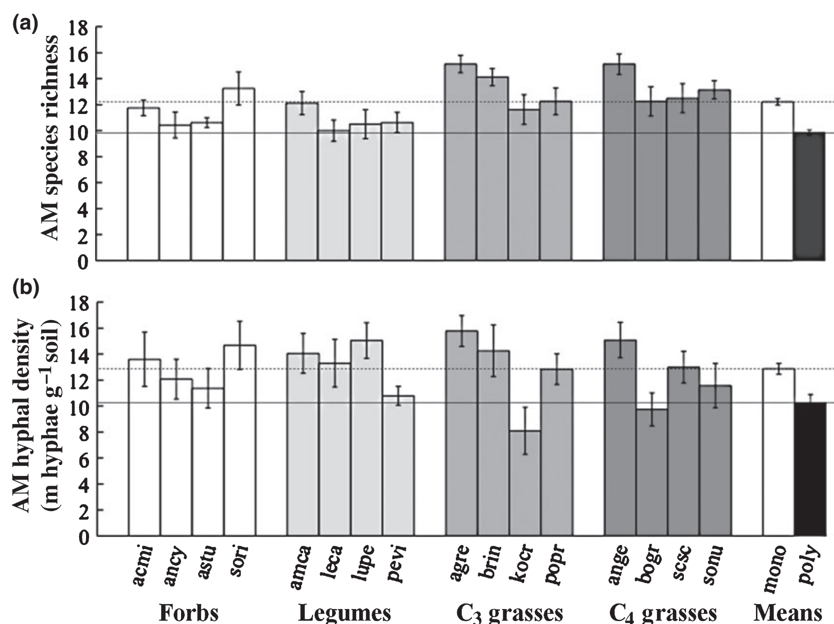

Fig. 4 (a) Mean richness of arbuscular mycorrhizal (AM) fungal spores and (b) mean density of AM fungal hyphae within monocultures of individual plant species is compared with the mean of all monocultures (grouped white bars) and the mean of all polycultures (black bars). Monocultures are grouped by functional group and color-coded from open bars to dark gray shading. Overall means of monoculture (open bar/dashed line) and polyculture (black bars/solid line) are shown on the far right and as lines across the graph for reference. Four letter species acronyms represent the first two letters of the genus and first two letters of the specific epithet of the species listed in the Materials and Methods section. Error bars represent one SE. Polyculture plant richness ranged from 5 to 13 total species.

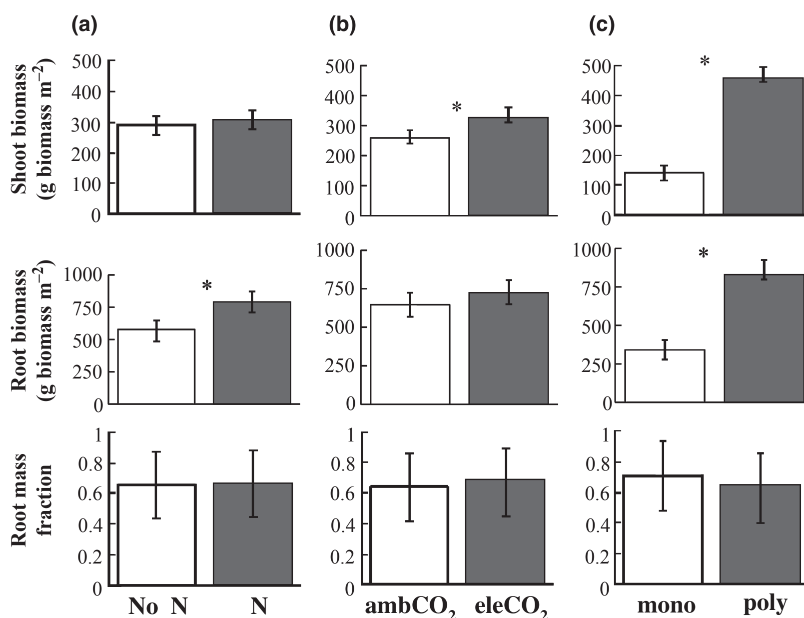

Fig. 5 Mean shoot biomass, root biomass and root fraction by: (a) nitrogen (N) treatment; (b) treatment with ambient (ambCO$_2$) and elevated carbon dioxide (eleCO$_2$); (c) plant richness within monocultures (mono) and polycultures (poly). Error bars represent one SE and different letters above bars indicate significant differences at the P = 0.05 level. Polyculture plant richness ranged from five to 13 total species.

without N fertilization (no N, 1125.1 ± 106.3; N, 1548.2 ± 156.6; Table 4b).

Structural equation model

Our hypothesized SEM (Fig. 1, Table 1a, S1) represented the data well (GFI, P = 0.99; Maximum likelihood χ^2 = 5.57, P = 0.35; RMSEA = 0.03, P = 0.57; Bollen–Stine bootstrap, P = 0.42). The parameters included in the model explained 44% of the variation in weed biomass, 66% in total plant biomass, 31% in AM spore richness and 22% in AM fungal biovolume in the soil (Fig. 1).

The path coefficients (λ) for direct effects are displayed on Fig. 1, and the direct, indirect and total effects on AM fungi and intermediate explanatory variables are given in Table 1(b), S1. The strongest predictors of AM fungal biovolume in soil were CO_2 enrichment (positive) and plant richness (negative; Table 1b). The strongest predictors of spore richness were plant richness (negative), fungal biovolume (positive), plant community composition, and weed biomass (negative; Table 1b). Nitrogen fertilization directly increased weed biomass and total plant biomass, and had a weak negative influence on AM fungal biovolume in the soil. Higher plant richness directly led to increased target plant

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biomass, lower fungal biovolume, lower spore richness and lower weed biomass. Weed biomass had no influence on AM fungal biovolume in the soil, but negatively influenced AM spore richness (Table 1b). Treatments also had many indirect effects on downstream variables via influences on other response variables (and Table S1).

Discussion

Our results demonstrate that resource availability structures AM fungal communities. As predicted by hypotheses 1 and 2 (see the Introduction), elevated CO_2 increased AM fungal biomass in the soil, whereas N fertilization decreased it. These findings are consistent with earlier discoveries that elevated CO_2 increases plant allocation of labile C below ground (Adair *et al.*, 2009); and that N fertilization reduces mycorrhizal biomass in the P-rich Cedar Creek soil (N. Johnson *et al.*, 2003b). Our findings also support the prediction that enriching atmospheric CO_2 should make soil nutrients relatively more limited and increase the importance of mycorrhizal uptake of these nutrients (Fitter *et al.*, 2000; Treseder & Allen, 2000; Treseder, 2004). The comparatively weaker response to CO_2 enrichment of root biomass (Fig. 5) than of EMH (Fig. 2) suggests that provisioning the EMH of AM fungi may be a more dynamic response to changes in carbon supply than changes in root biomass. Additional sampling dates would allow a better test of that hypothesis. It is noteworthy that Sanders *et al.* (1998) found that while intraradical colonization of AM fungi increased only proportionally to changes in root biomass, EMH densities increased fivefold under elevated CO_2 in *Prunella vulgaris*, suggesting that AM fungi shunt more plant-derived C to structures in the soil than to structures within plant roots. It is also interesting to note the considerable differences among plant functional groups in their mycorrhizal responses to resource enrichment (hypothesis 4; Fig. 3). This corroborates earlier glasshouse studies which show that the responses of AM fungi to CO_2 and N enrichment vary considerably among plants with different functional strategies (Johnson *et al.*, 2005). The community-scale outcome of differences in mycorrhizal responsiveness of plant taxa may contribute to the shifts in plant community composition that frequently occur when systems are fertilized (Johnson *et al.*, 2008).

Our results did not support the predictions of hypothesis 3 that polycultures would support greater diversity and biomass of AM fungi (Table 1). Although plant diversity significantly influenced the amount of AM fungi in the soil, EMH and the abundance and richness of AM spores were negatively correlated with plant richness, which was contrary to expectations because polyculture plots have higher productivity, and presumably a higher demand for the soil resources provided by mycorrhizal symbioses. We looked for complex responses and/or indirect effects that might account for this unexpected response. We suspected that the high abundance of hypothetically less mycorrhizal-dependent C_3 grasses that tend to dominate the polyculture plots might have reduced the abundance of AM fungi in the soil; but C_3 grasses had similar spore and hyphal averages as a group to the other plant functional groups (Fig. 4). Additionally, we found no statistically significant relationships between populations of individual plant species in polyculture or monoculture and AM fungal responses. We also hypothesized that weed densities in monoculture would be higher as a result of lower cover by target species, leading to a temporarily richer plant community and an ephemeral C source for AM fungi. However, while weed biomass was higher in monocultures than in polycultures, weed biomass was negatively correlated with AM spore richness. Furthermore, our species accumulation curves indicated that adequate sampling was conducted in both polyculture and monoculture and, at the plot scale, monocultures accumulated species at faster rates than polycultures. Based on these results, we conclude that the assembly of Glomeromycotan soil communities differs for monocultures and polycultures in this system.

While finding an inverse relationship between plant richness and AM spore richness is relatively uncommon, it is not unprecedented; other mycorrhizal studies have shown that monocultures can harbor remarkably diverse Glomeromycotan communities (Johnson & Wedin, 1997; Picone, 2000; Wirsel, 2004; D. Johnson *et al.*, 2003a). A recent study of publicly available Glomeromycotan DNA sequences from across the globe revealed no relationship between AM fungal richness and plant richness (Öpik *et al.*, 2010). Nevertheless, it is interesting to note that other studies conducted at the Cedar Creek Ecosystem Science Reserve found a positive relationship (Burrows & Pfleger, 2002) or no relationship between species richness of plants and richness of AM fungal spores (Wolf *et al.*, 2003). Burrows & Pfleger studied larger (11×11 m) plots in a biodiversity experiment (Tilman, 1997) adjacent to the BioCON experiment and found higher AM spore abundance and richness in polyculture than in monoculture. In comparing our results, we found that the species composition of our AM spore communities were different (e.g. the frequency and diversity of *Acaulospora* spores were greater in their study). It is possible that the different methods used to remove the seed-bank in the two experiments affected the Glomeromycotan 'spore-bank' and subsequent re-establishment of AM fungi. In the Burrows & Pfleger (2002) study, the top 5–10 cm of soil were physically removed and seedling establishment was slow. In BioCON, the topsoil was not removed, but was treated with methyl bromide, which eliminated the seed-bank, but likely also reduced the number of viable AM fungal propagules (Menge, 1982). Consequently, the two experiments may have had different AM fungal communities from inception, leading to different successional trajectories over time. Wolf *et al.* (2003) examined AM spores within BioCON after only 3 yr, compared with our 7 yr of

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treatment, and found more subtle treatment responses than in our study, and no relationship between the abundance or richness of AM spores and plant richness. We believe the stronger AM responses to CO_2, N and plant richness in our study result from the temporal dynamics of the BioCON system; Glomeromycotan responses to experimental treatments may take many years to manifest.

Unlike plant responses, we detected few interactive effects of the CO_2, N and plant richness treatments on AM fungi. Interactions among treatments are important drivers of plant community composition, diversity and productivity in BioCON (Reich *et al.*, 2006; Reich, 2009). This may indicate that drivers of Glomeromycotan community dynamics are different from drivers of plant communities, perhaps because plants respond directly to changing CO_2 availability, whereas AM fungi respond indirectly via plant hosts.

Our results demonstrate the difficulty of making generalizations about the relationship between plant diversity and Glomeromycotan communities. We showed that AM fungi responded differently to host richness in two experiments within the same research site. Additional experiments are planned which will combine microscopy methods with molecular methods to examine AM spore populations over time and also to compare their diversity inside and outside plant roots. There is reason to expect that AM fungal species partition niche space both temporally and spatially (Pringle & Bever, 2002). Furthermore, there is evidence that plants actively manage the diversity and composition of the AM fungi that they cultivate in their roots (Bever *et al.*, 2009), and in so doing, expand the diversity of resource-rich microsites that can be exploited in their rhizosphere (Maherali & Klironomos, 2007; Reinhardt, 2007). If it is true that intraspecific competition is stronger than interspecific competition (Yoda *et al.*, 1963; Linhart, 1988) and that a diverse flora of AM fungi can facilitate more complete exploitation of below-ground resources (Streitwolf-Engel *et al.*, 1997; Van der Heijden *et al.*, 2003), then we may hypothesize that plants grown in monocultures should cultivate a more diverse community of AM symbionts than those grown in polyculture. This 'niche-partitioning hypothesis' states that maintaining a diverse community of AM fungi in the soil may be a plant's mechanism to reduce the intensity of intraspecific competition in monocultures (Hartnett *et al.*, 1993; Moora & Zobel, 1996; Hart *et al.*, 2003). Individual plant hosts can select for a diversity of AM symbionts to create functional complementarity and greater host benefits (Koide, 2000). Hidden genotypic variation within Glomeromycotan species may generate considerable functional variation that will be undetected when morphospecies (Haas & Krikun, 1985; Bethlenfalvay *et al.*, 1989) or even phylospecies (Koch *et al.*, 2006; Ehinger *et al.*, 2009; Angelard *et al.*, 2010) are the taxonomic unit of study. Consequently, seemingly low taxonomic or phylogenetic diversity of AM fungi does not necessarily translate into low diversity of symbiotic functioning and this will need to be considered when testing the niche-partitioning hypothesis.

Structural equation modeling allows us to test our conceptual understanding of ecological systems by articulating causal relationships among variables and challenging these concepts with actual data. Our SEM indicates that among our experimental treatments, CO_2 had the strongest influence on AM biovolume in the soil, and plant richness had the strongest influence on AM spore richness. The fact that our SEM explained 31% of the variation in AM spore richness and 22% of the variation in AM fungal biovolume in soil indicates that additional factors not included in the model are important in shaping the Glomeromycotan soil community. Field-scale variation in soil bulk density and moisture is likely to account for a large proportion of this unexplained variation (Antoninka, 2011). There was also a direct path where higher AM fungal biovolume led to higher richness of AM spore communities (Fig. 1, Table 1), supporting hypothesis five that a more productive environment for fungi could have generated more potential niches for Glomeromycota.

In conclusion, Glomeromycotan communities are structured by resource availability, host community, time and spatial heterogeneity, among other influences. A better understanding of how these factors influence mycorrhizas will facilitate conservation of ecosystem function via appropriate management for Glomeromycotan fungi. We have shown that abiotic resource availability influences mycorrhizas in both predictable and unpredictable ways. Understanding the mechanisms of host community effects on Glomeromycotan communities remains a challenge for future studies.

Acknowledgements

Funding for this project was provided by National Science Foundation grants DEB-0322057, DEB-0080382, DEB-0218039, DEB-0219104, DEB-0217631, DEB-0322057 and Department of Energy Program for Ecological Research Grant DE-FG02-96ER62291, to P.R., and by NSF DEB-0316136 and 0842327 to N.C.J., and by PEO and the ARCS Foundation funding to A.A. We thank Jared Trost and Cedar Creek Field Crews for soil collection, Matthew Bowker for statistical help, and Andrea Redman, Matt Halldorson and Erica Lindsay for laboratory assistance. We also thank our three anonymous reviewers for comments that have greatly improved the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Species accumulation curves by plot (a) and number (b) of individual spores for monoculture and polycultures.

Table S1 Our hypothesized (a) and observed (b) outcomes for nontarget variables, from our structural equation model (SEM), including direct, indirect and total effect coefficients (λ) for hypothesized causal relationships

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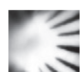

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