Species of plants and associated arbuscular mycorrhizal fungi mediate mycorrhizal responses to CO₂ enrichment

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Abstract

It has been suggested that enrichment of atmospheric CO₂ should alter mycorrhizal function by simultaneously increasing nutrient-uptake benefits and decreasing net C costs for host plants. However, this hypothesis has not been sufficiently tested. We conducted three experiments to examine the impacts of CO₂ enrichment on the function of different combinations of plants and arbuscular mycorrhizal (AM) fungi grown under high and low soil nutrient availability. Across the three experiments, AM function was measured in 14 plant species, including forbs, C₃ and C₄ grasses, and plant species that are typically nonmycorrhizal. Five different AM fungal communities were used for inoculum, including mixtures of Glomus spp. and mixtures of Gigasporaceae (i.e. Gigaspora and Scutellospora spp.). Our results do not support the hypothesis that CO₂ enrichment should consistently increase plant growth benefits from AM fungi, but rather, we found CO₂ enrichment frequently reduced AM benefits. Furthermore, we did not find consistent evidence that enrichment of soil nutrients increases plant growth responses to CO₂ enrichment and decreases plant growth responses to AM fungi.

Our results show that the strength of AM mutualisms vary significantly among fungal and plant taxa, and that CO₂ levels further mediate AM function. In general, when CO₂ enrichment interacted with AM fungal taxa to affect host plant dry weight, it increased the beneficial effects of Gigasporaceae and reduced the benefits of Glomus spp. Future studies are necessary to assess the importance of temperature, irradiance, and ambient soil fertility in this response. We conclude that the affects of CO₂ enrichment on AM function varies with plant and fungal taxa, and when making predictions about mycorrhizal function, it is unwise to generalize findings based on a narrow range of plant hosts, AM fungi, and environmental conditions.

Key words: AM fungi, arbuscular mycorrhizas, carbon dioxide enrichment, CO₂, experimental scale, Gigasporaceae, Glomus, mutualism, mycorrhizal function, nitrogen

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Introduction

Arbuscular mycorrhizal (AM) symbioses facilitate plant uptake of soil nutrients in most temperate and tropical ecosystems. The mutualistic effects of these associations are predicted to be sensitive to anthropogenic enrichment of atmospheric CO₂, because elevated CO₂ should simultaneously increase plants’ photosynthetic rates and soil nutrient requirements (O’Neill, 1994). From a plant perspective, AM function is determined by the balance between photosynthesize costs and nutrient benefits (Fitter, 1991). Allocation of photosynthesize to AM fungi represents a major C cost to plants (Koch & Johnson, 1984), and increased photosynthetic rates at elevated CO₂ should make more C available to support AM symbioses (Loveland et al., 1997; Jifton et al., 2002). Changing C acquisition costs through CO₂ enrichment is expected to increase the relative benefits of AM fungi.
uptake of soil resources (Hoeksema & Bruna, 2000; Sterner & Elser, 2002).

Several studies support the hypothesis that atmospheric CO2 enrichment will influence the balance between mycorrhizal costs and benefits. Carbon demands of AM fungi increase plant photosynthetic rates (Wright et al., 1998a), and C gained through AM enhancement of C assimilation is allocated to the fungus, not to increasing host plant biomass (Wright et al., 1998b; Miller et al., 2002). Furthermore, CO2 enrichment can mitigate plant growth depressions that are generated when C costs of AM fungi outweigh their nutrient uptake benefits (Jifton et al., 2002). Although many studies have shown that atmospheric CO2 enrichment increases percent root length colonization by AM fungi (Treseder, 2004), this effect generally disappears when the confounding effect of increased root biomass is factored out (Fitter et al., 2000). Nevertheless, elevated CO2 often increases allocation to AM hyphae that occur in the soil outside plant roots (Rillig, 2004; Staddon et al., 2004).

The idea that CO2 enrichment will decrease AM costs and/or increase their benefits for plants is clearly overly simplistic because many factors mediate AM function. Plant genotypes vary in mycorrhizal dependency (Graham & Eisenstat, 1994), AM fungal genotypes vary in mutualistic effects (Klironomos, 2003), and genotypes of associated host plants and AM fungi and environmental conditions interact to control the costs and benefits of AM symbioses (Johnson et al., 1997). Abiotic factors such as soil fertility (Johnson, 1993) and temperature (Gavito et al., 2003) can strongly impact mycorrhizal functioning. Plant taxa differ in their responses to both CO2 enrichment and AM symbioses. Fast-growing plant species with a C3 photosynthetic pathway have been shown to benefit more from elevated CO2 and less from mycorrhizae than slow-growing plants with a C4 photosynthetic pathway (Diaz, 1995; Luscher et al., 1998; Wilson & Hartnett, 1998; Johnson et al., 2003a). Taxa of AM fungi are also known to differ in their responses to CO2 enrichment (Klironomos et al., 1998; Treseder et al., 2003; Wolf et al., 2003).

Experimental assessment of mycorrhizal responses to CO2 enrichment has been examined in very few plant–fungus combinations; and in most cases has combined genotypes of plants and AM fungi that do not co-occur in natural systems. The applicability of such studies may be limited because ecotypes of plants and AM fungi are likely to become coadapted to each other and to local edaphic conditions (Bever et al., 2001; Schultz et al., 2001; Sanders, 2002).

To understand the variation in AM function associated with different plant and fungal taxa and resource availability, we conducted three experiments that compared the impacts of CO2 enrichment on the mycorrhizal function of many different plant–fungus combinations grown under high and low nutrient availability. The purpose of this research was to test the following hypotheses:

1. Enrichment of CO2 should increase mycorrhizal benefits for plant growth.
2. Enrichment of soil nutrients should increase plant growth responses to CO2 enrichment and decrease plant growth responses to AM fungi.

This individual-scale investigation focuses on plant biomass responses to AM symbioses. Other important benefits of AM associations such as interactions with root pathogens (Newsham et al., 1995) and soil structure (Miller & Jastrow, 2000) cannot be effectively examined in this series of experiments. However, these studies were designed to complement ecosystem-scale and community-scale experiments that do incorporate these higher-order AM functions. The ecosystem-scale experiment is a free-air CO2 enrichment (FACE) experiment at Cedar Creek Minnesota, USA (Reich et al., 2001) in which AM fungi were studied (Wolf et al., 2003), but not manipulated. The community-scale experiment is a mesocosm study that examined AM effects on plant community responses to CO2 enrichment (Johnson et al., 2003a).

Materials and methods

Experimental designs

Three separate experiments were conducted in 12 clear walled chambers (2.5 m × 1.3 m × 1.5 m, w × l × h) within a greenhouse at Northern Arizona University, Flagstaff Arizona, USA. Six chambers had ambient and six had elevated levels of atmospheric CO2 (set points of 465 and 720 ppm, respectively, daylight hours only). At Flagstaff’s atmospheric pressure (ca. 79 kPa), these concentrations provide CO2 partial pressures of 36.7 and 56.9 Pa, equivalent to sea level concentrations of 368 and 562 ppm, respectively. Soil used in these experiments was collected adjacent to the FACE experiment at Cedar Creek, Minnesota and transported to Flagstaff, Arizona. The sandy soil is a Typic Udipsamment (Grigal et al., 1974) with approximately 43 µg g−1 available P (Bray-1), and 95 µg g−1 available N. For all three experiments, 25 cm deep × 6.4 cm diameter Deeptots™ (Stuewe and Sons, Corvallis, OR, USA) were filled with 656 ml of soil (Experiments 2 and 3) or a soil–sand mixture (Experiment 1) that had been pasteurized by heating to 100°C for 8 h on 2 consecutive days.
The three experiments differ in the number of plant species studied, species composition of the AM fungal inoculum, soil media, and nutrient treatments (Table 1). All treatments were replicated six times. Experiment 1 examined 14 plant species × 2 CO₂ levels (aCO₂ and eCO₂) × 2 AM treatments (+ AM and −AM) × 6 replicates, for a total of 366 plants. Experiment 2 examined 4 plant species × 2 CO₂ levels (aCO₂ and eCO₂) × 3 AM treatments (Glomus spp., Gigasporaceae spp., and −AM) × 2 N levels (+ N and −N) × 2 P levels (+ P and −P) × 6 replicates, for a total of 576 plants. Experiment 3 examined 4 plant species × 2 CO₂ levels (aCO₂ and eCO₂) × 3 AM treatments (Glomus intraradices, Gigaspora gigantea and −AM) × 6 replicates, for a total of 144 plants.

Plants and AM fungi

The 14 plant species examined in Experiment 1 included representatives of five functional groups: C₄ grasses, C₃ grasses, composites, legumes, and putative nonmycorrhizal forbs (Table 2). These species co-occur at Cedar Creek and are common in mesic grasslands in North America. Experiments 2 and 3 examined only four plant species: Achillea millefolium, Koeleria cristata, Lespedeza capitata, and Schizachyrium scoparium. Except for Salsola kali, all of the seeds used in this experiment were acquired from the same seed sources used by Reich et al., 2001 at the Cedar Creek FACE site. Salsola seeds were collected from a roadside near Flagstaff, AZ.

Cultures of AM fungi were established on leeks and celery from fresh Cedar Creek soil following the methods of Morton et al. (1993). The inoculum used in Experiment 1 was a mixture of many AM fungal species isolated from Cedar Creek (Table 1). Experiment 2 used two different fungal genera: a mixture of Cedar Creek Glomus species and a mixture of Cedar Creek Gigasporaceae (i.e. Gigaspora and Scutellospora spp.). Experiment 3 used pure cultures of Glomus intraradices and Gigaspora gigantea kindly provided by John Klironomos at the University of Guelph, Canada.

Glomus inoculum consisted of colonized roots and spores and Gigasporaceae inoculum consisted of spores. In Experiment 1, a band of 5.5 g of fresh root fragments and AM spores was layered 6 cm from the top of each Deepot™ and covered with 3 cm of pasteurized soil. The −AM treatments were established in the same way except 5.5 g of autoclaved root fragments and AM spores were used. Communities of microorganisms (other than AM fungi) were equalized by adding a microbial wash to the −AM treatments. The microbial wash was prepared from water that had been used to extract spores and root fragments from the leek and celery cultures. This water was filtered through a 25 μm sieve four times, and 5 mL was applied to the −AM treatments. Inoculum and microbial wash were applied similarly in the two other experiments except that 32 g of colonized roots, soil, and spores were used in Experiment 2 and 4.7 g of perlite + colonized roots and spores were used in Experiment 3.

Growth conditions

Air temperature monitored at 10 min intervals within each of the 12 greenhouse chambers varied diurnally and among experiments with average daily minima and maxima (°C) of 17.1 and 32.2 in Experiment 1, 16.9 and 28.5 in Experiment 2, and 18.1 and 25.8 in Experiment 3 (Table 1). Maximum daytime PAR irradiance was about 1200 μmol m⁻² s⁻¹ at noon on clear days near the summer solstice, and as low as 400 μmol m⁻² s⁻¹ on clear days during winter. Plants were watered as needed with water that had been passed through a charcoal filter. Once each week, all of the plants in Experiment 1 were watered using a nutrient solution that was modified from Sylvia & Hubbell’s (1986) formulation for aeroponic culture of mycorrhizae, and contained: 433 mg L⁻¹ KNO₃, 8.4 mg L⁻¹ Ca(NO₃)₂.4H₂O, 199 mg L⁻¹ CaSO₄.2H₂O, 130 mg L⁻¹ K₂SO₄, 72 mg L⁻¹ MgSO₄, 0.06 mg L⁻¹ H₂BO₃, 0.54 mg L⁻¹ MnCl.4H₂O, 0.07 mg L⁻¹ ZnSO₄.7 H₂O, 0.03 mg L⁻¹ NaCl, and 0.02 mg L⁻¹ CuSO₄.5 H₂O. In Experiment 2, twice each week the + N plants were watered with a solution containing 433 mg L⁻¹ KNO₃, and + P plants received a solution containing 44 mg L⁻¹ KH₂PO₄. Plants in Experiment 3 received only water and no supplemental nutrients. Plants were harvested before they became root-bound. In Experiment 1, fast growing Echinochloa crusgalli was harvested at 8 weeks, the other plant species were harvested between 10.5 and 16 weeks. In Experiments 2 and 3, Schizachyrium scoparium was harvested at 10.5 weeks, Achillea millefolium, and Koeleria cristata, and Lespedeza capitata were harvested at 16 weeks.

Response variables

At harvest, plants were carefully removed from the soil and their roots were gently washed. Shoots were cut from roots, dried, and weighed. Roots were divided into two sub-samples and the fresh weight was determined for both. One subsample was stained using the technique of Koske & Gemma (1989), and examined for percent of root length colonized by AM fungi using the method of McGonigle et al. (1990). The other subsample was dried, and its fresh/dry mass ratio was used to calculated total root dry mass. Mycorrhizal
Table 1 Environmental conditions and materials used in Experiments 1, 2, and 3

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Plants</th>
<th>AM fungal inoculum</th>
<th>Soil media</th>
<th>Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td>14 species, 366 plants</td>
<td>One mixture of Cedar Creek <em>Glomus</em> and <em>Acaulospora</em> spp: <em>G. aggregatum/intraradices</em>, <em>G. clarum</em>, <em>G. constrictum</em>, <em>A. scrobiculata</em>, <em>A. spinosa</em>, and <em>A. trappei</em></td>
<td>One part Cedar Creek soil + three parts silica sand</td>
<td>All treatments received Sylvia and Hubbell's nutrient solution weekly</td>
</tr>
<tr>
<td>June 2001 through August/October 2001</td>
<td>Mean ºC: 23.2 ± 1.0</td>
<td>Min ºC: 17.1 ± 1.9</td>
<td>Max ºC: 32.2 ± 1.9</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td>Four species, 576 plants</td>
<td>Two mixtures of Cedar Creek spp: <em>Glomus</em> mixture: <em>Glomus aggregatum/intraradices</em>, <em>G. clarum</em>, <em>G. claridium</em>, <em>G. fasciculatum</em> Gigasporaceae mixture: <em>Gigaspora gigantea</em>, <em>G. margarita</em>, <em>Scutellospora calospora</em>, <em>Scutellospora</em> sp. (amber)</td>
<td>100% Cedar Creek soil</td>
<td>Factorial N and P treatments received N and P twice a week</td>
</tr>
<tr>
<td>August 2002 through December 2002</td>
<td>Mean ºC: 21.4 ± 2.4</td>
<td>Min ºC: 16.9 ± 2.0</td>
<td>Max ºC: 28.5 ± 2.9</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td>Four species, 144 plants</td>
<td>Two pure-cultures of non-endemic spp: <em>Glomus intraradices</em> and <em>Gigaspora gigantea</em></td>
<td>100% Cedar Creek soil</td>
<td>No nutrients, only filtered water</td>
</tr>
<tr>
<td>November 2002 through February 2003</td>
<td>Mean ºC: 20.4 ± 1.6</td>
<td>Min. 18.1 ± 1.3</td>
<td>Max. 25.8 ± 3.1</td>
<td></td>
</tr>
</tbody>
</table>

*Average daily mean, maximum and minimum (±SD) over entire experiment for all 12 chambers.
AM, arbuscular mycorrhizal.
responsiveness (MR) was calculated by comparing the total dry mass (TDW) of each species with and without mycorrhizae: 

\[ MR = \ln(\frac{\text{TDW}_{\text{AM plant}}}{\text{TDW}_{\text{non-AM plant}}}) \]

For these calculations, AM and non-AM plants were paired so that they shared the same greenhouse chamber, CO2, and nutrient treatments.

Total Kjeldahl N and P in dry shoots of Achillea, Koeleria, and Schizachyrium from Experiments 2 and 3 were determined colorimetrically by flow injection analysis using a Lachat Automated Ion Analyzer. Aboveground tissue samples were dried in a 60°C oven for 72 h and then ground on a Wiley Mill to pass through a 20 μm mesh sieve. Samples, weighing 0.05 g, were digested on a block digester for 5 h at 350°C. Digested samples were refrigerated until 1 day prior to analysis. Tissue N and P were not determined for Lespedeza or for plants fertilized with N and P (in Experiment 2).

Statistical analyses

All response variables were analyzed using ANOVA with the full model of all experimental treatments. This allowed us to test the main effects and two-way interactions of: CO2 level, N and P level (Experiment 2), mycorrhizal status, and the random blocking effect of chamber nested within CO2 level (no interactions tested). Root colonization data were arc-sine square root transformed prior to ANOVA. All statistical analyses were performed using JMP 4.0 (SAS, 1997).

Results

Root colonization

Roots of plants inoculated with AM fungi became colonized and roots of non-AM treatments remained uncolonized except for a few individuals which had very low levels of colonization (0.06–0.7%). Mycorrhizal inoculation of the nonmycorrhizal species in Experiment 1 did not generate colonization (Salsola and Berteroa) or only very low levels of colonization (<1% in Lupinus). Levels of AM colonization varied greatly among plant and fungal taxa and experiments. There were no consistent patterns in the responses of AM colonization to CO2 and nutrient treatments. Root colonization increased in response to CO2 enrichment in Poa (Experiment 1, \( F = 6.55, P = 0.03 \)) and Lespedeza.

Table 2  Results from Experiment 1

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Species abbreviation</th>
<th>CO2</th>
<th>AM</th>
<th>CO2 × AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4 grasses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andropogon gerardi Vitman</td>
<td>ANGE</td>
<td>ns</td>
<td>ns</td>
<td>4.25*</td>
</tr>
<tr>
<td>Bouteloua gracilis (Willd. ex Kunth) Lag. ex Griffiths</td>
<td>Bogr</td>
<td>∥ 4.92*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Echinochloa crus-galli L. (Beauv.)</td>
<td>ECCR</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Schizachyrium scoparium (Michaux) Nash</td>
<td>SCSC</td>
<td>ns</td>
<td>∥ 8.65**</td>
<td>ns</td>
</tr>
<tr>
<td>C3 grasses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koeleria cristata Pers</td>
<td>KOCR</td>
<td>∥ 8.08*</td>
<td>ns</td>
<td>4.12*</td>
</tr>
<tr>
<td>Poa pratensis L</td>
<td>POPR</td>
<td>∥ 17.68***</td>
<td>ns</td>
<td>10.94**</td>
</tr>
<tr>
<td>Composites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Achillea millefolium L</td>
<td>ACMI</td>
<td>∥ 5.37*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Helianthus annuus L.</td>
<td>HEHE</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Solidago rigida L</td>
<td>SORI</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Legumes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lespedeza capitata (Michaux)</td>
<td>LECA</td>
<td>ns</td>
<td>∥ 17.80**</td>
<td>ns</td>
</tr>
<tr>
<td>Lupinus perennis L</td>
<td>LUPE</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Petalostemum velosum Nutt.</td>
<td>PEVE</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Non-mycorrhizal forbs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berteroa incana L.</td>
<td>BEIN</td>
<td>ns</td>
<td>∥ 5.04*</td>
<td>9.14**</td>
</tr>
<tr>
<td>Salsola kali L.</td>
<td>SAKA</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Effect of CO2 enrichment and AM fungi on total plant biomass (TDW) as indicated by the F-ratios of two-way ANOVA. Arrows indicate factors that significantly increased or decreased the TDW of particular plant species. Significant CO2 × AM interactions are described in the text, ns indicates F-ratios were not significant.

*F-ratios were significant at \( P \leq 0.10 \); **F-ratios were significant at \( P \leq 0.05 \); ***F-ratios were significant at \( P \leq 0.01 \); ****F-ratios were significant at \( P \leq 0.001 \); Lupinus is both a legume and a nonmycorrhizal forb. AM, arbuscular mycorrhizal; TDW, total dry mass.
mean MR in ambient and elevated CO2 are significantly different at elevated CO2. Positive values indicate AM mutualism and negative values indicate AM parasitism.

Experiment 2 (cantly affect AM colonization with the exception that Nitrogen and phosphorus treatments did not significantly affect AM colonization with the exception that nitrogen enrichment increased colonization in Koeleria in Experiment 2 (F = 5.03, P = 0.03).

Experiment 1
The 14 plant species varied in their responses to CO2 enrichment and AM fungi (Table 2). Elevated CO2 significantly increased the TDW of both C3 grasses (Koeleria and Poa) and one of the composites (Achillea). In contrast, TDW of one of the C4 grasses (Bouteloua) was significantly reduced by CO2 enrichment. Inoculation with AM fungi increased the TDW of one C4 grass (Schizachyrium) and one legume (Lespedeza), and it decreased the TDW of Koeleria and the nonmycorrhizal forb Berteroa. Furthermore, there was a significant CO2 × AM interaction in the growth responses of Berteroa, Poa, and the C4 grass Andropogon (Table 2). MR was not significantly influenced by CO2 enrichment in 11 of the 14 species; however, it caused MR to become more negative in Berteroa and Poa and it increased MR of Petalostemum from negative to neutral (Fig. 1).

Experiment 2
Total biomass of three of the four plant species studied in Experiment 2 responded positively to CO2 enrichment (Table 3). None of the plant species responded directly to AM fungi, although there was a significant CO2 × AM interaction for TDW of Lespedeza and Schizachyrium. For both species, plants colonized with Gigasporaceae were larger at elevated than at ambient CO2, while those colonized with Glomus were larger at ambient than at elevated CO2. Nitrogen enrichment strongly increased TDW of Koeleria and it decreased TDW of Schizachyrium and Lespedeza. Also, there was a significant N × AM effect on TDW of Koeleria; at low N, −AM plants were significantly larger than those colonized by Gigasporaceae. None of the plants in Experiment 2 responded directly to P enrichment, however there was a significant CO2 × P interaction on TDW of Schizachyrium. At high P, Schizachyrium grown at ambient CO2 were significantly larger than those grown at elevated CO2. Also, there was a significant N × P interaction on TDW of Koeleria; among plants grown at low N, those given high P were significantly smaller than those given low P (Table 3).

MR of the four species was influenced differently by the experimental treatments. Enrichment of P decreased the MR of Achillea (F = 3.9, P = 0.05, data not shown). In contrast, enrichment of N increased MR of Achillea, Koeleria and Lespedeza (Fig. 2). Also, a CO2 × N interaction influenced the MR of Achillea and Koeleria. In both species, N enrichment caused MR to increase in elevated but not ambient CO2. The composition of the AM fungal inoculum interacted with CO2 in Lespedeza and Schizachyrium. In both species, CO2 enrichment increased the MR of plants inoculated with Gigasporaceae and decreased the MR of plants inoculated with Glomus (Fig. 2).

Fig. 1 The effects of CO2 enrichment on mycorrhizal responsiveness total dry mass (TDW); arbuscular mycorrhizal (AM) (MR = ln (TDW + AM plant/TDW−AM plant)) of the 14 plant species in Experiment 1. Species abbreviations are defined in Table 2. Bars represent mean values with standard error lines (N = 6), unshaded bars represent MR at ambient CO2 and shaded bars represent MR at elevated CO2. Positive values indicate AM mutualism and negative values indicate AM parasitism. *, indicate that within a species, mean MR in ambient and elevated CO2 are significantly different at P ≤0.10 and ≤0.05 respectively.
Unlike the previous two experiments, none of the plant species had a significant biomass response to CO₂ enrichment in Experiment 3 (Table 4). Like the previous experiments, the TDW of *Schizachyrium* was influenced by AM fungi or a CO₂ × AM interaction. *Schizachyrium* grew significantly larger when inoculated with Gigasporaceae vs. *Glomus*, and CO₂ enrichment further increased the benefits of this fungus. MR of *Schizachyrium* was significantly greater at elevated CO₂ than ambient, and in plants inoculated with Gigasporaceae than those inoculated with *Glomus* (Figs 3 and 4). In contrast, MR of *Lespedeza* inoculated with *Glomus* was significantly lower at elevated CO₂ than at ambient CO₂ (Fig. 3).

**Table 3**  Results from Experiment 2

<table>
<thead>
<tr>
<th>Plant species</th>
<th>F-ratios for main effects</th>
<th>F-ratios for interactions</th>
<th>CO₂ × AM</th>
<th>CO₂ × P</th>
<th>N × AM</th>
<th>N × P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achillea millefolium</em></td>
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<tr>
<td></td>
<td>[4.44[^*] ns</td>
<td></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><em>Koeleria cristata</em></td>
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<tr>
<td></td>
<td>[14.95[^**] ns</td>
<td></td>
<td>ns</td>
<td>ns</td>
<td>3.80[^*] 5.88[^**]</td>
<td></td>
</tr>
<tr>
<td><em>Lespedeza capitata</em></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>[5.26[^*] ns</td>
<td></td>
<td>ns</td>
<td></td>
<td>9.22[^***] ns</td>
<td>ns</td>
</tr>
<tr>
<td><em>Schizachyrium scoparium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.65[^<em>] 5.02[^</em>]</td>
<td>ns</td>
</tr>
</tbody>
</table>

Effect of CO₂ enrichment, AM fungi, N, and P enrichment on total plant biomass (TDW) as indicated by the F-ratios of two-way ANOVA. Arrows indicate factors that significantly increased ↑ or decreased ↓ the TDW of particular plant species. Significant interactions are described in the text, ns indicates F-ratios were not significant.

[^*]F-ratios were significant at P≤0.10;[^**]F-ratios were significant at P≤0.05;[^***]F-ratios were significant at P≤0.01;[^****]F-ratios were significant at P≤0.001. ¹None of the CO₂ × N or P × AM interactions were significant.

AM, arbuscular mycorrhizal; TDW, total dry mass.

**Fig. 2**  The effects of CO₂ enrichment and arbuscular mycorrhizal (AM) taxa on mycorrhizal responsiveness (MR, ln(TDW + AM plant/TDW – AM plant)) of the four plant species studied in Experiment 2. Bars represent mean values with standard error lines (N = 6). Unshaded bars represent MR at ambient CO₂ and shaded bars represent MR at elevated CO₂; striped bars represent high nitrogen treatments. Positive values indicate AM mutualism and negative values indicate AM parasitism. The significance levels of relevant treatments and interactions, as detected by ANOVA, are listed for each response,[^*] and[^*] indicate P≤0.10 and ≤0.05, respectively.

**Experiment 3**

Unlike the previous two experiments, none of the plant species had a significant biomass response to CO₂ enrichment in Experiment 3 (Table 4). Like the previous experiments, the TDW of *Schizachyrium* was influenced by AM fungi or a CO₂ × AM interaction. *Schizachyrium* grew significantly larger when inoculated with Gigasporaceae vs. *Glomus*, and CO₂ enrichment further increased the benefits of this fungus. MR of *Schizachyrium* was significantly greater at elevated CO₂ than ambient, and in plants inoculated with Gigasporaceae than those inoculated with *Glomus* (Figs 3 and 4). In contrast, MR of *Lespedeza* inoculated with *Glomus* was significantly lower at elevated CO₂ than at ambient CO₂ (Fig. 3).
Across the three plant species that were analyzed, foliar %N and %P were significantly lower in plants from Experiment 2 than from Experiment 3 (for N: $F = 228.7$, $P < 0.0001$; for P: $F = 121.4$, $P < 0.0001$). Foliar %N was significantly lower in plants grown at elevated CO$_2$ ($F = 15.5$, $P = 0.05$), but CO$_2$ enrichment had no effect on foliar %P. There was no significant effect of AM inoculation on either %N or %P in any of the species in either experiment.

**Discussion**

Our results indicate that enrichment of CO$_2$ does not uniformly increase mycorrhizal benefits for plant growth. To the contrary, there is variability in responses and when the AM fungal community is dominated by
Glomus species, CO₂ enrichment may actually reduce the beneficial effects of mycorrhizae on plant biomass (e.g., in Lespedeza). Mechanisms for this result are unknown; however, studies show that members of Glomaceae and Gigasporaceae differ in their mutualistic exchange of carbon and nutrients (Douds & Schenck, 1990; Pearson & Jakobsen, 1993; Smith et al., 2004). It is possible that differences in the C sink-strength among AM fungal taxa influence their responses to changes in host plant physiology that accompany CO₂ enrichment. Future studies are necessary to assess this possibility.

It is well known that root colonization levels are often not related to mycorrhizal function (McGonigle, 1988; Smith et al., 2004). Consequently, in our experiments, the absence of a consistent response of AM colonization to CO₂ or nutrient enrichment despite significant functional responses is not unexpected, and it further underscores the importance of measuring AM variables other than root colonization. Quantification of functional changes in mycorrhizal associations can only be measured using a functional parameter such as MR, not through measuring a structural parameter such as root colonization.

Our hypothesis, that enrichment of soil nutrients should increase plant growth responses to CO₂ enrichment and decrease plant growth responses to AM fungi, was also not supported by our data. In Experiment 2, none of the plant species exhibited a significant TDW response to the N × CO₂ interaction and only Schizachyrium showed a significant P × CO₂ interaction. However, the outcome of this interaction was opposite to that predicted by the hypothesis: P enrichment increased the TDW of plants grown at ambient CO₂ significantly more than those grown at elevated CO₂. As expected, P enrichment reduced the MR of Achillea, but contrary to expectations, enrichment of N increased rather than decreased MR of three of the four species studied in Experiment 2. Also, there were significant N × CO₂ interactions on MR of Achillea and Koeleria. In both species, N enrichment increased MR at elevated but not ambient CO₂. These findings suggest that plants and AM fungi may be competing for N when C is not in limiting supply. This idea is supported by the fact that the Cedar Creek soil used in this experiment has very low levels of N (Johnson et al., 2003b). Cedar Creek soil also has very high levels of P, and that may help account for the unexpected observation that AM colonization did not increase the percent tissue P in Experiments 2 and 3.

We observed considerable interexperiment variability in the responses of individual plant species. Differences between the three experiments could result from differences in fungal inoculum or environmental conditions. Different AM fungi were used in each experiment, ranging from a diverse community of many species in Experiment 1 to genus-specific consortia in Experiment 2 to single isolates of Glomus intraradices and Gigaspora gigantea in Experiment 3. Given these

Fig. 4 Photograph of Schizachyrium that has been grown at ambient (left) or elevated (right) CO₂ in the absence of arbuscular mycorrhizal (AM) fungi (− AMF), with Gigaspora gigantea (Gig marg.) or with Glomus intraradices (Gl. intra.) in Experiment 3.
differences, we find it striking that in both Experiments 2 and 3 the MR of *Schizachyrium* inoculated with Gigasporaceae increased in response to CO₂ enrichment and the MR of *Lespedeza* inoculated with Glomaceae decreased in response to CO₂ enrichment. This finding suggests that Glomaceae and Gigasporaceae may have fundamentally different carbon exchange relationships with their host plants.

Different temperature and light conditions among the three experiments could also explain some of the interexperiment variability. Both of these environmental factors have been shown to influence mycorrhizal development in field experiments (Heinemeyer et al., 2003; Staddon et al., 2003, 2004). Furthermore, Gavito et al. (2003) showed that temperature influences AM function more strongly than CO₂ enrichment. It is not possible to determine whether our interexperiment differences were generated by differences in the species composition of the AM fungal inocula, differences in the greenhouse conditions, or interactions among these biotic and abiotic factors. However, we can safely conclude that when making predictions about mycorrhizal function, it is unwise to extrapolate findings from experiments that are conducted using a narrow range of plant hosts, AM fungi, and environmental conditions.

Plant responses observed in these three individual-scale experiments corroborate those observed in our community-scale companion study (Johnson et al., 2003a), in which all 14 species were grown together in experimental mesocosms at elevated and ambient levels of CO₂ and soil nitrogen. In both studies, plant species varied considerably in their responses to atmospheric CO₂ levels and the presence or absence of AM fungi. Elevated CO₂ often enhanced growth of C₃ species (e.g., *Koeleria*, *Poa*, and *Achillea*) but not C₄ species (e.g., *Bouteloua* and *Schizachyrium*), and plants with coarse roots such as *Lespedeza* benefited greatly from AM fungi, while plants with fine root systems such as *Koeleria* and *Berteroa* often grew more slowly in the presence of AM fungi. Our ecosystem-scale companion study (Wolf et al., 2003) showed that AM fungal species differ in their responses to CO₂ enrichment and plant diversity. In plant monocultures, spore densities of *Glossum clarum* increased while densities of *G. fasciculatum* decreased in response to CO₂ enrichment but these responses disappeared in polycultures composed of 16 plant species. These findings, combined with those of the present study, indicate that species of plants and AM fungi are individualistic in their responses to CO₂ enrichment, and the functioning of pairs of plants and AM fungi also respond differently to CO₂ enrichment. Furthermore, some patterns are sensitive to the scale of the experimental system (i.e. individual, community, or ecosystem) while other patterns are not.

Future studies should be designed to dove-tail multiple experimental scales and account for biotic and abiotic mediators of mycorrhizal function. Efforts should be made to grow naturally co-occurring plant and AM fungal genotypes in their native soil under realistic temperature and light regimes because mycorrhizal function is controlled by interactions among plants, mycorrhizal fungi, and environmental factors, particularly factors related to resource availability (Johnson et al., 1997). This complexity must be considered when making predictions of community responses to atmospheric CO₂ enrichment.

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