

REPORT

Interactions among mycorrhizae, atmospheric CO₂ and soil N impact plant community composition

Nancy Collins Johnson,* Julie Wolf and George W. Koch
Departments of Environmental Sciences, Biological Sciences, and the Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ 86001-5694, USA
*Correspondence: E-mail: nancy.johnson@nau.edu

Abstract

We examined plant community responses to interactions between arbuscular mycorrhizal (AM) fungi and availability of atmospheric CO₂ and soil N. Communities of 14 plant species were grown in mesocosms containing living or killed AM fungal inoculum, ambient or elevated atmospheric CO₂ and low or enriched soil N. After one growing season, significantly different plant communities existed in the different treatments. Plant species richness was lowest in +N mesocosms and highest in +AM + CO₂ mesocosms. At ambient CO₂, AM fungi reduced richness but at elevated CO₂ they increased it. This was caused by changes in mortality rates of several C₃ forbs and may suggest that CO₂ enrichment ameliorates the carbon cost of some AM symbioses. Soil moisture was higher in +CO₂ mesocosms but +AM counteracted this effect. These results suggest that AM symbioses may be important mediators of plant community responses to anthropogenic CO₂ and N enrichment.

Keywords

Arbuscular mycorrhizae, CO₂ enrichment, community composition, evapotranspiration, grassland, mesocosm, mycotrophy, nitrogen eutrophication.

Ecology Letters (2003) 6: 532–540

INTRODUCTION

Biotic communities are expected to respond to anthropogenic enrichment of atmospheric carbon dioxide (CO₂) and soil nitrogen (N); however, complex interactions among ecosystem components generate uncertainty about the nature of these responses. Arbuscular mycorrhizae are ubiquitous fungus–plant symbioses that enhance uptake of soil nutrients and water, protect plants from pathogens, and stimulate photosynthesis through enhanced sink strength for carbon (Smith & Read 1997; Wright *et al.* 1998). These associations help structure plant communities because arbuscular mycorrhizal (AM) fungi improve the fitness and productivity of some plant species more than others (Grime *et al.* 1987; Hartnett & Wilson 1999; Klironomos *et al.* 2000). Altered mycorrhizal function is predicted to influence plant community responses to CO₂ enrichment because AM fungi are obligate biotrophs and generate a substantial carbon sink for their host plant (Diaz 1996; Sanders 1996; Miller *et al.* 2002). In plants that are dependent upon mycorrhizae, the AM carbon sink has been shown to facilitate increased biomass production under elevated CO₂ (Gavito *et al.* 2000; Jiffton *et al.* 2002). Arbuscular mycorrhizae may also mediate

plant community responses to anthropogenic N enrichment because the species composition and mutualistic function of AM fungal communities are impacted by N fertilization (Johnson 1993; Egerton-Warburton & Allen 2000; Corkidi *et al.* 2002). Understanding the interactions between CO₂ and N availability and mycorrhizal function will help us better predict the responses of plant communities to the anticipated global enrichment of these resources.

Plant taxa vary in their responses to atmospheric CO₂ enrichment and mycorrhizae. Plant photosynthetic physiology is often a good predictor of responsiveness to both atmospheric CO₂ enrichment and mycorrhizae. C₃ plants have been shown to benefit more from CO₂ enrichment (Poorter *et al.* 1996; Reich *et al.* 2001) and less from mycorrhizae (Hetrick *et al.* 1990; Wilson & Hartnett 1998) than C₄ plants. Among species of C₃ and C₄ prairie grasses, there is evidence for an inverse relationship between plant responsiveness to CO₂ enrichment and mycorrhizal dependency (Reyes *et al.* 2002). Legumes have been shown to respond both positively and negatively to CO₂ enrichment (Körner *et al.* 1996). Most legumes benefit from AM colonization (Wilson & Hartnett 1998); however, the genus *Lupinus* is a notable exception because it often does not

form AM associations (O'Dell & Trappe 1992). Reich *et al.* (2001) found that grouping plants according to photosynthetic physiology, growth form (grass or forb), and N-fixation capability is useful but not sufficient to understand plant and ecosystem responses to elevated CO₂ and N availability.

Both CO₂ enrichment and mycorrhizae can indirectly influence soil moisture via effects on stomatal conductance. Soil moisture can increase under elevated atmospheric CO₂ because of the commonly observed reduction in stomatal conductance, particularly in C₃ species (Field *et al.* 1995). The increased soil moisture generated by elevated CO₂ has been shown to increase rates of microbial metabolism and nutrient cycling (Hungate *et al.* 1997). Stomatal conductance and transpiration rates are generally higher in AM compared to non-AM plants, in both mesic and drought conditions (Auge 2000). Increased N availability typically stimulates plant growth, stomatal conductance, and whole-plant water use (Hunsaker *et al.* 2000). Because elevated CO₂, AM fungi, and N availability have contrasting effects on plant growth, water use, and soil moisture, the interactive effects of these factors may have unexpected effects on plant community structure.

Species and genera of AM fungi vary in their responses to elevated CO₂ (Klironomos *et al.* 1998) and N enrichment (Eom *et al.* 1999; Egerton-Warburton & Allen 2000). Field studies have shown that spore abundances of certain species of AM fungi increase while others decrease with elevated CO₂ (Wolf *et al.* 2003). Also, CO₂ enrichment has been shown to increase the density of extraradical mycelium of AM fungi under some plant communities but not under others (Rillig & Allen 1999; Rillig *et al.* 2000; Wolf 2001). In soils that are not severely phosphorus limited, anthropogenic N enrichment typically reduces species richness and changes the community composition in favour of fast growing, highly competitive plant (Berendse *et al.* 1993) and AM fungal species (Egerton-Warburton & Allen 2000). Enrichment of atmospheric CO₂ and soil N can act synergistically on communities of plants and soil organisms (Klironomos *et al.* 1997; Schenk *et al.* 1997). However, the impact of *simultaneous* enrichment of both atmospheric CO₂ and soil N on mycorrhizal function has not been well studied (Egerton-Warburton & Allen 2001).

AM associations are so ubiquitous in terrestrial ecosystems that it is difficult to find an experimental system without them in which their role in structuring plant communities can be assessed. Mesocosms provide a viable method to manipulate AM fungi and quantify their effects on community and ecosystem structure and function (Wilson & Hartnett 1997; Van der Heijden *et al.* 1998). This report describes plant community responses to enrichment of atmospheric CO₂ and soil N in the presence and absence of AM fungi using mesocosms of prairie

communities. This greenhouse study was designed to complement a Free Air CO₂ Enrichment (FACE) experiment at Cedar Creek Minnesota, USA (Reich *et al.* 2001) in which AM associations were studied (Wolf 2001; Wolf *et al.* 2003), but not manipulated. Our study addressed the following questions: (1) Do AM associations mediate plant community responses to enrichment of atmospheric CO₂ and soil N? (2) Do AM fungi impact C₃ and C₄ plants differently at elevated and ambient CO₂? (3) Do AM fungi and availability of atmospheric CO₂ interact to impact soil moisture?

MATERIALS AND METHODS

Experimental design

This study was conducted in 12 greenhouse chambers (2.5 m × 1.3 m × 1.5 m tall) at Northern Arizona University in Flagstaff Arizona, USA. Six chambers had ambient and six had elevated levels of atmospheric CO₂ (set points of 450 and 688 p.p.m. respectively daylight hours only). At Flagstaff's elevation, these CO₂ levels are equivalent to 368 and 560 p.p.m. at sea level, which are the concentrations used at the Cedar Creek FACE experiment. Atmospheric CO₂ level, presence or absence of AM fungi, and soil N availability were manipulated using a 2 × 2 × 2 factorial experiment replicated six times, for a total of 48 mesocosms. Each mesocosm (48 cm × 38 cm × 43 cm deep) was filled with approximately 61 L of soil and planted with 42 seedlings: three seedlings each of 14 plant species within five functional groups. The C₄ grasses included: *Andropogon gerardii* Vitman, *Schizachyrium scoparium* (Michaux) Nash, and *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths; C₃ grasses included: *Agropyron repens* L., *Koeleria cristata* Pers, and *Poa pratensis* L.; C₃ composites included: *Achillea millefolium* L., *Heliopsis belianthoides* L., and *Solidago rigida* L.; C₃ legumes included: *Lespedeza capitata* (Michaux), *Lupinus perennis* L., and *Petalostemum villosum* Nutt.; putative non-mycotrophic C₃ forbs included *Berteroa incana* L. and *Salsola kali* L. *Lupinus* is both a legume and a non-mycotrophic forb. These species co-occur at Cedar Creek and are common in mesic grasslands in North America.

Temperatures in the chambers varied diurnally and across the growing season ranging from 18 to 36 °C. During the first 2 weeks of the experiment, mesocosms were watered daily with water filtered through a charcoal cartridge. After 2 weeks, soil moisture content was measured three times per week using time domain reflectometry (TDR, Jones *et al.* 2002), and filtered water was added to each mesocosm in the quantity necessary to return the moisture level to 4.9% by mass. This target moisture level was equal to 75% of the estimated total water-holding capacity of the soil, and did not result in leakage from the mesocosm's drainages. During

weeks 3, 7, and 11, each +N mesocosm received 0.701 g of NH_4NO_3 dissolved in 500 mL of filtered water, which provided a seasonal total of 0.245 g total N per mesocosm. This level is equivalent to 4 g total N m^{-2} , the level used at the Cedar Creek FACE experiment. Because the mesocosms were never watered to above field capacity, this N can be assumed to have remained in the systems.

Soil, plants and fungi

Soil was collected adjacent to the FACE experiment at Cedar Creek Minnesota and transported to Flagstaff, AZ. Except for *Salsola*, seeds of the experimental species were acquired from the same sources that were used in the Cedar Creek FACE experiments (Reich *et al.* 2001). *Salsola* seeds were collected from a roadside near Flagstaff, AZ. Mesocosms were filled with a 1 : 3 mixture of Cedar Creek soil and silica sand that had been steam-sterilized (8 h on two consecutive days), and 2.30 g of Osmocote® slow release fertilizer (18–6–12 NPK) was scattered over the soil surface. Also, 0.127 g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 2.51 g $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 13.61 g MgSO_4 , 0.023 g NaCl, 0.650 g H_3BO_3 , 0.408 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.053 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.015 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and 0.0083 g FeEDTA were dissolved in 3 L of deionized water and mixed throughout the lower soil profile. The quantity of these amendments approximated the nutrient levels that would occur if the mesocosms were filled with 100% Cedar Creek soil. Mixed cultures of endemic Cedar Creek AM fungi were grown on leeks and celery using the methods of (Morton *et al.* 1993). *Glomus aggregatum/intraradices*, *G. clarum*, *G. constrictum*, *Gigaspora gigantea*, *Gi. margarita*, *Acaulospora spinosa*, *A. scrobiculata*, and *A. trappei* were present in the AM fungal inoculum. All of these species were observed in the field at the Cedar Creek FACE site (Wolf *et al.* 2003). Living spores, hyphae and fragments of colonized leek roots (16 g fresh weight per mesocosm) were added to the 24 + AM mesocosms and the same amount of killed (autoclaved) spores, hyphae and roots were added to the 24 – AM mesocosms. To equalize the microbial communities of the two treatments, each –AM mesocosm received 175 mL of a microbial slurry that had been rinsed from the +AM inoculum and filtered through a 20- μm sieve to remove AM fungal propagules. All 48 mesocosms received 250 mL of microbial slurry that was prepared by mixing 5 kg of fresh Cedar Creek soil with 20 L of deionized water and filtering it through a 20- μm sieve. Seedlings of each of the 14 species were grown in sterilized vermiculite for 1–8 weeks (depending upon their growth rates), at elevated or ambient CO_2 , and were transplanted into mesocosms in corresponding CO_2 treatments. Plants were transplanted when their shoots were between 2.5 and 3.5 cm tall. Seedlings were planted into randomized positions on a 6 \times 7 grid placed over the mesocosm surface.

Each mesocosm was individually randomized to generate 48 unique planting patterns. Any seedlings that died within 3 weeks of transplantation were replanted. After that time, dead plants were not replaced. After 16 weeks, plant roots were collected from each mesocosm and checked for AM colonization. The +AM mesocosms had an average of 35% root length colonized and the –AM mesocosms had no colonization. There was no effect of CO_2 or N enrichment on the percentage of AM colonization. Low levels (*ca.* 2% root length colonized) of non-mycorrhizal fungi occurred with equal frequency across all treatments. The three legume species formed root nodules in all treatments. After 20 weeks, the plants had fully matured, many had produced flowers and seed, and all were beginning to senesce. Shoots of each plant were individually cut 3 cm above the soil surface, dried and weighed.

Statistical analyses

Total aboveground biomass, plant species richness and evenness, volume of water added per mesocosm and biomass of each of the 14 species were compared using ANOVA with the full model of all experimental treatments. This allowed us to test the main effects and interactions of: CO_2 level, N level, mycorrhizal status, and the random blocking effect of chamber nested within CO_2 level (no interactions tested). Mortality of individual plants within the functional groups was analysed by logistic regression of likelihood of mortality under the different treatments. ANOVA and logistic regression were performed using the JMP 4.0 statistical package (SAS 1997).

Plant communities within the different experimental treatments were compared with Multi-Response Permutation Procedure (MRPP; Mielke 1984) using PC-ORD (Version 4.14; McCune & Mefford 1999). This non-parametric resampling method accommodates the multivariate nature of community response data, and calculates the probability (*P*) that detected differences between treatment groups are due to random chance alone (Zimmerman *et al.* 1985). Shoot biomasses of conspecific plants within each mesocosm were summed to obtain total biomass per mesocosm for each of the 14 plant species. These specific biomasses were used to describe and compare plant communities under the experimental treatments.

The location of the air-cooling system generated a temperature gradient from the front to the back of the greenhouse. Therefore, MRPP analyses were run as a blocked design, with adjacent pairs of chambers (one ambient and one elevated chamber) as blocks. Median alignment of the blocks was used in all analyses. This removes between-block variation from the analysis, allowing treatment differences to be assessed independently from

spatial variation between chambers caused by the temperature gradient.

For all MRPPs, the chance-corrected within-group agreement (\mathcal{A}) is presented. The value of \mathcal{A} represents the degree of within-group homogeneity and is analogous to 'effect size'. If all communities within each treatment-level group are identical, \mathcal{A} takes a maximum value of 1; $\mathcal{A} = 0$ when within-group heterogeneity equals that expected by random chance (i.e. no 'effect' of treatments). Values of \mathcal{A} between 0.1 and 0.3 are often meaningful in ecological community data (McCune & Mefford 1999).

RESULTS

Across the 48 mesocosms, total aboveground biomass was not influenced by CO_2 level, but it was significantly higher in mesocosms with enriched soil N ($F = 38.1$, $P < 0.0001$), and in those with AM fungi present ($F = 7.3$, $P = 0.011$ Fig. 1). Mean plant species richness was significantly lower in mesocosms with high N (12 species) than with low N (13.2 species, $F = 31.3$, $P < 0.0001$), and higher in

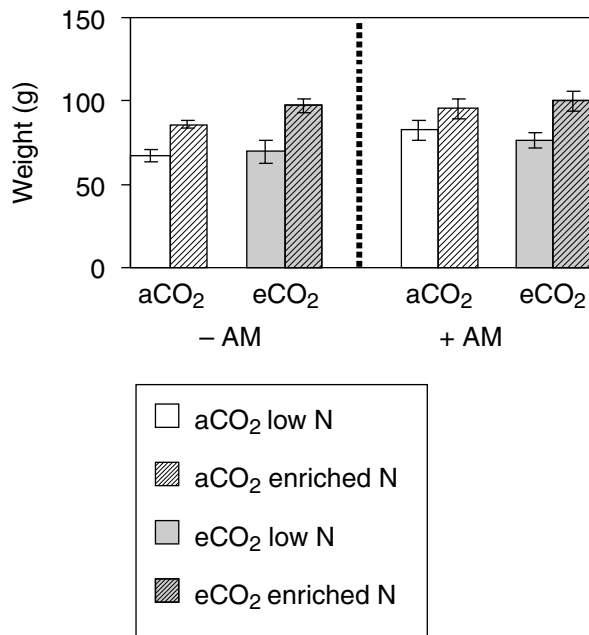


Figure 1 Total mean dry shoot biomass per mesocosm, \pm one standard error ($n = 48$). Open bars represent mesocosms with low soil N, hatched bars denote those with enriched N. Unshaded bars represent mesocosms grown under ambient CO_2 (aCO_2) and shaded bars denote those grown under elevated CO_2 (eCO_2). The left-hand side of each histogram shows non-mycorrhizal mesocosms ($-AM$) and the right-hand side shows mycorrhizal mesocosms ($+AM$). Total shoot biomass was significantly affected by the presence of AM fungi ($P = 0.011$) and addition of N ($P < 0.0001$).

mesocosms with elevated CO_2 (13 species) than with ambient CO_2 (12.2 species, $F = 4.7$, $P = 0.05$). There was a significant interactive effect of $AM \times \text{CO}_2$ on plant species richness ($F = 16.4$, $P = 0.0003$); $+AM$ mesocosms in elevated CO_2 retained more plant species than the other treatments (Fig. 2a). Evenness of the communities was not significantly influenced by any of the experimental treatments. Mesocosms with different experimental treatments dried out at different rates. The presence of AM fungi increased evapotranspiration ($F = 4.3$, $P = 0.05$) while elevated CO_2 decreased it ($F = 6.8$, $P = 0.03$). Compared to the other treatments, significantly less water was lost from the elevated CO_2 , $-AM$ mesocosms (Fig. 2b). Enrichment of N strongly increased evapotranspiration ($F = 81.6$, $P < 0.0001$), a mean of 33.6 L of water was added to the low N mesocosms and 41.1 L of water was added to the high N mesocosms.

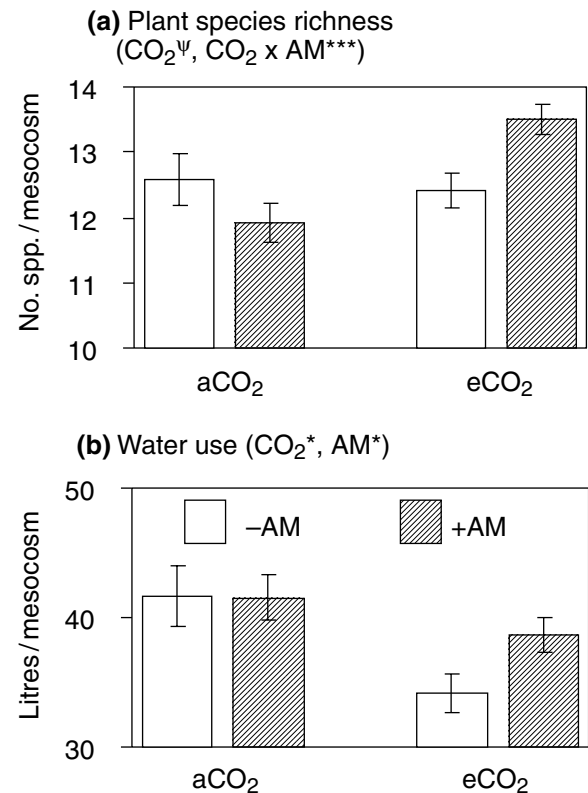


Figure 2 (a) Plant species richness and (b) total amount of water added to maintain uniform soil moisture in mesocosms under ambient (aCO_2) or elevated (eCO_2) CO_2 and without ($-AM$) or with AM mycorrhizal fungi ($+AM$). Means \pm one standard error are shown ($n = 48$). The significance levels of relevant treatments and interactions, as detected by ANOVA, are listed for each response ($^{\psi}0.10 \geq P > 0.05$; $*0.05 \geq P > 0.01$; $**0.01 \geq P > 0.001$; $***0.001 \geq P$). Addition of N uniformly decreased plant species richness and increased water usage (not shown).

Plant community composition was influenced by CO₂ ($\zeta A = 0.18$, $P = 0.03$), and the two-way interactions: N \times AM ($\zeta A = 0.25$, $P = 0.00009$), CO₂ \times N ($\zeta A = 0.22$, $P = 0.0003$), and CO₂ \times AM ($\zeta A = 0.18$, $P = 0.0007$; Fig. 3). Plant species responded individually to the experimental treatments. Aboveground biomass of two C₃ species responded positively to elevated CO₂; shoots of *Koeleria* and *Achillea* were significantly larger at high CO₂ (Fig. 4a and c). Nine of the 14 species responded significantly to N. Enrichment of soil N was associated with higher aboveground biomass of five species: *Agropyron*, *Poa*, *Bouteloua*, *Salsola*, and *Berteroa* (Fig. 4a, b and e), and lower biomass of four species: *Andropogon*, *Solidago*, *Petalostemum*, and *Lespedeza* (Fig. 4b–d). Eight species responded significantly to AM fungi. Five species had greater biomass in +AM mesocosms: *Andropogon*, *Schizachyrium*, *Solidago*, *Lespedeza*, and *Salsola* (Fig. 4b–e), and three species had greater biomass in –AM mesocosms: *Poa*, *Achillea*, and *Lupinus* (Fig. 4a, c and d). There was no *Salsola* mortality and almost no mortality among the C₃ and C₄ grasses; however, mortality was quite high among several of the forb species and rates varied with treatment. The likelihood of mortality of C₃ composites was significantly higher in mesocosms with high N ($P < 0.0001$), and significantly lower in +AM

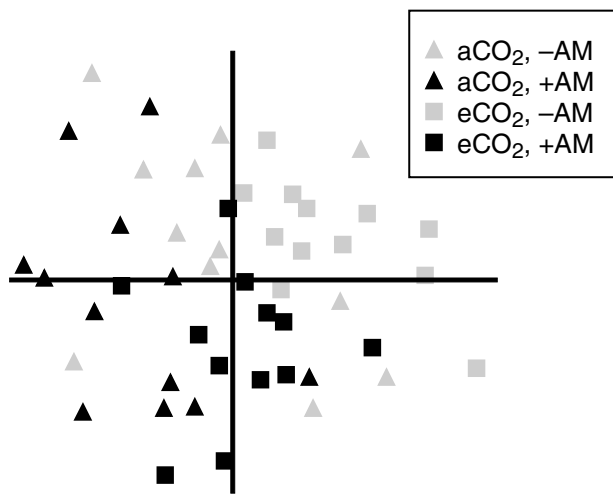


Figure 3 Non-metric multidimensional scaling ordination of the 48 mesocosm plant communities. Mesocosms were initiated with identical (but randomly arranged) plant communities composed of 42 seedlings, three seedlings each of 14 plant species. Community composition was described by the total shoot biomass of each of the 14 plant species, 20 weeks after transplantation. The axes serve to delineate the ordination space and are not directly correlated with treatments. Each symbol represents one mesocosm plant community. Grey symbols represent non-mycorrhizal (–AM) and black represent mycorrhizal (+AM) mesocosms, triangles represent mesocosms under ambient CO₂ (aCO₂) and squares represent those under elevated CO₂ (eCO₂).

mesocosms at elevated CO₂ ($P = 0.027$, Fig. 5a). Mortality of mycorrhizal legumes (i.e. not including *Lupinus*) was significantly more likely in high N ($P < 0.0001$) and under elevated CO₂ ($P = 0.002$), and less likely in the presence of AM fungi ($P < 0.0001$, Fig. 5b). Mortality of the putative non-mycotrophic forb *Berteroa* plus the non-mycotrophic legume *Lupinus* was significantly less likely under elevated CO₂ ($P = 0.0001$), more likely in high N ($P = 0.047$), and more likely in the presence of AM fungi ($P = 0.020$, Fig. 5c).

DISCUSSION

Plant species composition in our experimental prairie communities diverged significantly within one growing season. Interactions among mycorrhizae and the availability of atmospheric CO₂ and soil N account for a significant amount of the variability in this divergence. As expected, many C₃ plants grew largest *without* AM fungi at elevated CO₂ and high N, while many C₄ grasses grew largest *with* AM fungi (e.g. Fig. 4a and b). However, some notable exceptions illustrate the shortcomings of the standard functional groups, and indicate that it may be impossible to distil the full diversity of prairie plant growth strategies into a few functional groups. Unexpectedly, *Salsola* was significantly larger in the presence of AM fungi (Fig. 4e). This is contrary to the findings of others (Allen *et al.* 1989; Johnson 1998) and we currently have no explanation for this result. The C₃ species *Solidago* and *Lespedeza* both grew largest in mesocosms with AM fungi at low soil N (Fig. 4c and d). This suggests that these two relatively slow growing prairie forbs require AM associations to establish as seedlings among faster growing members of the community. Nearly all of the species that grew significantly better in the presence of AM fungi also grew best at low soil N. This pattern suggests that mycotrophic plants compete less effectively at high soil N and/or that N fertilization has a detrimental effect on the maintenance of mutualistic AM associations. With the exception of the C₄ *Bouteloua*, all of the species that grew significantly larger with enriched soil N were C₃ species that also grew best at elevated CO₂. In summary, nitrophily was often associated with a positive response to elevated CO₂ and a negative response to AM fungi. These results suggest that plant growth rate (fast or slow) and mycorrhizal dependency (high or low) may be useful characteristics to consider when grouping plants according to their functional responses to resource enrichment (Diaz 1995).

Our findings support Reynolds' (1996) suggestion that differences in species' abilities to compete for potentially limiting resources can have large effects on community responses to elevated CO₂, and that root symbioses like AM fungi could mediate these responses. If our greenhouse mesocosms are an accurate proxy for real prairie communities, then we can predict that in the absence of mycorrhizae,

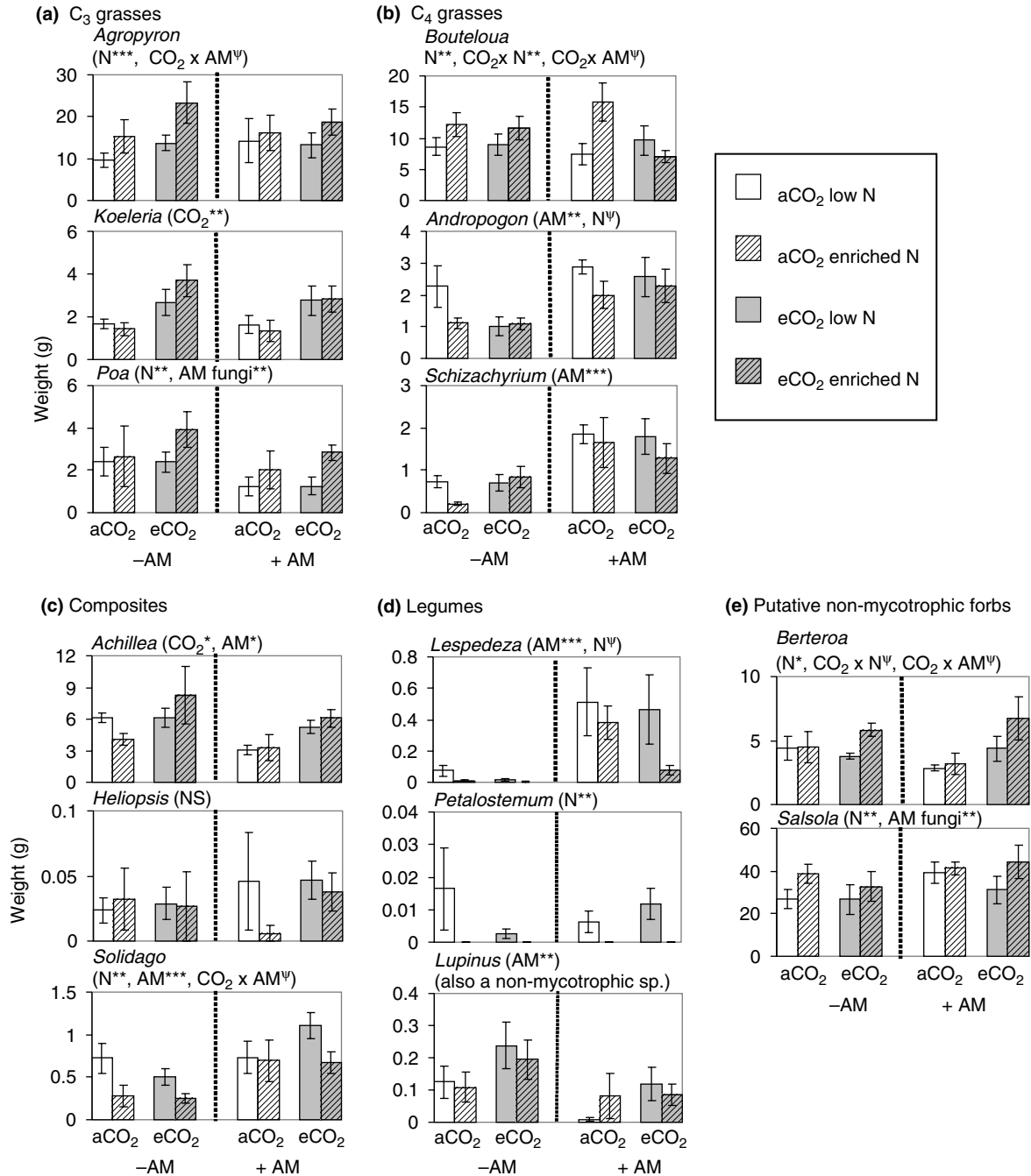


Figure 4 Shoot biomasses of individual species within the following functional groups (means \pm one standard error are shown, $n = 48$): (a) C₃ grasses, (b) C₄ grasses, (c) composites, (d) legumes, and (e) putative non-mycotrophic forbs. Open bars represent mesocosms with low soil N, hatched bars denote those with enriched N. Unshaded bars represent mesocosms grown under ambient CO₂ (aCO₂), and shaded bars denote those grown under elevated CO₂ (eCO₂). The left-hand side of each histogram shows non-mycorrhizal mesocosms (-AM) and the right-hand side shows mycorrhizal mesocosms (+AM). The significance levels of relevant treatments and interactions, as detected by ANOVA, are listed for each response (^ψ0.10 $\geq P > 0.05$; *0.05 $\geq P > 0.01$; **0.01 $\geq P > 0.001$; ***0.001 $\geq P$).

a world enriched with CO₂ and N should become dominated by fast growing C₃ nitrophilic plant species. However, CO₂ enrichment of systems with intact AM associations and/or

low soil N are just as likely to be dominated with C₄ grasses and slow growing mycotrophic forbs. Interactions between AM fungi and soil moisture availability may further mediate

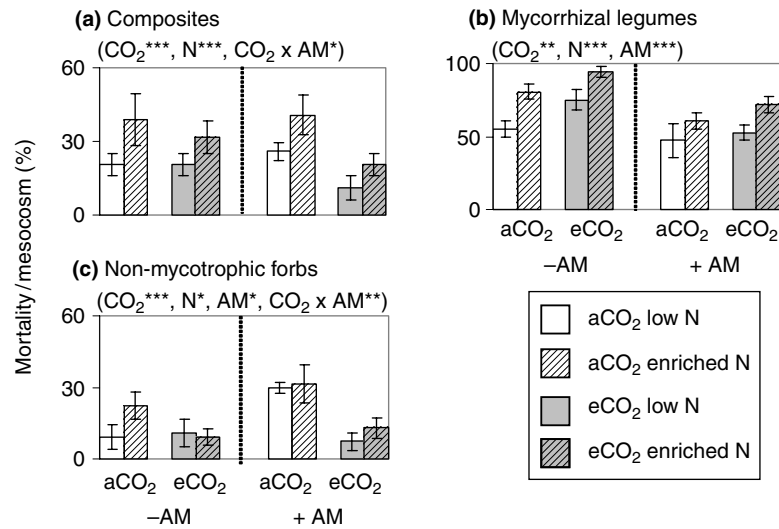


Figure 5 Percent mortality per mesocosm varied with treatments for three plant functional groups (means \pm one standard error are shown, $n = 48$): (a) composites (*Achillea*, *Heliopsis*, *Solidago*), (b) mycorrhizal legumes (*Lepedeza*, *Petalostemum*), (c) putative non-mycotrophic species (only *Berteroa*, because there was no *Salsola* mortality, plus *Lupinus*). Group mortality was analysed with logistic regression; mortality was associated with treatment (P values) using Wald chi-squared tests. Open bars represent mesocosms with low soil N, hatched bars denote those with enriched N. Unshaded bars represent mesocosms grown under ambient CO_2 (aCO_2) and shaded bars denote those grown under elevated CO_2 (eCO_2). The left-hand side of each histogram shows non-mycorrhizal mesocosms ($-AM$) and the right shows mycorrhizal mesocosms ($+AM$). The significance levels of relevant treatments and interactions are listed for each group (* $0.05 \geq P > 0.01$; ** $0.01 \geq P > 0.001$; *** $0.001 \geq P$).

plant community responses to CO_2 enrichment. We observed that elevated CO_2 only generated higher soil moisture in the absence of AM fungi. This indirect abiotic impact of mycorrhizae is likely to be important, because C_4 plants generally do better than C_3 plants in dry soils.

Plant species richness was highest in mesocosms with elevated CO_2 , $+AM$ fungi, and low soil N because mortality of many C_3 forbs was lowest in this combination of treatments. This pattern cannot be explained by differences in soil moisture, because if water availability was a significant driver of mortality, then we would expect that the treatment combinations with the highest evapotranspiration would have the highest mortality rates. However, this was not the case. Except for the non-mycotrophic forbs, mortality was consistently higher in the wetter elevated CO_2 , $-AM$ treatment than in the drier elevated CO_2 , $+AM$ treatment (Figs 2b and 5). It is possible that the carbon cost of AM associations is involved in this reversal, because CO_2 enrichment can ameliorate AM-induced growth depressions (Gavito *et al.* 2000; Jifon *et al.* 2002). Thus, elevated CO_2 may increase the mutualistic quality of some of the AM associations in our experimental community. Future studies that harvest both above and belowground biomass will test the hypothesis that CO_2 enrichment increases the mutualistic value of AM associations for mycotrophic plant species.

Diaz *et al.* (1993) hypothesized that plants species that do not form mycorrhizal associations should benefit less from

CO_2 enrichment than mycotrophic plant species because they lack the belowground carbon sink necessary to prevent photosynthetic down-regulation or accumulation of carbohydrates in the rhizosphere. Our findings do not support this hypothesis; two of the non-mycotrophic species in our community (*Lupinus* and *Berteroa*) had lower mortality under elevated CO_2 , and two strong mycotrophs (*Schizachyrium* and *Lepedeza*) grew best under ambient CO_2 . However, our findings do support the generalization that fast growing plants typically show the strongest growth response to elevated CO_2 (Diaz 1995). All four of the species that grew best with enriched CO_2 and N have relatively fast growth rates and two of them are classified as noxious weeds (USDA 2002).

Synthesis

Plant species responded individually to the presence of AM fungi and the availability of CO_2 and N. Significantly different plant communities arose under different treatment combinations. As expected, fast growing C_3 plants responded most positively to enriched atmospheric CO_2 and soil N. However, the presence of AM fungi was deleterious to many of these C_3 plants, and thus, mycorrhizae could reduce or eliminate the C_3 advantage in CO_2 and N enriched ecosystems. We found that at elevated CO_2 , the presence of AM fungi increased evapotranspiration rates to

levels equal to those found at ambient CO₂. Consequently, water balance is an indirect mechanism by which AM fungi may mediate plant community responses to CO₂ enrichment. Although our findings do not support the hypothesis that mycotrophic plant species benefit more from CO₂ enrichment than non-mycotrophic plant species, they do suggest that in some plant species elevated CO₂ can increase the net benefits of mycorrhizae by reducing their relative carbon cost. This was manifested in reduced mortality of several C₃ forbs when grown in the presence of AM fungi at elevated CO₂. Consequently, in our experimental system, plant species richness was greatest when AM fungi were present, soil N was low and atmospheric CO₂ was elevated. We conclude that mycorrhizae can be important mediators of plant community responses to atmospheric CO₂ enrichment, and that soil N further regulates these responses.

ACKNOWLEDGEMENTS

The National Science Foundation funded this study (DEB: 9806529). We are grateful to Tom Acker and his students in Applied Fluid Dynamics, Graydon Bell, Brad Blake, Cheri Church, Sally Evans, Nathan Glover, Michael Greene, Chas Jones, Michael Kearsley, Adam Langley, Matthew Loeser, Ted Martinez, Kyle Nelson, Melissa Reyes, Diane Rowland, and Jeff Voorhees for their assistance with this project. The Center for Data Insight at Northern Arizona University generously provided invaluable assistance with complex data management.

REFERENCES

- Allen, M.F., Allen, E.B. & Friese, C.F. (1989). Responses of the non-mycotrophic plant *Salsola kali* to invasion by vesicular-arbuscular mycorrhizal fungi. *New Phytol.*, 111, 45–49.
- Auge, R.M. (2000). Stomatal behavior of arbuscular mycorrhizal plants. In: *Arbuscular Mycorrhizas: Physiology and Function* (eds Kapulnik, Y. & Douds, D.D. Jr.). Kluwer Academic, Dordrecht, pp. 201–237.
- Berendse, F., Aerts, R. & Bobbink, R. (1993). Atmospheric nitrogen deposition and its impact on terrestrial ecosystems. In: *Landscape Ecology of a Stressed Environment* (eds Vos, C. & Opdam, C.P.). Chapman & Hall, London, pp. 104–121.
- Corkidi, L., Rowland, D.L., Johnson, N.C. & Allen, E.B. (2002). Nitrogen fertilization alters the functioning of arbuscular mycorrhizae at two semiarid grasslands. *Plant Soil*, 240, 299–310.
- Diaz, S. (1995). Elevated CO₂ responsiveness, interactions at the community level and plant functional types. *J. Biogeog.*, 22, 289–295.
- Diaz, S. (1996). Effects of elevated [CO₂] at the community level mediated by root symbionts. *Plant Soil*, 187, 309–320.
- Diaz, S., Grime, J., Harris, J. & McPherson, E. (1993). Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature*, 364, 616–617.
- Egerton-Warburton, L.M. & Allen, E.B. (2000). Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecol. Appl.*, 10, 484–496.
- Egerton-Warburton, L.M. & Allen, E.B. (2001). Mycorrhizal fungal communities: their organization, role and conservation in ecosystems under elevated atmospheric CO₂ and anthropogenic nitrogen deposition. In: *Microorganisms in Plant Conservation and Biodiversity* (eds Sivasithamparam, K. & Dixon, K.W.). Kluwer Academic, New York.
- Eom, A., Hartnett, D. & Wilson, W. (1999). The effect of fire, mowing and fertilizer amendment to arbuscular mycorrhizas in tallgrass prairie. *Am. Midl. Nat.*, 142, 55–70.
- Field, C.B., Jackson, R.B. & Mooney, H.A. (1995). Stomates responses to increased CO₂: implications from the plant to the global scale. *Plant, Cell Environ.*, 18, 1214–1225.
- Gavito, M.E., Curtis, P.S., Mikkelsen, T.N. & Jakobsen, I. (2000). Atmospheric CO₂ and mycorrhiza effects on biomass allocation and nutrient uptake of nodulated pea (*Pisum sativum* L.) plants. *J. Exp. Bot.*, 51, 1931–1938.
- Grime, J.P., Mackey, J.M.L., Hillier, S.H. & Read, D.J. (1987). Mechanisms of floristic diversity: evidence from microcosms. *Nature*, 328, 420–422.
- Hartnett, D.C. & Wilson, G.W.T. (1999). Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, 80, 1187–1195.
- Hetrick, B.A.D., Wilson, G.W.T. & Todd, T.C. (1990). Differential responses of C₃ and C₄ grasses to mycorrhizal symbiosis, phosphorus fertilization, and soil microorganisms. *Can. J. Bot.*, 68, 461–467.
- Hungate, B.A., Chapin, F.S. III, Zhong, H., Holland, E.A. & Field, C.B. (1997). Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia*, 109, 149–153.
- Hunsaker, D.J., Kimball, B.A., Pinter, P.J.J., Wall, G.W., LaMorte, R.L., Adamsen, F.J. *et al.* (2000). CO₂ enrichment and soil nitrogen effects on wheat evapotranspiration and water use efficiency. *Agri. For. Meteorol.*, 104, 85–105.
- Jifton, J.L., Graham, J.H., Drouillard, D.L. & Syvertsen, J.P. (2002). Growth depression of mycorrhizal *Citrus* seedlings grown at high phosphorus supply is mitigated by elevated CO₂. *New Phytol.*, 153, 133–142.
- Johnson, N.C. (1993). Can fertilization of soil select less mutualistic mycorrhizae? *Ecol. Appl.*, 3, 749–757.
- Johnson, N.C. (1998). Responses of *Salsola kali* and *Panicum virgatum* to mycorrhizal fungi, phosphorus and soil organic matter: implications for reclamation. *J. Appl. Ecol.*, 35, 86–94.
- Jones, S.B., Wraith, J.M. & Or, D. (2002). Time domain reflectometry measurement principles and applications. *Hydrol. Process.*, 16, 141–153.
- Klironomos, J., Rillig, M., Allen, M., Zak, D., Kubiske, M. & Pregitzer, K. (1997). Soil fungal–arthropod responses to *Populus tremuloides* grown under enriched atmospheric CO₂ under field conditions. *Global Change Biol.*, 3, 473–478.
- Klironomos, J., Ursic, M., Rillig, M. & Allen, M. (1998). Interspecific differences in the response of arbuscular mycorrhizal fungi to *Artemisia tridentata* grown under elevated atmospheric CO₂. *New Phytol.*, 138, 599–605.
- Klironomos, J.N., McCune, J., Hart, M. & Neville, J. (2000). The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecol. Lett.*, 3, 137–141.

- Körner, Ch., Bazzaz, F.A. & Field, C.B. (1996). The significance of biological variation, organism interactions, and life histories in CO₂ research. In: *Carbon dioxide, Populations, and Communities* (eds Körner, Ch. & Bazzaz, F.A.). Academic Press, New York, pp. 443–456.
- McCune, B. & Mefford, M.J. (1999). *Multivariate Analysis of Ecological Data*, Version 4.14. MjM Software, Gleneden Beach, OR, USA.
- Mielke, P.W.J. (1984). Meterological applications of permutation techniques based on distance functions. In: *Handbook of Statistics* (eds Krishnaiah, P.R. & Sen, P.K.). Elsevier Science, Amsterdam, Vol. 4, pp. 813–830.
- Miller, R.M., Miller, S.P., Jastrow, J.D. & Rivetta, C.B. (2002). Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in *Andropogon gerardii*. *New Phytol.*, 155, 149–162.
- Morton, J.B., Bentivenga, S.P. & Wheeler, W.W. (1993). Germ plasm in the international collection of arbuscular and vesicular-arbuscular mycorrhizal fungi (INVAM) and procedures for culture development, documentation and storage. *Mycotaxon*, 48, 491–528.
- O'Dell, T.E. & Trappe, J.M. (1992). Root endophytes of lupin and some other legumes in northwestern USA. *New Phytol.*, 122, 479–485.
- Poorter, H., Roumet, C. & Campbell, B.D. (1996). Interspecific variation in the growth response of plants to elevated CO₂: a search for functional types. In: *Carbon dioxide, Populations, and Communities* (eds Körner, Ch. & Bazzaz, F.A.). Academic Press, New York, pp. 375–412.
- Reich, P.B., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M.G., Knopw, J. *et al.* (2001). Do species and functional groups differ in acquisition and use of C, N, and water under varying atmospheric CO₂ and N availability regimes? A field test with 16 grassland species. *New Phytol.*, 150, 435–448.
- Reyes, M.A., Johnson, N.C. & Wolf, J.E. (2002). Effects of CO₂ enrichment on mycorrhizal responsiveness of tallgrass prairie species: an inverse relationship. In: *Abstracts of the Ecological Society of America Meetings*, Tucson, AZ, USA, August, p. 414.
- Reynolds, H.L. (1996). Effects of elevated CO₂ on plants grown in competition. In: *Carbon dioxide, Populations, and Communities* (eds Körner, Ch. & Bazzaz, F.A.). Academic Press, New York, pp. 273–286.
- Rillig, M.C. & Allen, M.F. (1999). What is the role of arbuscular mycorrhizal fungi in plant to ecosystem responses to elevated atmospheric CO₂? *Mycorrhiza*, 9, 1–8.
- Rillig, M.C., Hernandez, G.Y. & Newton, P.C. (2000). Arbuscular mycorrhizae respond to elevated atmospheric CO₂ after long-term exposure: evidence from a CO₂ spring in New Zealand supports the resource balance model. *Ecol. Lett.*, 3, 475–478.
- Sanders, I. (1996). Plant–fungal interactions in a CO₂ rich world. In: *Carbon dioxide, Populations, and Communities* (eds Körner, Ch. & Bazzaz, F.A.). Academic Press, New York, pp. 265–272.
- SAS (1997). *JMP Statistical Discovery Software*. SAS Institute Inc., Cary, NC, USA.
- Schenk, U., Jager, H.-J. & Weigel, H.-J. (1997). The response of perennial ryegrass/white clover swards to elevated atmospheric CO₂ concentrations. 1. Effects on competition and species composition and interaction with N supply. *New Phytol.*, 135, 67–79.
- Smith, S.E. & Read, D.J. (1997). *Mycorrhizal Symbiosis*, 2nd edn. Academic Press, New York.
- USDA (2002). National PLANTS Database (WWW document). URL: <http://plants.usda.gov>.
- Van der Heijden, M., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engle, R., Boller, T. *et al.* (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69–72.
- Wilson, G.W.T. & Hartnett, D.C. (1997). Effects of mycorrhizas on plant growth and dynamics in experimental tallgrass prairie microcosms. *Am. J. Bot.*, 84, 478–482.
- Wilson, G. & Hartnett, D. (1998). Interspecific variation in plant responses to mycorrhizal colonization in tall grass prairie. *Am. J. Bot.*, 85, 1732–1738.
- Wolf, J.E. (2001). Seasonal and host-specific responses of arbuscular-mycorrhizal fungi to elevated carbon dioxide and nitrogen. Masters thesis, Northern Arizona University, Flagstaff, AZ.
- Wolf, J., Johnson, N.C., Rowland, D.L. & Reich, P.B. (2003). Elevated carbon dioxide and plant species richness impact arbuscular mycorrhizal fungal spore communities. *New Phytol.*, 157, 579–588.
- Wright, D.P., Scholes, J.D. & Read, D.J. (1998). Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. *Plant Cell Environ.*, 21, 209–216.
- Zimmerman, G.M., Goetz, H. & Mielke, P.W.J. (1985). Use of an improved statistical method for group comparisons to study effects of prairie fire. *Ecology*, 66, 606–611.

Manuscript received 31 December 2002

First decision made 11 February 2003

Manuscript accepted 5 March 2003