Oecologia (1998) 117:496–503 

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# Increased allocation to external hyphae of arbuscular mycorrhizal fungi under $CO_2$ enrichment

Received: 28 March 1998 / Accepted: 27 August 1998

**Abstract** *Prunella vulgaris* was inoculated with different arbuscular mycorrhizal fungi (AMF) and grown at two concentrations of $CO_2$ (ambient, 350 μl $l^{-1}$, and elevated, 600 μl $l^{-1}$) to test whether a plants response to elevated $CO_2$ is dependent on the species of AMF colonizing the roots. Using compartments accessible only to AMF hyphae but not to roots, we also tested whether elevated $CO_2$ affects the growth of external AMF hyphae. Plant biomass was significantly greater at elevated than at ambient $CO_2$; the biomass of the root system, for example, increased by a factor of 2. The colonization of AMF inside the root remained constant, indicating that the total AMF inside the root system also increased by a factor of 2. The length of external AMF hyphae at elevated $CO_2$ was up to 5 times that at ambient $CO_2$, indicating that elevated $CO_2$ promoted allocation of AMF biomass to the external hyphae. The concentration and content of phosphorus in the stolons differed significantly between ambient and elevated $CO_2$ but this resulted in either an increase or a decrease, according to which AMF isolate occupied the roots. We hypothesized that an increase in external hyphal growth at elevated $CO_2$ would result in increased P acquistion by the plant. To test this we supplied phosphorus, in a compartment only accessible to AMF hyphae. Plants did not acquire more phosphorus at elevated $CO_2$ when phosphorus was added to this compartment. Large increases in AMF hyphal growth could, however, play a significant role in the movement of fixed carbon to the soil and increase soil aggregation.

**Key words** Arbuscular mycorrhizal symbiosis · Belowground respiration · Elevated $CO_2$ · Soil aggregation · Soil carbon

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## Introduction

There is currently great interest in how natural ecosystems respond to rising atmospheric $CO_2$ concentrations. Many plant species show higher rates of photosynthesis at elevated than at ambient $CO_2$ and accumulate more biomass, particularly non-structural carbohydrates (Stitt 1991). Despite this, in natural ecosystems large growth responses of plants to elevated $CO_2$ are seldom observed (Díaz et al. 1993; Leadley and Stöcklin 1995; Wolfenden and Diggle 1995; Leadley and Körner 1996) and this has partly been explained by mineral nutrient limitation (Zangerl and Bazzaz 1984; Díaz et al. 1993). Even so, exposure to elevated $CO_2$ can still lead to a great increase in rates of carbon uptake by ecosystems (Drake and Leadley 1991; Stocker et al. 1997). A significant portion of this additional fixed carbon is re-released to the soil as $CO_2$ as a result of increased belowground respiration. This has been attributed to an increase in root respiration (Hungate et al. 1997). However, it could also be connected to increased respiration of rhizosphere micro-organisms, in particular, plant symbionts.

In grasslands, the roots of most plants form symbiotic associations with arbuscular mycorrhizal fungi (AMF; Class Zygomycetes, Order Glomales) (Smith and Read 1997). AMF grow at the expense of carbohydrates supplied by the plant, and are able to acquire essential nutrients such as phosphorus from the soil, transporting them to the plant through their hyphae.

The effect of elevated $CO_2$ on the AM symbiosis is of interest because any increase in host carbohydrate supply as a result of elevated $CO_2$ could promote the growth of external AMF hyphae, thus increasing the volume of soil from which nutrients could potentially be acquired. This would also result in an additional carbon flux into the soil, in terms of biomass and $CO_2$ released as a product of increased AMF hyphal respiration.

In ectomycorrhizal systems, increased growth of external hyphae has indeed been observed at elevated $CO_2$

(Ineichen et al. 1995). Little is known about AMF responses to elevated $CO_2$, although it has been observed that elevated $CO_2$ can cause increased growth of native AMF inside the roots of plants from annual grasslands in California, north American prairies and calcareous grasslands in Switzerland (Monz et al. 1994; Sanders 1996; Rillig et al. in press). One study has linked plant response to elevated $CO_2$ with the mycorrhizal status of the plants. Díaz et al. (1993) observed a detrimental effect of $CO_2$ on the growth of non-mycorrhizal plants while mycorrhizal plants were either unaffected by elevated $CO_2$ or grew larger than at ambient $CO_2$. They hypothesized that non-mycorrhizal plants increased their carbon exudation into the soil, causing an expansion of the non-symbiotic soil microflora and a concomitant increase in microbial nutrient sequestration, lowering nutrient availability for the plant. Sanders (1996) proposed an alternative hypothesis; namely that external AMF hyphae originating in the roots of the coexisting plant species could be exploiting the soil more efficiently at elevated $CO_2$, thereby limiting nutrient availability to non-mycorrhizal plants.

It has recently been shown that different species of AMF which co-occur in calcareous grasslands differentially affect the clonal growth of *Prunella vulgaris* L., *P. grandiflora* (L.) Scholler and the growth of several other calcareous grassland species (Streitwolf-Engel et al. 1997; van der Heijden 1998). Differential effects of elevated $CO_2$ on different AMF species could, therefore, also potentially determine a plant's response to elevated $CO_2$.

Here we describe experiments designed to test directly whether: (1) elevated atmospheric $CO_2$ promotes the growth of AMF in the soil; (2) this leads to improved phosphorus acquisition; and (3) the response of mycorrhizal plants to elevated $CO_2$ may be determined by the response of the particular AMF species colonizing its roots.

## Methods

### Biological material

All biological material (seeds of *P. vulgaris* and AMF isolates) originated from a study site, Nenzlinger Weide, a calcareous grassland, which is described elsewhere (Stocker et al. 1997). *P. vulgaris* seeds were collected in the field from many randomly selected individuals and mixed. We therefore assume that the seeds used for the experiment are not genetically uniform. Three cultures of AMF from Nenzlinger Weide were established and are described in detail elsewhere (Streitwolf-Engel et al. 1997); namely, *Glomus geosporum* (Nicol. & Gerd.) Walker (BEG 18), *Glomus* sp. (BEG 19), morphologically similar to *G. constrictum* Trappe. and to *G. botryoides* Rothwell & Victor, and *Glomus* sp. (isolate Basle Pi), similar in morphology to *G. microcarpum* Tulasne & Tulasne. All AMF cultures are maintained in the Botanical Institute of the University of Basle. Further information regarding the availability of these cultures can be accessed through the *Banque Européen des Glomales* (http://biont.ukc.ac.uk/beg/asp/default.asp).

### Plant growth conditions

#### *Experiment 1*

*P. vulgaris* seeds were planted in a tray containing a sterile mixture of quartz sand and loamy soil (1:1 v:v) on 29 June 1994. After 4 weeks, 40 seedlings were transplanted singly into pots in 750 $cm^3$ of an autoclaved calcareous soil and quartz sand mixture (1:1 v:v). Seedlings of the same size were selected for transplantation. A cylindrical compartment (14 cm long × 8.3 cm diameter) was attached to the side of the pot and the compartment was filled with sterilized quartz sand. Analysis of the sand revealed that there was no detectable phosphorus. It was separated from the pot by a 30-μm nylon mesh which could not be penetrated by *P. vulgaris* roots but which could be penetrated by external AMF hyphae. Hereafter, this compartment is called the hyphal compartment.

Twenty plants were kept in each of two controlled climate chambers with a simulated summer climate of 25 °C day (16 h) and 16°C night (8 h), with natural lighting, supplemented by two 1000-W daylight halogen lights when the natural light level fell below 180 μmol $m^{-2}$ $s^{-1}$. Chambers contained an atmosphere of either 350 μl $l^{-1}$ or 600 μl $l^{-1}$ $CO_2$ concentration. Pots and $CO_2$ treatments were swapped between chambers and randomized every 2 weeks. Plants were watered every 2 days with 50 ml water and no nutrients were added.

Thirty plants were inoculated with 5 ml of one of the AMF cultures, or, for the non-AMF treatment, given 5 ml of an autoclaved mixture of the three inocula. Each pot received a sieved (32 μm sieve) washing of a mixture of field soil and all three inocula (Koide and Li 1989) containing natural bacteria and fungi but no AMF propagules. Plants were harvested on 30 November 1994, 18 weeks after AMF and $CO_2$ treatments were started.

#### *Experiment 2*

Seeds of *P. vulgaris* were germinated and seedlings were transplanted singly into 40 pots on 11 April 1996. Plants were grown in the same substrate, in the same type of pots and environmental conditions (temperature, light, and $CO_2$ concentrations) as those described in experiment 1, except where indicated below. Only two AMF isolates, *Glomus* sp. (BEG 19) and *Glomus* sp. (isolate Basle Pi) were used to inoculate the seedlings and there was no non-AMF treatment. Four extra pots which were not inoculated with AMF were set up in order to estimate the growth of non-AMF hyphae in the hyphal compartment. Each pot was watered every 2 days with 30 ml of deionized water. From 6 May, all pots were given 25 ml of 12.5% of full strength of Hoaglands nutrient solution, lacking phosphorus, every 2 weeks. The concentration of K in the Hoaglands solution was adjusted with KCl. Ten weeks after transplantation, half the hyphal compartments received 10 ml of $KH_2PO_4$ solution, equivalent to 100% of the concentration in full strength Hoaglands solution. The $KH_2PO_4$ solution was injected into the hyphal compartment at four positions, equidistant along the length of the compartment. This phosphorus treatment was repeated every 2 weeks until the plants were harvested, 20 weeks after transplantation.

### Measurement of plants and fungi

During the course of the experiments, leaf area, leaf number and stolon branch number were measured every 2 weeks from the start of the experiment (day following transplantation) until the plants were harvested. At harvest, leaf and stolon branch number of each plant were recorded. The roots were washed to remove soil and fresh weight was determined. A small subsample of each root system of a known fresh weight was stained with trypan blue (Phillips and Hayman 1970) and AMF colonization was estimated by a line intersection method (McGonigle et al. 1990). All other plant material was weighed, dried, weighed again and ground. The total dry weight of the roots was estimated by determining the fresh

weight:dry weight ratio of the main root sample and adding the expected dry weight of the subsample, calculated on the basis of this ratio. Phosphorus analysis was carried out on ashed stolon, root and leaf tissue using the molybdate blue reaction (Watanabe and Olsen 1965).

External AMF hyphal length was estimated at two positions in the hyphal compartment in experiment 1. Samples were harvested at a distance of 1–2 cm and 13–14 cm from the nylon mesh using a manifold filtration technique (Jakobsen et al. 1992). A third sample point, 6.5–7.0 cm from the nylon mesh, was made in experiment 2. Samples were stained with trypan blue and hyphal length estimated by a gridline intersection method under 200 × magnification. In experiment 1, the diameter of the hypha was also measured where it crossed an intersection of the grid so that hyphal length could be calculated separately for four different size classes of hyphal diameter. A background control count of hyphal length was made from all pots of the non-AMF treatment in experiment 1 and from the additional pots with no AMF in experiment 2. This value, albeit small, was subtracted from the mean values of hyphal length from all the AMF before analysis. Hyphal lengths were also used to estimate hyphal biomass following Jakobsen et al. (1992).

### Experimental design and statistical analysis

Pots were arranged in the growth chambers in a block design where each growth chamber contained an equal number of replicates of each of the AMF treatments (four in experiment 1 and two in experiment 2) and each block contained one replicate of each AMF treatment or combination of AMF treatment and phosphorus treatment. Position in the block was randomized and each week when pots were switched between chambers the blocks and the pots within blocks were also randomized.

Data from experiment 1 were analysed by ANOVA with AMF treatment (four levels) and $CO_2$ treatment (two levels), as main factors, with five replicates for each treatment combination. In experiment 2, the ANOVA included AMF treatment (two levels), phosphorus treatment (two levels) and $CO_2$ treatment (two levels), as main factors with five replicates of each treatment combination. ANOVAs of all plant growth and fungal growth parameters were performed using JMP (SAS Institute Inc., N.C., USA). All proportional data were arcsin square root transformed before ANOVA and data which did not exhibit a normal distribution were transformed accordingly (Zar 1984). For each variable, Bartlett's test was used to determine whether variances were equal between treatments and ANOVA was only performed on variables with equal variance. Means comparisons were carried out using the least significant difference (LSD) test and were only performed where a significant $CO_2$, phosphorus or AMF treatment effect was shown by ANOVA. In the case of AMF treatment effects in experiment 1, the data were analysed both with and without the non-AMF treatment. ANOVA was performed without the non-AMF treatment to establish whether significant differences were due to the different AMF isolates or to a AMF versus non-AMF effect. This was necessary because for many variables the values for non-mycorrhizal plants were very low or unmeasurable. Where a variable was unmeasurable on the majority of non-mycorrhizal plants, the non-AMF treatment was entirely removed from the analysis.

## Results

### Main effects of AMF treatment

Hyphal lengths in the hyphal compartment of the non-AMF treatment were 0.085 m $g^{-1}$ (SE ± 0.023) and 0.033 m $g^{-1}$ (SE ± 0.014), respectively, in experiment 1 at 1–2 cm and 13–14 cm distances from the nearest roots, and 0.092 m $g^{-1}$ (SE ± 0.025), 0.081 m $g^{-1}$ (SE ± 0.019) and 0.021 m $g^{-1}$ (SE ± 0.012), respectively, in experiment 2 at 1–2 cm, 6.5–7.0 cm and 13–14 cm distances from the nearest roots. External hyphal length differed between isolates in experiment 1 at a distance of 1–2 cm from the nearest root (Fig. 1a). External hyphal length of *Glomus* sp. (Basle Pi) was significantly greater than that of *Glomus* sp. (BEG 19) and the differences among the isolates were mainly due to differences in the length of hyphae of 2.5 and 5.0 μm diameter. *Glomus* sp. (Basle Pi) also produced thicker external hyphae of 7.5 and 10 μm diameter, which were not formed by the other two isolates. At a distance of 13–14 cm from the nearest roots hyphal length did not

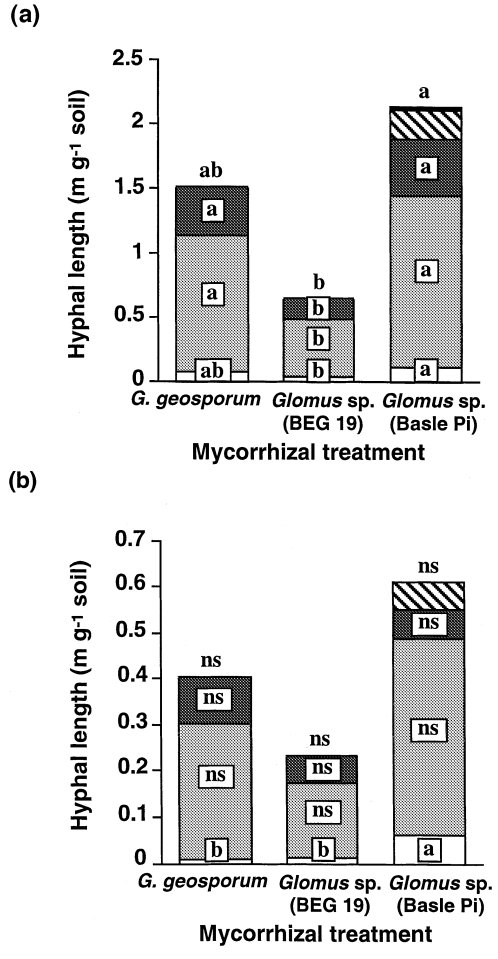


**Fig. 1** Mean external hyphal length (m $g^{-1}$ soil) of three arbuscular mycorrhizal fungi (AMF) isolates harvested from a root-free chamber in experiment 1 **a** 1–2 cm and **b** 13–14 cm from the nearest root. *Shading* represents size classes of hyphae as measured by hyphal diameter. 10 μm, 7.5 μm, 5.0 μm, 2.5 μm, 1.25 μm. Different *letters above bars* indicate a significant difference ($P \leq 0.05$) according to the LSD test for mean hyphal length averaged over all size classes. Different *letters in squares within bars* indicate a significant difference ($P \leq 0.05$) according to the LSD test for each size class of hyphae. *F*-ratios of ANOVA, with total hyphal length and size classes 5.0, 2.5 and 1.25 μm diameter (1–2 cm from root) and hyphal length of size class 1.25 μm (13–14 cm from root) as dependent variables, were $F_{(1,24)} = 6.17$ ($P \leq 0.007$), $F_{(1,24)} = 4.34$ ($P \leq 0.024$), $F_{(1,24)} = 4.82$ ($P \leq 0.017$), $F_{(1,24)} = 6.17$ ($P \leq 0.007$), $F_{(1,24)} = 8.55$ ($P \leq 0.002$), respectively

differ significantly among isolates except that *Glomus* sp. (Basle Pi) produced a significantly greater length of fine hyphae of 1.25 μm diameter, although this constitutes a small amount of total hyphal length (Fig. 1b). In experiment 2, there were no significant differences in hyphal length between the two AMF isolates.

In experiment 1, plant growth, as expressed in total dry weight at the end of the experiment, was strongly and significantly promoted by all of the AMF treatments as compared to the non-mycorrhizal treatment, both at ambient and elevated $CO_2$. Furthermore, in both experiments, percentage root length colonized by AMF, percentage root length occupied by arbuscules or vesicles, total plant dry weight, stolon dry weight, leaf dry weight, stolon branch number and leaf area were all significantly and strongly affected by AMF treatment, i.e. by the type of AMF colonizing the roots, independently of either $CO_2$ or phosphorus treatments. These effects are presented in Streitwolf-Engel et al. (1997) and are, therefore, not presented in this manuscript.

## Main effects of $CO_2$ treatment

Plants were larger in both experiments when grown at 600 μl $l^{-1}$ $CO_2$ than at 350 μl $l^{-1}$ $CO_2$ independently of AMF treatment (Fig. 2a; Table 1). The $CO_2$ effects on dry weight were mainly due to $CO_2$ effects on stolon dry weight (Fig. 2b; Table 1) and on root dry weight (Fig. 2c; Table 1). Leaf dry weight was significantly greater at elevated $CO_2$ than at ambient $CO_2$ in experiment 2 (Table 1) and leaf weight followed the same trend in experiment 1, although this was not a significant effect. Leaf phosphorus concentration was significantly reduced at elevated $CO_2$ concentration compared to ambient $CO_2$ concentration in experiment 1 (Fig. 2d). Total plant, stolon, leaf and root P concentrations were significantly lower at elevated $CO_2$ than at ambient $CO_2$ in experiment 2 (Table 1). Total plant, leaf and root P contents were, however, significantly higher at elevated $CO_2$ than at ambient $CO_2$ in experiment 2 (Table 1) indicating that plants could acquire more phosphorus when growing at elevated $CO_2$. All the $CO_2$ effects on plant growth, P content and P concentration were independent of P treatment to the hyphal compartment. There were no $CO_2$ effects on stolon branching of *P. vulgaris* in either experiment. There were also no main $CO_2$ effects on the percentage of root length colonized

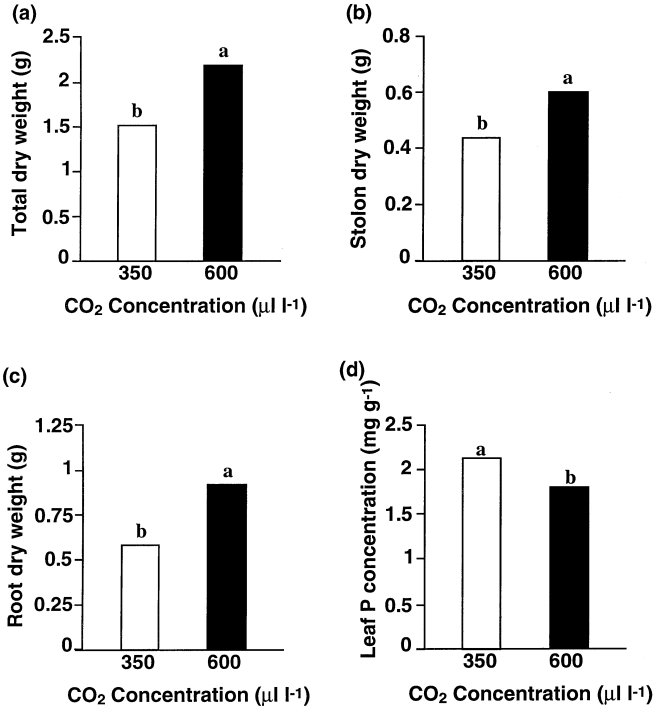


**Fig. 2** Mean **a** total dry weight, **b** stolon dry weight, **c** root dry weight and **d** leaf P concentration in *P. vulgaris* grown at 350 and 600 μl $l^{-1}$ $CO_2$ in experiment 1. *F*-ratios of ANOVA, for data presented in **a–d** as dependent variables, were $F_{(1,31)} = 6.86$ ($P \leq 0.014$), $F_{(1,24)} = 6.41$ ($P \leq 0.018$), $F_{(1,29)} = 5.74$ ($P \leq 0.023$), $F_{(1,27)} = 32.92$ ($P \leq 0.0001$), respectively. Different *letters above bars* indicate a significant difference ($P \leq 0.05$) according to the LSD test

**Table 1** Mean values of plant growth at 350 and 600 μl $l^{-1}$ $CO_2$ in experiment 2 (*AMF* arbuscular mycorrhizal fungi). Values in parentheses represent ±1 SE. *F* ratios are for a main $CO_2$ treatment effect in ANOVA with 1 and 31 *df*

| Variable | 350 μl $l^{-1}$ $CO_2$ | 600 μl $l^{-1}$ $CO_2$ | *F*-ratio | *P* |
|---|---|---|---|---|
| Total plant weight (g) | 8.03 (0.98) | 14.95 (1.33) | 19.22 | *** |
| Stolon weight (g) | 1.36 (0.13) | 1.99 (0.12) | 14.06 | *** |
| Leaf weight (g) | 2.57 (0.20) | 4.53 (0.35) | 23.48 | *** |
| Root weight (g) | 4.10 (0.68) | 8.43 (0.96) | 11.63 | ** |
| Stolon branch number | 26.47 (2.09) | 29.45 (2.05) | 1.85 | ns |
| Total plant P content (mg) | 14.17 (1.20) | 18.58 (1.54) | 9.50 | ** |
| Stolon P content (mg) | 4.06 (0.30) | 4.86 (0.48) | 3.27 | ns |
| Leaf P content (mg) | 4.85 (0.33) | 6.75 (0.77) | 5.60 | * |
| Root P content (mg) | 4.06 (0.57) | 5.99 (0.61) | 5.36 | * |
| Total plant P concentration (mg $g^{-1}$) | 1.76 (0.09) | 1.24 (0.07) | 19.49 | *** |
| Stolon P concentration (mg $g^{-1}$) | 3.13 (0.11) | 2.52 (0.15) | 11.28 | ** |
| Leaf P concentration (mg $g^{-1}$) | 1.96 (0.09) | 1.50 (0.11) | 10.14 | ** |
| Root P concentration (mg $g^{-1}$) | 1.11 (0.06) | 0.76 (0.04) | 21.04 | *** |
| % Root length colonized by AMF | 56.00 (7.8) | 62.00 (8.6) | 1.44 | ns |

***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$, *ns* not significant

by AMF or occupied by either hyphae, arbuscules or vesicles of AMF in either experiment (Table 1).

At a distance of 1–2 cm from the nearest roots external hyphal length was not significantly affected by the $CO_2$ treatment in experiment 1 (Fig. 3a). However, at a distance of 13–14 cm from the nearest root, external hyphal length was significantly greater at elevated $CO_2$ than at ambient $CO_2$ concentration (Fig. 3b). Although each size class of hyphae was greater in length at elevated $CO_2$ than at ambient $CO_2$ concentration, this difference was only significant for hyphae with a diameter of 2.5 μm. In experiment 2, hyphal lengths were 3.8, 3.9 and 5.2 times greater at elevated $CO_2$ than at ambient $CO_2$ at distances of 1–2 cm, 6.5–7.5 cm and 13–14 cm from the nearest root, respectively (Fig. 4). This increase in external hyphal growth was proportionally greater than that observed for root weight which was 2.1 times greater at elevated $CO_2$ than at ambient $CO_2$ (Table 1).

## AMF × $CO_2$ and AMF × P treatment interactions

There were significant AMF × $CO_2$ treatment interactions on stolon P concentration and stolon P content in experiment 1 (Fig. 5). A significant increase in stolon P concentration occurred in plants which were inoculated

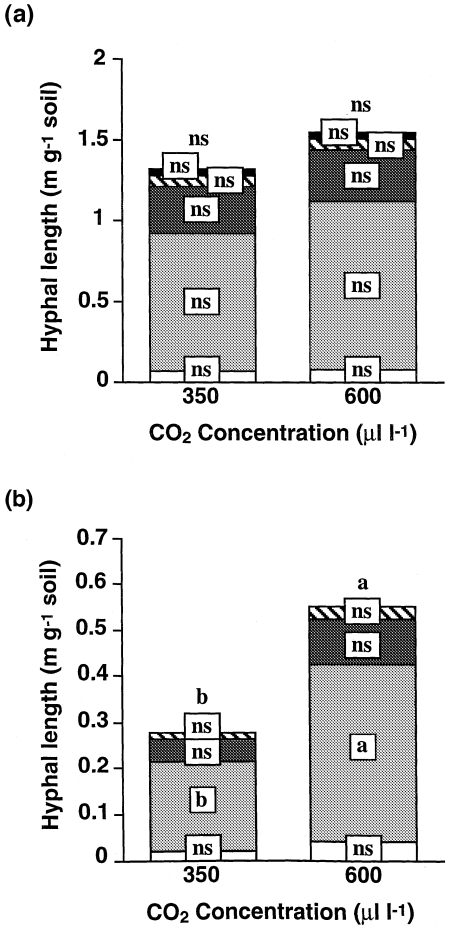


**Fig. 3** Mean external hyphal length (m $g^{-1}$ soil) of AMF in experiment 1, grown at 350 and 600 μl $l^{-1}$ $CO_2$ and harvested from a hyphal compartment **a** 1–2 cm and **b** 13–14 cm from the nearest root. *Shading* represents size classes of hyphae as measured by hyphal diameter (see Fig. 1 for legend). Different *letters above bars* indicate a significant difference ($P \leq 0.05$) according to the LSD test for mean hyphal length averaged over all size classes. Different *letters in squares within bars* indicate a significant difference ($P \leq 0.05$) according to the LSD test for each size class of hyphae. *F*-ratios of ANOVA, with total hyphal length and 2.5 μm diameter size class (13–14 cm from root) as dependent variables, were $F_{(1,24)} = 4.34$ ($P \leq 0.048$) and $F_{(1,24)} = 4.49$ ($P \leq 0.044$), respectively

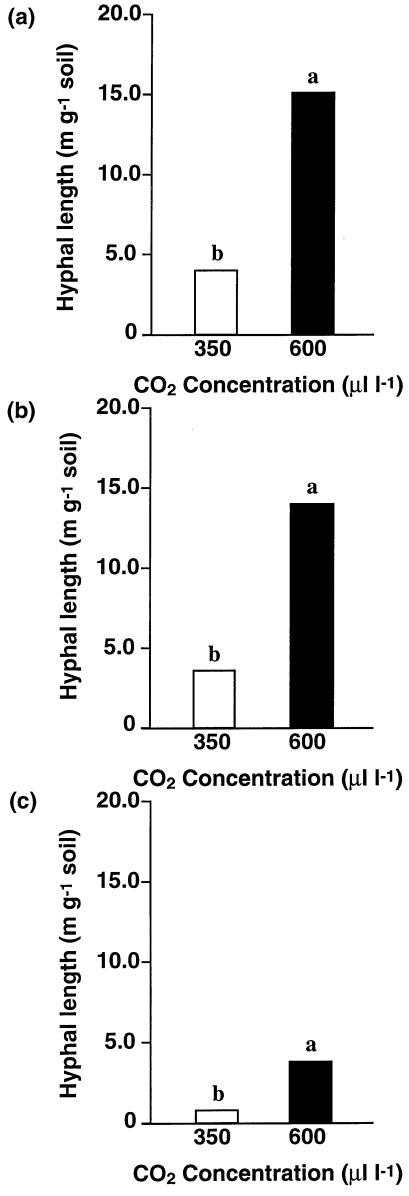


**Fig. 4** Mean external hyphal length (m $g^{-1}$ soil) of AMF in experiment 2, grown at 350 and 600 μl $l^{-1}$ $CO_2$ and harvested from a hyphal compartment **a** 1–2 cm, **b** 6.5–7.5 cm and **c** 13–14 cm from the nearest root. Different *letters above bars* indicate a significant difference ($P \leq 0.05$) according to the LSD test. *F*-ratios of ANOVA on hyphal length at 1–2 cm, 6.5–7.5 cm and 13–14 cm from the root as dependent variables, were $F_{(1,29)} = 34.54$ ($P \leq 0.0001$), $F_{(1,29)} = 8.55$ ($P \leq 0.007$) and $F_{(1,29)} = 7.68$ ($P \leq 0.009$), respectively

with G. *geosporum* and treated with the elevated $CO_2$ concentration compared to those treated with the ambient $CO_2$ concentration (Fig. 5a). The opposite effect occurred with plants inoculated with *Glomus* sp. (BEG 19) where stolon P concentration was reduced in plants grown at elevated $CO_2$ concentrations compared to those grown at ambient $CO_2$ concentrations. The size of these effects on P concentration were relatively small, although, owing to both AMF and $CO_2$ main effects on stolon dry weight (Fig. 2b) the difference in stolon P content among plants inoculated with different AMF isolates at elevated $CO_2$ is considerably greater than at ambient $CO_2$ concentrations (Fig. 5b). There was also a significant AMF × $CO_2$ treatment interaction on the proportion of root length occupied by hyphae and vesicles but without the presence of arbuscules in experiment 1 (Fig. 5c). Although there were no significant AMF × $CO_2$ treatment interactions in experiment 2, they would not have been expected on the basis of the results of experiment 1 because there was no significant interaction in experiment 1 following removal of the *G. geosporum* treatment from the data set. Experiment 2 lacks the *G. geosporum* treatment. There were no other significant AMF × $CO_2$ treatment interactions for any of the other variables measured in experiments 1 and 2.

There were no significant main effects of P treatment on any plant or fungal variables in experiment 2. There was, however, a significant AMF × P treatment interaction on plant P concentration ($F_{(1,31)} = 6.12$, $P \leq 0.01$). The P concentration of plants inoculated with *Glomus* sp. (BEG 19) was higher in the treatment with no added P than in the treatment with added P. In contrast, the P concentration of plants inoculated with *Glomus* sp. (Basle Pi) were higher in the treatment with added P than in the treatment with no added P (data not shown). There were no other significant AMF × P treatment interactions for any of the other variables.

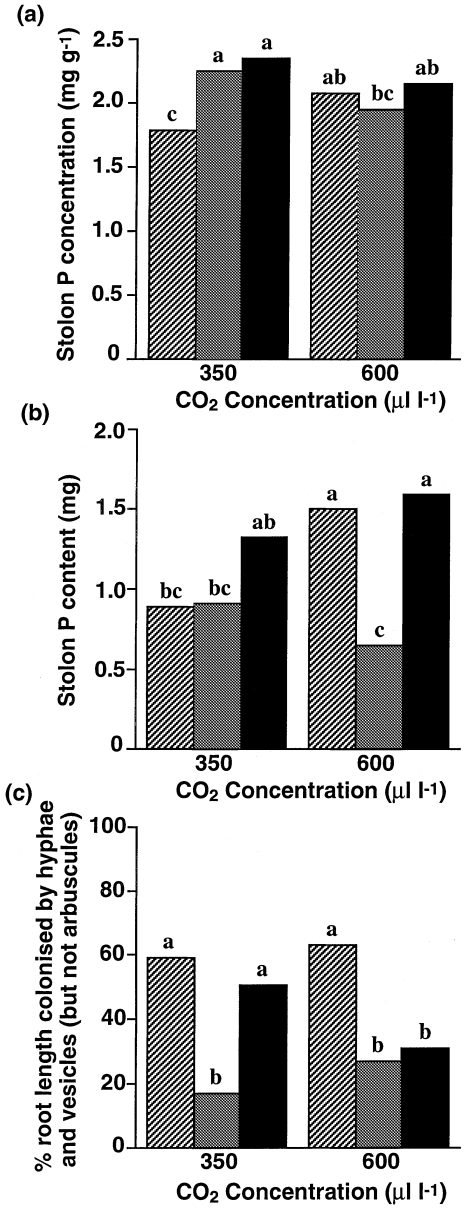


**Fig. 5** Mean **a** stolon P concentration, **b** stolon P content and **c** colonization by hyphae and vesicles (but not arbuscules) of AMF in *P. vulgaris* in experiment 1, grown at 350 and 600 µl $l^{-1}$ $CO_2$ and inoculated with one of three AMF isolates. *Glomus geosporum* (BEG 18), *Glomus* sp. (BEG 19), = *Glomus* sp. (Basle Pi). *F*-ratios of ANOVA, for data presented in **a–c** as dependent variables, were $F_{(2,22)} = 4.43$ ($P \leq 0.048$), $F_{(2,22)} = 5.35$ ($P \leq 0.013$) and $F_{(2,24)} = 3.89$ ($P \leq 0.034$), respectively. Different *letters above bars* indicate a significant difference ($P \leq 0.05$) according to the LSD test

## Discussion

### Effects of elevated $CO_2$ on the growth of *P. vulgaris*

In the present investigation, *P. vulgaris* reached a significantly larger biomass at elevated $CO_2$ than at ambient $CO_2$ (Fig. 2; Table 1). Another study found a negative effect of elevated $CO_2$ on the growth of *P. vulgaris* (Leadley and Stöcklin 1995). The two studies were conducted in the same soil, although in the study of Leadley and Stöcklin (1995), *P. vulgaris* plants were grown in microcosms with other species and inoculated with unsterilized soil from the study site (probably containing a mixture of several AMF). The differences in the two studies could, therefore, be due to interactions with other plant species or differential effects of $CO_2$ on AMF which were not used in our study.

### Effects of elevated $CO_2$ on the mycorrhizal symbiosis

In the earlier study in microcosms mentioned above, where *P. vulgaris* did not grow better at elevated $CO_2$ the AMF colonization of its roots was significantly higher at elevated than at ambient $CO_2$ (Sanders 1996). In the present study there were no significant main effects of $CO_2$ treatment on the percentage root length colonized

by AMF, although the root systems of *P. vulgaris* were approximately twice as large at elevated than at ambient $CO_2$. This means that the AMF species also grew more inside the roots at elevated than at ambient $CO_2$ but that this response was proportionally the same as that of the plants. Growth of AMF was also stimulated in the root-free soil: external hyphal length of all AMF isolates was increased by elevated $CO_2$ (Figs. 3, 4). It is likely that the stronger effect of elevated $CO_2$ on external hyphal growth observed in experiment 2 compared to experiment 1 was due to the longer duration of this experiment. In experiment 2, elevated $CO_2$ promoted hyphal growth outside the root much more than growth of the plant's root system. This indicates that AMF grew more overall at elevated $CO_2$ but that there was also greater allocation of their biomass to external hyphae.

## Consequences of increased AMF hyphal growth for ecosystems

### *Nutrient acquisition*

The increase in growth of the external hyphae is expected to considerably increase the potential volume of soil from which the plant could acquire nutrients. In experiment 1, the root-free tube from which the hyphae were harvested contained quartz sand and no available nutrients. Therefore, investment by the plant into AMF hyphal production in this compartment could not be beneficial for the plant, although this was unlikely to have been a great cost because $CO_2$ increased plant growth. It has been shown in other studies that phosphorus uptake is correlated with the volume of soil exploited by the external hyphae (Jakobsen et al. 1992). Although in experiment 2 external hyphal length increased considerably and plants took up more P at elevated than at ambient $CO_2$, this was not significantly affected by adding P to the hyphal compartment. Thus, we could not demonstrate an increased uptake of P directly, perhaps due to a lack of P limitation in the soil.

Our results suggest, as an alternative to the hypothesis of Díaz et al. (1993), that the observed difference in the reaction of mycorrhizal and non-mycorrhizal plants to elevated $CO_2$ could be due to increased growth of external AMF hyphae. It is clear from the results of our experiments that external AMF hyphae have the potential to grow more in the rhizosphere of non-mycorrhiza-forming neighbouring plants at elevated $CO_2$ than at ambient $CO_2$. This could either increase the direct competition for nutrient acquisition between AMF hyphae and non-mycorrhizal roots or lead to additional fixed carbon deposited in the rhizosphere of non-mycorrhizal plants by increased AMF hyphal growth, which subsequently may cause an expansion of soil microflora and consequently competition for nutrients between non-mycorrhizal roots and the soil microflora.

### *Movement of fixed carbon to the soil*

The increase in external hyphal length could also result in a significant increase in the amount of fixed carbon moving from the plant to the soil. Our estimates of the increase in hyphal biomass at elevated $CO_2$ range from 2.7 to 110.9 $\mu g\ g^{-1}$ soil, which is in the range of approximately 2.63–108.13 mg increase in hyphal biomass per plant, as compared to a root biomass increase of about 4–8 g. Thus, external AMF hyphae might contribute substantially to C flux, particularly since they might have much more rapid turnover and higher respiration than the roots.

### *Root respiration*

Recent investigations have indicated that a significant portion of the additional carbon that is fixed in ecosystems is re-released by root respiration, although the estimates of increases in root respiration are proportionally higher than increases in root biomass, root turnover or root exudation would predict (Hungate et al. 1997). This and other similar investigations do not take into account the role that external AMF hyphae could play in the process of respiratory $CO_2$ release to the soil. Root and microbial respiration are separated by measuring total soil respiration and subtracting soil microbial respiration (which is measured by $CO_2$ release from incubated soil, following removal of roots). Estimated values of root respiration probably include the respiration from active AMF hyphae. Since our results show that increased AMF hyphal growth at elevated $CO_2$ is proportionally higher than that of roots, this would suggest that AMF hyphal respiration could contribute significantly to the disproportionate increase in $CO_2$ release which has previously been attributed to root respiration (Hungate et al. 1997).

### *Soil aggregation*

External AMF hyphal length has been shown to be positively correlated with the percentage of water-stable soil aggregates (Tisdall and Oades 1979). Considering the relationship between hyphal length and soil aggregation measured by Tisdall and Oades (1979), the increases in hyphal length observed in this experiment as a result of elevated $CO_2$ could potentially lead to an increase of approximately 62% in water-stable soil aggregates.

## Differential effects of AMF species on plant responses to elevated $CO_2$

The result of a previous study with the same experimental system showed that growth and growth form of *P. vulgaris* were strongly dependent on which AMF

occupied the roots (Streitwolf-Engel et al. 1997). Exactly the same effects of the different AMF isolates on the growth and growth form of *P. vulgaris* were observed in both experiment 1 and 2 of the present study. Our present results indicate that the effects of the different AMF on the growth and growth form are indeed robust since under an altered environment these main AMF effects were unchanged. The only plant responses to elevated $CO_2$ that were differentially affected by AMF isolates were stolon P content and concentration (Fig. 5a, b). Whether stolon P concentration in *P. vulgaris* increased or decreased at elevated $CO_2$ depended on which isolate of AMF the plants were inoculated with. Changes in stolon P concentration as a result of this treatment were relatively small. However, because the effect of elevated $CO_2$ was to increase plant size, irrespective of AMF isolate, the final amount of P accumulated in the stolons differed greatly among the AMF isolate treatments at elevated $CO_2$ compared to ambient $CO_2$. This effect cannot, however, easily be explained by the AMF $\times$ $CO_2$ treatment interactions on AMF colonization (Fig. 5c). If similar effects occurred in natural conditions then colonization by different AMF isolates from the AMF community could result in a larger variation in P content in a population of *P. vulgaris* growing at elevated $CO_2$ than at ambient $CO_2$.

## Conclusions

We conclude that in elevated $CO_2$ plant interactions with AMF could be important for nutrient acquisition, soil carbon, soil respiration and soil aggregation in environments through it's effects on the growth of external AMF hyphae. We also conclude that although the different AMF co-occurring in natural communities could potentially be strong determinants of the structure of *P. vulgaris* populations (Streitwolf-Engel et al. 1997) the majority of such differential effects of the fungi are unlikely to be altered at elevated $CO_2$. The differential effects of these fungi could, however, lead to an increase or decrease and greater variation in some traits, e.g. stolon P allocation, at elevated $CO_2$.

**Acknowledgements** We thank P.W. Leadley for critical comments on an earlier version of this manuscript, two anonymous reviewers for their helpful comments and M. Alt, C. Erdin, F. Ehrsam, M. Schneider and I. Steiner for technical assistance. This research was supported by a grant from the Priority Programme Environment of the Swiss National Science Foundation.

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