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Arbuscular mycorrhiza infection enhances the growth response of *Lolium perenne* to elevated atmospheric pCO_2

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Abstract

Elevated atmospheric pCO_2 increases the C-availability for plants and thus leads to a comparable increase in plant biomass production and nutrient demand. Arbuscular mycorrhizal fungi (AMF) are considered to play an important role in the nutrient uptake of plants as well as to be a significant C-sink. Therefore, an increased colonization of plant roots by AMF is expected under elevated atmospheric pCO_2. To test these hypotheses, *Lolium perenne* L. plants were grown from seeds in a growth chamber in pots containing a silica sand/soil mixture for 9 weeks with and without inoculation with *Glomus intraradices* (Schenck and Smith). The growth response of plants at two different levels of N fertilization (1.5 or 4.5 mM) combined with ambient (35 Pa) and elevated atmospheric pCO_2 (60 Pa) was compared. The inoculation with *G. intraradices*, the elevated atmospheric pCO_2 and the high N fertilization treatment all led to an increased plant biomass production of 16%, 20% and 49%, respectively. AMF colonization and high N fertilization increased the plant growth response to elevated atmospheric pCO_2; the plant growth response to high N fertilization was also increased by AMF colonization. The root/shoot ratio was reduced by high N fertilization or elevated atmospheric pCO_2, but was not affected by AMF colonization. The unchanged specific leaf area indicated that if AMF colonization represented an increased C-sink, this was fully covered by the plant. Elevated atmospheric pCO_2 strongly increased AMF colonization (60%) while the high N fertilization had a slightly negative effect. AMF colonization neither improved the N nor P nutrition status, but led to an improved total P uptake. The results underline the importance of AMF for the response of grassland ecosystems to elevated atmospheric pCO_2.

Key words: Elevated carbon dioxide, mycorrhiza, ryegrass.

Introduction

The atmospheric partial pressure of carbon dioxide (pCO_2) has increased continuously over the past decades, mainly as a result of the consumption of fossil fuel and changes in land use; it is expected that the atmospheric pCO_2 will have risen from the current 360 p.p.m. to about 500 p.p.m. within the next decade (Houghton *et al.*, 1995). At present, photosynthesis of C_3 plants is limited by the availability of atmospheric pCO_2 (Drake *et al.*, 1997). Experiments have shown that the growth and development of plants are often stimulated by an increase in atmospheric pCO_2, especially at high nutrient availability. It has been found that the CO_2 effect depended greatly on the growth conditions, the inter- and intraspecific competition, the plant growth stage, and the duration that plants were exposed to elevated atmospheric pCO_2 (Poorter *et al.*, 1996; Lüscher *et al.*, 1998).

In field experiments conducted in fertile soil, grassland legumes showed a stronger increase in yield at elevated atmospheric pCO_2 than grasses (Hebeisen *et al.*, 1997; Lüscher *et al.*, 1998) and there is evidence that the growth response to elevated atmospheric pCO_2 of grasses grown in the field is particularly limited by nitrogen (Soussana *et al.*, 1996; Zanetti *et al.*, 1997; Fischer *et al.*, 1997; Isopp *et al.*, 2000; Daepp *et al.*, 2001). A stimulation of plant growth by elevated atmospheric pCO_2, therefore, entails a matching increase in the demand of plants for mineral nutrients.

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Located at the plant–soil interface, arbuscular mycorrhizal fungi (AMF) are a potentially important link in the chain response of ecosystems to elevated atmospheric pCO_2 (Fitter *et al*., 2000; Staddon *et al*., 1999*a*). *Glomus intraradices* has recently been found to be one of the very abundant AMF in the Swiss FACE experiment (H Gamper and A Leuchtmann, personal communication). As obligate symbionts, these fungi depend on the plant as C source (Smith and Read, 1997) and thus form an additional C-sink significant enough to enhance carbon fixation under elevated atmospheric pCO_2 (Poorter *et al*., 1996) and to alleviate the photosynthetic down-regulation (Staddon *et al*., 1999*b*). On the other hand, the external hyphae of AMF extend into the soil matrix beyond the root depletion zone and considerably improve the plants' exploration of the soil for nutrients (i.e. in fine pores), thus promoting the availability and the uptake of mineral nutrients (Powell, 1975; Newsham *et al*., 1995). The extent of AMF colonization of plant roots, however, depends on the developmental stage of plants as well as on their nutrient status; high N or P fertilization can inhibit AMF infection (Klironomos *et al*., 1996, 1997; Hodge *et al*., 1999).

Thus, under elevated atmospheric pCO_2, AMF colonization could be enhanced due to (i) an increased C-availability from the plant and (ii) an increased demand for nutrients by the plant. Consequently, the CO_2 and N growth response of plants colonized by AMF is expected to be stronger compared to non-mycorrhizal plants. To test these hypotheses and therefore to understand the possible role AMF play in grassland ecosystems under elevated atmospheric pCO_2, a growth chamber experiment with *Lolium perenne* in a factorial design with AMF inoculation, N fertilization and atmospheric pCO_2 was conducted.

Materials and methods

Preparation of mycorrhizal inoculum

To produce mycorrhizal inoculum, sterilized and pregerminated seeds of *Plantago lanceolata* L. (three seeds per pot) and *Zea mays* L. (one seed per pot) were sown in pots containing a 3 : 1 mixture of sterilized sand (0.7–3 mm) and autoclaved soil (121 °C, 20 min). The autoclaved soil was supplemented with a bacterial mycorrhiza-free filtrate (10 ml l^{-1} soil). The filtrate was prepared by mixing 100 g fresh soil with 300 ml of sterile water. After sedimentation of the soil, the supernatant was filtered twice (W1, Whatman, Maidstone, UK). The prepared sand/soil mixture was incubated at room temperature for 23 d before use.

The substrate containing inoculum of the arbuscular mycorrhizal fungus *Glomus intraradices* (BEG 75, lot 1999; Federal Research Station, Wädenswil, Switzerland) was added to each pot; no inoculation was used for non-mycorrhizal pots. Plants were watered daily and fertilized once a month with a commercial nutrient solution (Type A; Hauert, Grossaffoltern, Switzerland). After 72 d, roots of the plants were taken and cut into 1 cm pieces, mixed with their respective pot content and used as inoculum for the experiment. This way, equivalent mycorrhizal and non-mycorrhizal root/soil material was obtained for later inoculation of the experimental plants.

Plant material and growth conditions

Seedlings (one per pot) of perennial ryegrass (*Lolium perenne* L. cv. Bastion) were cultivated in sterile pots (18 cm height, 10 cm diameter) containing a 9 : 1 mixture of sterilized silica sand (diameter 0.7–1.2 mm) and bacterial filtrate-inoculated soil prepared as described above. To each pot, 50 ml of mycorrhizal or non-mycorrhizal inoculum was added and then covered with a 2 cm layer of the sand/soil mixture. Before planting, the seeds were surface-sterilized in 70% (v/v) ethanol for 5 min and pregerminated on water agar (15 g l^{-1}; Fluka Chemie AG, Buchs, Switzerland) at room temperature for 8 d. Plants were cultivated in growth chambers (PGR-15, Conviron Instruments Co., Winnipeg, Canada) one under ambient [35 Pa] and one elevated [60 Pa] atmospheric partial pressure of carbon dioxide [pCO_2]) at 20/15 °C (day/night) and a relative humidity of 80/90%. To prevent the effects of CO_2 to be confounded by chambers, pots and CO_2 treatments were rotated between the chambers at weekly intervals. The photoperiod was set with periods of 16/8 h (day/night) and a photosynthetically active photon flux density of 560 μmol m^{-2} s^{-1}. Plants were irrigated twice a day with a commercial nutrient solution (Hauert, Grossaffoltern, Switzerland) that was diluted ten times more than recommended by the manufacturer. The nutrient solution was supplemented with NH_4NO_3 to final concentrations of 1.5 mM and 4.5 mM N, respectively. Once a week, all pots were rinsed with deionized water to prevent salt accumulation. Pots were rearranged within each growth chamber once per week.

Plants supplemented with non-mycorrhizal inoculum (myc−) were never found to develop AMF colonization (50% of all myc− pots were checked).

Plant sampling

Half of the plants were harvested 42 d and the other half 60 d after sowing. The roots of the unearthed plants were washed immediately and the plants were divided into root, shoot, leaf, and necrotic material and the fresh weight of all fractions was recorded. Leaf number and area (Model LI-3000 A, Li-Cor Inc., Licoln, NE, USA) were determined. For determining mycorrhizal colonization, a sample (approximately 1 g fresh mass) was taken over the whole root length and stored in KOH solution (10% w/w) for later staining. All plant fractions were oven-dried at 65 °C for 48 h and their dry mass recorded.

Chemical analyses

Chemical analyses were carried out with the plant material harvested at the final harvest (60 d after sowing). Aliquots of the dried plant fractions were first ground with a sample mill (Cyclotec 1093, Tectator, Sweden) followed by grinding with a ball mill (type MM2; Retsch GmbH & CO, Haan, Germany). Total N content of the above-ground material was determined from 2 mg aliquots sealed in tin caps (0.04 ml; Brechbühler AG, Schlieren, Switzerland) using an Elemental Analyser (Europa Scientific, Cambridge, UK). Total N content of the root material was determined from 50 mg ground material sealed in tin caps (Leco Instruments GmbH, Mönchengladbach, Germany) using an elemental analyser (Leco CHN 1000, Leco Instruments GmbH, Mönchengladbach, Germany).

Determination of fungal colonization

Samples of washed roots were macerated in KOH solution (10%, w/w) for 1 h at 90 °C in a water bath. After rinsing with tap water, the samples were acidified in HCl (1%) for 1 h and rinsed again with tap water. Thereafter, the samples were stained in a hot water bath at 90 °C for 1 h in a solution containing lactic acid, glycerol and distilled water (1 : 1 : 1, by vol.) supplemented with trypan blue and methylene-blue (both 0.05% w/w; Fluka AG, Buchs Switzerland). After staining, the samples were washed in deionized water and stored in water at 4 °C in the dark.

The extent of mycorrhizal colonization was determined with the 'Gridline intersect method' (Ambler and Young, 1977; Giovannetti and Mosse, 1980) using a stereo microscope at 50× magnification. One hundred intersections were investigated out of a subsample of root pieces of one plant and the presence of at least one mycorrhizal hypha was counted as infection.

Statistical analysis

The experiment was set up in a fully randomized design with eight replicates (pots) and the statistical analyses were performed using SAS (Statistical Analysis System, SAS Institute Inc. Carry, North Carolina, USA). Analyses of variance were carried out using the General Linear Model procedure using $P < 0.05$ as the critical level of significance (see also Table 1). Normal distribution of the data and homogeneity of variance were tested with the univariate procedure of SAS and with residual versus predicted plots.

Results

Effects of AMF colonization, N fertilization and elevated atmospheric pCO_2 *on plant growth*

All three treatments significantly increased the total plant biomass (pCO_2, N, AMF; $P < 0.0001$) with AMF colonization (16%) causing the weakest and high N fertilization (49%) the strongest increase, while the increase at elevated atmospheric pCO_2 (20%) was comparable to that with AMF colonization (Table 1, 2). Colonization by AMF doubled the increase in plant biomass under elevated atmospheric pCO_2 as compared to the increase without AMF colonization (myc×pCO_2, $P < 0.05$). Likewise, AMF colonization significantly raised the plant biomass increase at high N fertilization from 40% to 58% (myc×N, $P < 0.01$).

The root : shoot ratio (Table 2) was significantly reduced at high N fertilization (20%; $P < 0.0001$) whereas elevated atmospheric pCO_2 caused only a slight reduction (11%) and only at the first harvest ($P < 0.01$). AMF colonization had no effect on the root : shoot ratio (AMF; ns).

The specific leaf area (Table 2) was unaffected by AMF colonization (AMF, ns). At elevated atmospheric pCO_2

Table 1. *Significance probabilities resulting from the analysis of variance of growth parameters of* L. perenne *subjected to treatments of two different levels of atmospheric* pCO_2 *(35 or 60 Pa), N fertilization (1.5 mM and 4.5 mM) and AMF colonization (myc+, myc−)*

Analysis was made over both harvests for Tables 2 and 3 and for the last harvest for Table 4. Three-way interactions were never statistically significant, thus data not shown. Levels of significance were as follows: ns = $P > 0.5$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

	pCO_2	N	AMF	pCO_2×N	pCO_2×AMF	N×AMF
Plant biomass	****	****	****	***	*	**
Root/shoot ratio	*	****	ns	ns	ns	ns
Specific leaf area	****	****	ns	ns	ns	ns
AMF colonization	****	ns	–	ns	–	–
Shoot N concentration	****	****	ns	ns	ns	ns
Root N concentration	*	****	**	***	ns	ns
Shoot P concentration	****	****	**	***	ns	ns
Root P concentration	ns	****	ns	ns	ns	ns
Total plant P content	*	***	****	****	*	*

Table 2. *Effect of N fertilization (1.5 or 4.5 mM), AMF colonization (myc+, myc−) and atmospheric* pCO_2*(35 or 60 Pa) on total plant biomass (g DW plant*$^{-1}$*), root : shoot ratio (g g*$^{-1}$*) and specific leaf area (cm*^{2} *g*$^{-1}$*) of* Lolium perenne*, 42 d (harvest 1) and 60 d (harvest 2) after sowing*

Means of eight replicates and SE are shown.

Growth parameter	Harvest	1.5 mM N				4.5 mM N				SE
		myc−		myc+		myc−		myc+		
		35 Pa CO_2	60 Pa CO_2	35 Pa CO_2	60 Pa CO_2	35 Pa CO_2	60 Pa CO_2	35 Pa CO_2	60 Pa CO_2	
Total plant biomass	1	2.46	2.90	2.73	3.38	3.31	3.62	3.41	4.45	0.32
	2	5.71	6.28	6.23	6.40	8.02	9.38	8.99	12.70	0.32
Root : shoot	1	0.75	0.71	0.78	0.70	0.60	0.47	0.59	0.56	0.044
	2	0.82	0.86	0.85	0.77	0.64	0.52	0.64	0.63	0.044
Specific leaf area	1	96.2	78.2	107.1	82.0	132.1	106.6	120.8	103.1	5.27
	2	109.1	84.3	102.9	72.2	133.4	87.9	123.5	100.6	5.27

it was significantly reduced by 23% (pCO_2, $P < 0.0001$), whereas high N fertilization clearly increased the specific leaf area by 24% (N, $P < 0.0001$).

The effect of elevated atmospheric pCO_2 *and N fertilization on AMF colonization*

The extent of AMF colonization (Tables 1, 3) was significantly enhanced (56%) under elevated atmospheric pCO_2 (pCO_2, $P < 0.0001$), whereas high N fertilization tended to have a negative effect (15%) on the AMF colonization (N, ns).

Effects of elevated atmospheric pCO_2 *and N fertilization on N and P concentration of shoot and root and on the total plant P content*

AMF colonization had no effect on the N concentration (Tables 1, 4) in shoot or root of *L. perenne* (AMF, ns). Elevated atmospheric pCO_2 lowered the N concentration in the shoot to a greater extent (23%; N, $P < 0.0001$) than in the roots (10%; N, $P < 0.05$), while high N fertilization led to a significantly higher increase in the N concentration in the shoot (82%; N, $P < 0.0001$) than in the roots (47%; $P < 0.0001$).

The P concentration (Table 4) in the shoot was reduced by AMF colonization (8%; AMF, $P < 0.01$) and elevated atmospheric pCO_2 (14%; pCO_2, $P < 0.0001$) whereas the P concentration in the roots remained unaffected by both treatments (AMF, pCO_2, ns). N fertilization had the strongest effect on the P concentration of the plants and led to a similar decrease of 28% in the shoot (N, $P < 0.0001$) and 26% in the roots (N, $P < 0.0001$). The decrease in the shoot P concentration due to N fertilization was significantly decreased from 33% at ambient pCO_2 to 21% at elevated pCO_2 ($pCO_2 \times$N, $P < 0.001$) while the effect of N fertilization on the P concentration of roots was unaffected by elevated pCO_2 ($pCO_2 \times$N, ns).

The total plant P content was increased by N fertilization, AMF colonization and elevated atmospheric pCO_2 by 19%, 10% and 7% (Tables 1, 4). Colonization by AMF affected the CO_2 response of P uptake positively ($pCO_2 \times$AMF, $P < 0.05$). The total plant P content was increased by AMF, but mostly under high N fertilization (N$\times$AMF, $P < 0.05$). Elevated atmospheric pCO_2 led to a slight reduction in P uptake under low N fertilization, but to a strong increase under high N fertilization ($pCO_2 \times$N, $P < 0.0001$). AMF colonization was the only factor that always led to an increased P uptake.

Discussion

The results of these experiments clearly demonstrated that all three treatments, AMF colonization, elevated atmospheric pCO_2 and N fertilization, promoted plant growth in the *G. intraradices*/*L. perenne* symbiosis. The significantly positive $pCO_2 \times$AMF interaction is particularly striking and suggests an additive effect of the two treatments. This is in contrast to the results with *Plantago lanceolata* (Staddon *et al*., 1999*b*). However, interspecific

Table 3. *Effect of N fertilization (1.5 or 4.5 mM) and atmospheric* pCO_2 *(35 or 60 Pa) on the extent of AM colonization (percentage root length infected) of roots of* Lolium perenne*, 42 d (harvest 1) and 60 d (harvest 2) after sowing*

Means of eight replicates and SE are shown.

Parameter	Harvest	1.5 mM N		4.5 mM N		SE
		35 Pa CO_2	60 Pa CO_2	35 Pa CO_2	60 Pa CO_2	
Percentage root colonization	1	12.00	23.75	11.25	20.63	2.29
	2	17.63	25.75	16.38	19.00	2.29

Table 4. *Effect of N fertilization (1.5 or 4.5 mM), AMF colonization (myc+, myc–) and atmospheric* pCO_2 *(35 or 60 Pa) on N concentration (mg N* g^{-1}*DW), P concentration (mg P* g^{-1}*DW) of shoots and roots, and total plant P content (mg plant*$^{-1}$*) of* Lolium perenne *(60 d after sowing)*

Means of eight replicates and SE are shown.

Parameter	1.5 mM N				4.5 mM N				SE
	myc–		myc+		myc–		myc+		
	35 Pa CO_2	60 Pa CO_2	35 Pa CO_2	60 Pa CO_2	35 Pa CO_2	60 Pa CO_2	35 Pa CO_2	60 Pa CO_2	
N concentration of shoot	1.16	0.86	1.17	0.83	2.04	1.69	2.04	1.57	0.05
N concentration of root	0.89	0.73	0.88	0.86	1.29	1.20	1.31	1.15	0.06
P concentration of shoot	2.38	1.86	2.16	1.78	1.58	1.51	1.48	1.36	0.06
P concentration of root	1.89	1.68	1.70	1.89	1.44	1.43	1.46	1.40	0.09
Total plant P content	12.16	11.00	12.38	11.72	12.17	13.76	13.14	17.16	0.50

differences in the response of plants to elevated atmospheric pCO_2 and AMF colonization as well as in the response of AMF species to elevated atmospheric pCO_2 are well known (Monz *et al*., 1994; Rillig *et al*., 1998*a*; Klironomos *et al*., 1998). By contrast to the stimulatory effect of AMF colonization on the CO_2 response of plant biomass production found here, an increased CO_2 response at high N fertilization had already been shown in an earlier experiment with *Lolium perenne* (Daepp *et al*., 2001).

The significant increase in plant biomass and the changes in the nutrient concentration of the plant tissue observed in these experiments, are consistent with the model of a positive feedback loop as suggested earlier (Fitter *et al*., 2000). The increased C fixation due to elevated atmospheric pCO_2 promotes the growth of AMF which improve the nutritional status of the host plant. This, in turn, leads to a better ability of the plant to respond to elevated atmospheric pCO_2. Such a positive feedback loop would eventually be constrained by other deficiencies such as water supply or temperature.

The fact that AMF colonization was roughly double as high under elevated atmospheric pCO_2 compared to ambient pCO_2 at both harvests, while plant biomass production was increased to a much lesser extent, suggests that the CO_2-mediated stimulation of AMF colonization was not simply the result of faster plant development (Staddon and Fitter, 1998); though, this can never be entirely excluded. However, the fact that under field conditions (after 7 years of growth in the Swiss FACE) the same CO_2 stimulation of AMF colonization was observed (data not shown) would support the present result.

The increase in the total plant biomass observed in this experiment was mostly due to an increase in the shoot biomass which represents the harvested yield of *L. perenne*. Although in similar experiments with *Artemisia tridentata*, the colonization by *Glomus* spp. was shown to favour shoot growth to a greater extent than other mycorrhizal species, root growth was only enhanced at elevated atmospheric pCO_2 (Klironomos *et al*., 1998). No effect on the root/shoot ratio was observed in this experiment due to AMF colonization. Only high N fertilization and, to a smaller extent, elevated atmospheric pCO_2 promoted shoot growth above that of root growth. A comparison of the specific leaf areas revealed distinct morphological differences in the response to high N or high pCO_2. Whereas high N fertilization clearly increased the specific leaf area, thus investing in leaf area for photosynthesis, elevated atmospheric pCO_2 led to a significant decrease in the leaf area per unit dry matter suggesting an accumulation of non-structural carbohydrates. This may result from a persistent C-sink limitation in the plants, especially with low levels of N supply (Fischer *et al*., 1997; Isopp *et al*., 2000) and/or from a lack of acclimation of photosynthesis to elevated atmospheric pCO_2 (Drake *et al*., 1997).

The N concentration of shoots and roots were most affected by N fertilization whereas AMF colonization showed no effect. The effect of high N fertilization on the N concentration of the shoot was opposed to that of elevated atmospheric pCO_2, but both treatments increased the N concentration of the roots although the increase due to high N fertilization was 5-fold that due to elevated atmospheric pCO_2. Similar to the N concentration, the P concentration of shoot and roots were most influenced by the high N fertilization treatment which caused a similar reduction in both tissues although the reduction was clearly attenuated under elevated atmospheric pCO_2. Contrary to other results (Staddon *et al*., 1999*b*), elevated atmospheric pCO_2 caused a significant reduction in the P concentration of the shoot, whereas the concentration in the roots remained unaffected, consistent with Staddon *et al*'s results. The effect of AMF on the P concentration of the shoot and roots was comparable to that of elevated atmospheric pCO_2, although it was less pronounced. The decrease in the P concentration of the shoot is again in line with the positive feed-back loop proposed earlier (Fitter *et al*., 2000) in which the increased availability of C for metabolic processes enhances AMF growth, leading to increased P metabolism and photosynthetic rates in leaves. However, it has to be emphasized that, given the large increase in biomass production due to AMF infection, total P uptake per plant was increased.

Consistent with similar experiments conducted with *Bromus hordeaceus* (Rillig *et al*., 1998*b*) the increased C availability for plants under elevated atmospheric pCO_2, clearly promoted the colonization of the *L. perenne* roots by *Glomus intraradices* which, as an obligate symbiont, relies entirely on the plant as a source for carbon. Nevertheless, the increased AMF colonization did not result in a detectable C drain of the plant (unchanged specific leaf area), indicating either that (i) the rate of photosynthesis was adjusted to the increased C-sink prompted by increased AMF colonization, that (ii) increased AMF colonization was counteracted by reduced external fungal mass/activity or that (iii) an increased C-sink was very small; the latter possibility may be supported by a relatively low infection rate in the present experiment. Likewise, in an earlier study (Gavito *et al*., 2000), AMF had no effect on the photosynthetic rate in pea, also indicating that the AMF-prompted C-sink was relatively small. Thus, it is not clear whether the promotion of AMF growth was directly due to an increased C availability or indirectly related to an increased nutrient demand of the faster growing plants under elevated atmospheric pCO_2 (Staddon *et al*., 1999*b*).

Some other reports state that root colonization by AMF is strongly influenced by soil nutrient availability

(Klironomos *et al*., 1996, 1997; Rillig *et al*., 1998*b*). The fact that high N fertilization did not lead to a significant reduction in AMF colonization argues against a regulatory mechanism induced by mineral N availability in the present experiment. However, with respect to P, a large increase in uptake was observed under elevated atmospheric pCO_2, high N fertilization and AMF colonization, suggesting that the level of AMF colonization may have been controlled by the relative availability of P or other micro-nutrients. This may have overridden a possible down-regulation of AMF colonization caused by high N fertilization and thus confounded a judgement of the effect of nutrient supply on AMF colonization.

Conclusion

The significant increase in plant biomass observed in *L. perenne* upon inoculation with the inoculum of *G. intraradices* used here, particularly under elevated atmospheric pCO_2 and high N fertilization, clearly suggests that AMF are beneficial to the plants, particularly if growth conditions improve, but the availability of certain nutrients do not. Furthermore, the results underline the importance AMF may play in the response of grassland ecosystems to elevated atmospheric pCO_2.

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