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Mycelial production, spread and root colonisation by the ectomycorrhizal fungi *Hebeloma crustuliniforme* and *Paxillus involutus* under elevated atmospheric CO_2

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Abstract Effects of elevated atmospheric carbon dioxide (CO_2) levels on the production and spread of ectomycorrhizal fungal mycelium from colonised Scots pine roots were investigated. *Pinus sylvestris* (L.) Karst. seedlings inoculated with either *Hebeloma crustuliniforme* (Bull:Fr.) Quél. or *Paxillus involutus* (Fr.) Fr. were grown at either ambient (350 ppm) or elevated (700 ppm) levels of CO_2. Mycelial production was measured after 6 weeks in pots, and mycelial spread from inoculated seedlings was studied after 4 months growth in perlite in shallow boxes containing uncolonised bait seedlings. Plant and fungal biomass were analysed, as well as carbon and nitrogen content of seedling shoots. Mycelial biomass production by *H. crustuliniforme* was significantly greater under elevated CO_2 (up to a 3-fold increase was observed). Significantly lower concentrations and total amounts of N were found in plants exposed to elevated CO_2.

Keywords Elevated CO_2 · Mycelium · Ectomycorrhiza · Root colonisation · Mycelial spread

Introduction

Boreal forest trees rely on symbiotic ectomycorrhizal (ECM) fungi for their nutrient uptake. The extraradical fungal mycelium grows out from the mycorrhizal root tips into the soil surrounding the tree root system. Increased production of extraradical mycelium in response to elevated, atmospheric carbon dioxide (CO_2) has been reported from laboratory experiments with ECM fungi (Ineichen et al. 1995; Rouhier and Read 1998a) and arbuscular mycorrhizal (AM) fungi (Klironomos et al. 1998; Rouhier and Read 1998b; Sanders et al. 1998). However, for AM fungi, several authors found no effects of elevated CO_2 on mycorrhizal development and phosphorous (P) uptake, and suggested that fungal responses to CO_2 could simply be plant-size-dependant (Gavito et al. 2000, 2002; Staddon et al. 1999). Field observations indicate that late successional species (with higher C demand) may become more common when their host plants are exposed to elevated CO_2 levels (Rey and Jarvis 1997). ECM morphotypes forming extensive extraradical mycelia and rhizomorphs also appeared to increase in response to elevated CO_2 in a study using saplings and soil collected from the field (Godbold and Berntson 1997; Godbold et al. 1997). Increased fungal biomass production and ECM metabolic activity in response to elevated CO_2 have also been observed in field studies (Klamer et al. 2002; Wiemken et al. 2001). In a changing environment in which atmospheric levels of CO_2 continue to rise (Wigley and Raper 1992), these findings are of considerable ecological importance. The mycorrhizal fungal mycelium is intimately involved in the sequestration and partitioning of carbon (C) into the soil, and plays an important role in the mobilisation and acquisition of nutrients from the soil (Leake and Read 1997). Carbon allocation to mycorrhizal fungi is substantial and has been estimated at 15-20% of photosynthetically fixed C (Finlay and Söderström 1992; Smith and Read 1997). Under increased CO_2 levels the photosynthetic rate of plants increases (e.g. Norby et al. 1999), most likely affecting the flow of C in the plant-fungus system. Although down-regulation, or acclimation, of plant photosynthesis has been reported on a number of occasions for short-term pot experiments (reviewed by e.g. Curtis and Wang 1998; Ward and Strain 1999), long-term exposure to elevated CO_2 can have different effects (reviewed by e.g. Gunderson and Wullschleger 1994). Some authors have reported that down-regulation is not usually seen in

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longer-term experiments (Curtis and Wang 1998; Norby et al. 1999; Saxe et al. 1998), and the productivity response of a forest may persist for several years (Finzi et al. 2002; Norby et al. 2002). Others have reported a downward adjustment (Egli et al. 2001; Oren et al. 2001; G. Wallin, personal communication). However, the amount of C available for the symbiotic fungus may increase as a result of increased CO_2 fixation by plants under elevated atmospheric CO_2 levels.

The C allocated to the fungus will ultimately be partitioned mainly between biomass and respiration, and there may be considerable differences between fungal species in this respect (Cairney 1999). The extraradical mycelium can constitute a large fraction of the below-ground fungal biomass. Wallander et al. (2001) estimated the total amount of ECM mycelium (including mantles) in the humus layer to be between 700–900 kg ha^{-1}. In semi-hydroponic systems, Wallander and Nylund (1992) reported extraradical mycelial biomass to be 4% and 30%, respectively, of total fungal biomass of *Hebeloma crustuliniforme* and *Laccaria bicolor* associated with *Pinus sylvestris* seedlings. Miller et al. (1989) reported that up to 30% of total root and fungal biomass was extraradical mycelium in Ponderosa pine seedlings grown in microcosms.

Mycelial growth form, spread and mycorrhiza formation can differ markedly among ECM species (Agerer 2001; Wu et al. 1999). The colonisation of root tips will depend on the production of mycelium, its spread through the soil and its ability to establish on uncolonised root tips or to out-compete other ECM fungi already established on colonised root tips. The importance of the extraradical mycelium, in particular the rhizomorphs, in colonisation of roots has been stressed by a number of authors (Chilvers and Gust 1982; Read 1984). In an early investigation into competition between ECM fungi, Garbaye (1983) found that inoculum density determined the success or failure of colonisation by ECM fungi. Under elevated atmospheric CO_2, ECM fungal species that respond to increased C from the host plant by producing more extraradical mycelium could have a competitive advantage over species that do not respond.

In summary, it is not only the production of fungal mycelium, but also its spread and ability to colonise root tips that are important when considering the response of ECM fungi colonising plants growing under increased CO_2 levels. The aim of the present study was to investigate possible effects of elevated atmospheric CO_2 levels on the production, spread of and colonisation by ECM fungi associated with Scots pine seedlings, and to examine growth and nutrient status of the plant seedlings. The following questions were addressed: (1) do ECM fungi respond to elevated atmospheric CO_2 under limited nutrient availability by producing more extraradical mycelium, (2) are ECM fungi able to spread a greater distance from colonised plants under elevated CO_2, (3) will ECM fungi infect more uncolonised plant roots under elevated CO_2, and (4) will the nutrient status of Scots pine seedlings grown under elevated CO_2 change? Two different fungi, *H. crustuliniforme* and *Paxillus involutus*, which both produce large amounts of extraradical mycelium were chosen for the study.

Materials and methods

Mycorrhizal synthesis

In both experiments, ectomycorrhizal seedlings were synthesised under ambient atmospheric CO_2 levels following the method described by Duddridge (1986) with some modifications; the ratio of the peat:vermiculite:modified Melin-Norkrans (MMN) agar was changed to 1:4:2 and half-strength MMN medium (both C and nutrients) was used. We used 4-week-old *Pinus sylvestris* (L.) Karst. seedlings germinated on water agar. Fungal isolates of *H. crustuliniforme* (UP181) and *Paxillus involutus* (BL1) from the Department of Forest Mycology and Pathology, Uppsala, were used for the syntheses. After 8 weeks, heavily ECM colonised seedlings (>90% colonisation level) were moved from synthesis dishes to experimental vessels.

Experiment 1—mycelial production

To investigate mycelial production of ECM fungi associated with seedlings grown under two different CO_2 levels, square, black plastic flower pots (55×55×65 mm) filled with 200 ml washed perlite (<5 mm diameter) were used. The bottom of the pots was covered with a mesh to retain the perlite, a growth medium from which extraction of fungal biomass is relatively easy. The pots were watered to field capacity and left for 1 day. On the 2nd day, a single 8-week-old ECM seedling was transferred from synthesis dishes to each pot. After the seedlings were planted, the surface of the perlite was covered with gravel (<1 cm diameter) in order to decrease evaporation during the experiment. The pots were weighed, and each pot was maintained at its initial weight throughout the experiment. Three times a week a nutrient solution (Ingestad 1979) was added, with a N concentration of 50 mg l^{-1} and proportions of the other elements by weight to N (N=100) of: K=65, P=15, Ca=6, Mg=6 and S=9. Each pot received a total of 100 ml (5 mg N) nutrient solution over a 6-week period during which CO_2 treatment was applied (see below). Nutrient addition was supplemented with distilled water to maintain initial weights.

Experiment 2—mycelial spread and root colonisation

To investigate mycelial spread and root colonisation, Perspex boxes (172×105×38 mm) were used. These were divided into three lanes by plastic barriers with the edges sealed with silicon. Each lane was filled with 175 ml washed perlite. A total of four seedlings, enclosed in separate, 4 ml mesh bags (mesh size 100 μm) containing perlite, were planted 45 mm apart in each lane (Fig. 1). The mesh bags allowed the mycelia to grow out and spread through the perlite, but seedling roots were contained. The first seedling had been inoculated 8 weeks previously with either *H. crustuliniforme* or *P. involutus*, and is referred to as d_0 (Fig. 1). Seedling numbers 2–4 were uninoculated and 4 weeks old upon transfer to the boxes. These acted as bait seedlings and are referred to in relation to their distance from the inoculated seedling (d_0), as seedling d_{45}, d_{90} and d_{135} (Fig. 1). Only two, randomly chosen lanes in each box were used, and the numbers of replicates were eight for *H. crustuliniforme*, and five or six for *P. involutus* in ambient and elevated treatment, respectively. The edge of the mesh bags projected above the perlite surface in order to restrict root growth to within the bag, and the surface of the perlite was covered with gravel to decrease evaporation. The outside of the boxes was covered with aluminium foil to prevent entry of light in order to reduce growth of algae. Nutrient solution (40 ml) was added to the lanes after transferring the seedlings, and the weight of each box

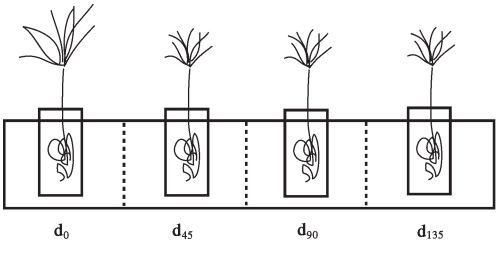

Fig. 1 Microcosm system for investigating effects of elevated, atmospheric CO_2 levels on mycelial spread and root colonisation in Scots pine seedlings colonised with *Hebeloma crustuliniforme* or *Paxillus involutus*. A three-lane box system showing sections d_0, d_{45}, d_{90} and d_{135} (number corresponding to distance in millimetres from inoculated seedling), which contained seedlings enclosed in individual mesh bags. Seedling d_0 was an inoculated seedling, and seedlings d_{45}, d_{90} and d_{135} were bait seedlings

was noted. Nutrient solution was added three times a week throughout the experiment, giving a total of 200 ml solution (10 mg N). Distilled water was added on a gravimetric basis, to compensate for uneven evaporation among boxes. The experiment was run for 4 months during which CO_2 treatment was applied (see below).

Experimental conditions

Both experiments were conducted in growth chambers receiving either ambient (350 ppm) or elevated (700 ppm) levels of CO_2. To maintain high relative humidity inside the chambers, air was pumped into the chambers after passing through distilled water. The growth chambers were maintained at a photon flux density of 200 μmol m^{-2} s^{-1} under an 18/6 h day/night cycle. Experimental systems were removed from the chamber during watering and replaced in a new position.

Harvest

When harvesting experiment 1 (mycelial production), seedlings were extracted from the pots and the root systems were gently washed in distilled water. At harvest of experiment 2 (mycelial spread), each lane was divided into four equal sections at the mid-point between seedlings d_0, d_{45}, d_{90} and d_{135} (Fig. 1). Seedlings were removed from the mesh bags and their root systems were gently washed. The perlite from each mesh bag was combined with the perlite belonging to the corresponding section. Fresh and dry weights of roots and shoots and number of ECM root tips were recorded for all plants. Colonisation of roots and size of root systems were variable within treatments, and the number of ECM roots was related to the size of the root system. The perlite was stored in individual bags at –40°C until analysis of the fungal biomass. Total C and N contents of all plant shoots from the mycelial spread experiment were analysed using an Elemental Analyzer, 2400 CHN (Perkin Elmer, Norwalk, Conn.).

Determination of fungal biomass

The amount of fungal biomass in the samples (roots and growth substrate) was determined using chitin analysis as described by Vignon et al. (1986). Measurement of fungal biomass therefore includes both living and dead material. Perlite was prepared for chitin analysis by grinding 10 ml from each sample. Standard curves were prepared from both pure D-(+)-glucosamine and from dried fungal mycelia of *H. crustuliniforme* and *P. involutus* grown on MMN medium. Conversion factors [µg glucosamine/mg dry weight (dw)] for calculating the fungal biomass derived from these standard curves were 40 µg and 43 µg glucosamine/mg dw for *H. crustuliniforme* and *P. involutus*, respectively. Fungal biomass results are reported as total amounts (mg dw) per root system (i.e. root-associated mycelium) and as amounts per 10 ml growth substrate (i.e. extraradical mycelium) for both experiments. Also, for the production experiment, total amounts of fungal biomass per growth substrate (or pot) is given, and for the spread experiment, fungal biomass per 10 ml perlite for each of the four sections is given.

Statistical analysis

One-way analysis of variance (ANOVA) was performed for each fungal species to test for possible treatment effects of elevated CO_2 on total plant, root and shoot fresh weights and dry weights, root/shoot ratios, number of ECM root tips and fungal biomass. Treatment effects on total amount of fungal mycelium and amount of fungal mycelium per unit dry weight of root and plant, as well as total number of ECM roots and amount of ECM roots per unit dry weight root and plant, were examined separately. In experiment 2, comparisons between seedlings in corresponding sections were also performed using ANOVA, to compare seedlings of equal size. Two-way analysis of variance was also performed on experiment 2 (mycelial spread) to investigate possible interactions between CO_2 treatment and distance to the inoculated seedling.

Results

Mycelial production experiment

The total fungal biomass produced by *H. crustuliniforme* was significantly greater ($F_{(1,8)}$=7.32, P=0.03) in the elevated than in the ambient CO_2 atmosphere (Fig. 2a). The mean total fungal biomass of *H. crustuliniforme* in colonised roots was 2.20±0.95 mg for the ambient treatment and 6.52±1.2 mg for the elevated treatment. Corresponding values for the growth substrate were 0.15±0.02 mg/10 ml perlite (3.1±0.3 mg in total per pot) for the ambient treatment and 0.34±0.07 mg/10 ml (6.7±1.35 mg in total per pot) for the elevated treatment. A similar trend was seen for fungal biomass expressed per unit root dw, with a mean 2 times higher than that for the control treatment, although this difference was not statistically significant ($F_{(1,8)}$=4.68, P=0.067). There were no significant differences between treatments in root, shoot and total plant fresh/dry weights, root/shoot ratios or ECM root tips (Fig. 2). The extraradical mycelium corresponds to ca. 0.5–1.2% of the total plant biomass (i.e. the weight of shoot and root system, including ECM tips) and approximately 50% of the total fungal biomass (root-associated and extraradical) irrespective of treatment. *Paxillus involutus* inoculated plants grew poorly, the mean shoot and root dry weights were 36±10 mg and 66±9 mg, respectively, for the ambient CO_2 treatment, and 37±30 mg and 73±20 mg, respectively, for the elevated CO_2 treatment (fungal data not shown).

Mycelial spread experiment

No treatment differences in fungal parameters were found, with two exceptions (see below). There was high

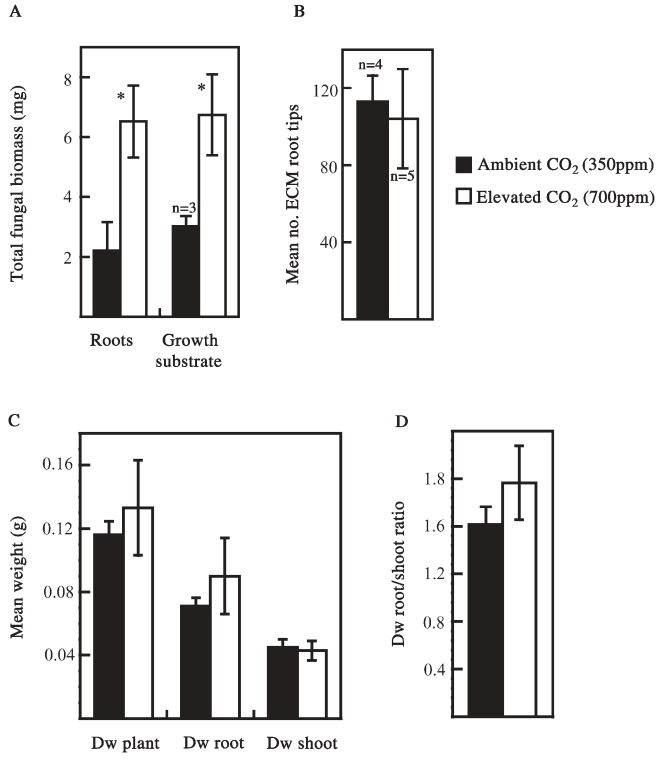

Fig. 2A–D Plant and fungal biomass in a mycelial production experiment with mycorrhizal Scots pine seedlings. Mean values ±standard error for seedlings inoculated with *H. crustuliniforme* grown in ambient (350 ppm) or elevated (700 ppm) CO_2 levels. **A** Total amount of fungal biomass for roots and growth substrate, **B** number of ECM root tips, **C** plant dry weight yields, and **D** dry weight root/shoot ratios. n=4 for ambient treatment, n=5 for elevated treatment unless otherwise indicated. Significances (P-values of the ANOVA test) are indicated as *, P<0.05

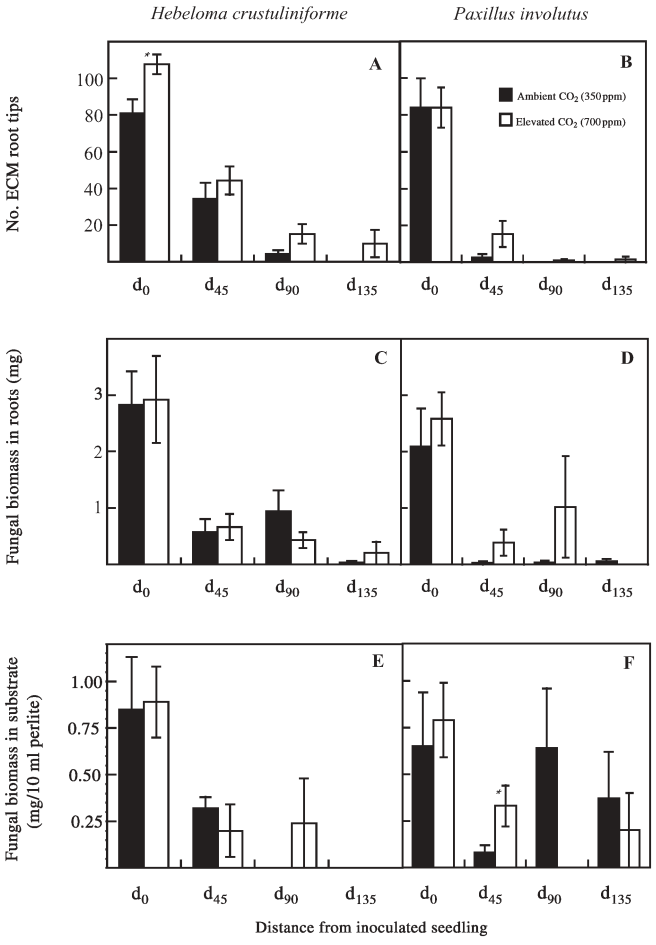

Fig. 3A–F Fungal biomass in an experiment investigating mycelial spread from ECM seedlings and root colonisation of uninoculated Scots pine seedlings under CO_2-enrichment. The total number of ECM root tips for *H. crustuliniforme* (**A**) and *P. involutus* (**B**), total amount of fungal biomass in roots for *H. crustuliniforme* (**C**) and *P. involutus* (**D**), and amount of fungal biomass/10 ml growth substrate for *H. crustuliniforme* (**E**) and *P. involutus* (**F**). Mean values ±standard error are given. *H. crustuliniforme*: n=8, *P. involutus*: n=5 for ambient treatment, n=6 for elevated treatment. Significances (P-values of the ANOVA test) are indicated as *, P<0.05

variation among replicates (Fig. 3). *Hebeloma crustuliniforme*-inoculated seedlings grown at elevated levels of CO_2 had a mean fungal biomass on roots (2.9±0.8 mg) similar to seedlings grown at ambient CO_2 levels (2.8±0.6 mg) (Fig. 3c). For *P. involutus*-inoculated seedlings the mean fungal biomass on roots was 2.1±0.7 mg and 2.6±0.5 mg for ambient and elevated CO_2 levels, respectively (Fig. 3d). As expected, the highest amounts of fungal biomass were associated with the inoculated seedlings (d_0) (Fig. 3a–f). The amount of fungal biomass in the growth substrate did not differ significantly between treatments when corresponding sections were compared, with one exception. Significantly more fungal biomass ($F_{(1,10)}$=4.71, P=0.048) was found in the growth substrate for *P. involutus* seedlings (d_{45}) growing 45 mm from the inoculated seedlings under elevated CO_2 treatment. For *H. crustuliniforme*-inoculated plants, fungal biomass in the growth substrate was detected up to 45 mm from the inoculated seedling in the ambient treatment. In the elevated CO_2 treatment, fungal biomass was detected up to 90 mm from the inoculated seedling (Fig. 3e).

The total numbers of ECM root tips found for *H. crustuliniforme*-inoculated seedlings were 80.8±7.7 at ambient CO_2 levels but significantly ($F_{(1,15)}$=8.07, P=0.013) higher (107.6±5.5) at elevated CO_2 levels (Fig. 3a). When the number of *H. crustuliniforme* mycorrhizal root tips was divided by plant biomass (fresh and dry weights) there were also significantly more ECM root tips per unit biomass on the inoculated seedlings in the elevated CO_2 treatment compared to the ambient CO_2 treatment (P<0.001). For *H. crustuliniforme*, no ECM root tips were found on seedling d_{135} (growing 135 mm from the inoculated seedling) in the ambient CO_2 treatment, but in the elevated CO_2 treatment there were mycorrhizal roots (Fig. 3a).

For *P. involutus*-inoculated seedlings the total numbers of ECM root tips were similar, 84.0±15.9 at ambient CO_2 levels and 84.0±10.8 at elevated CO_2 levels. Elevated CO_2 had no effect on ECM root tips per unit biomass in *P.*

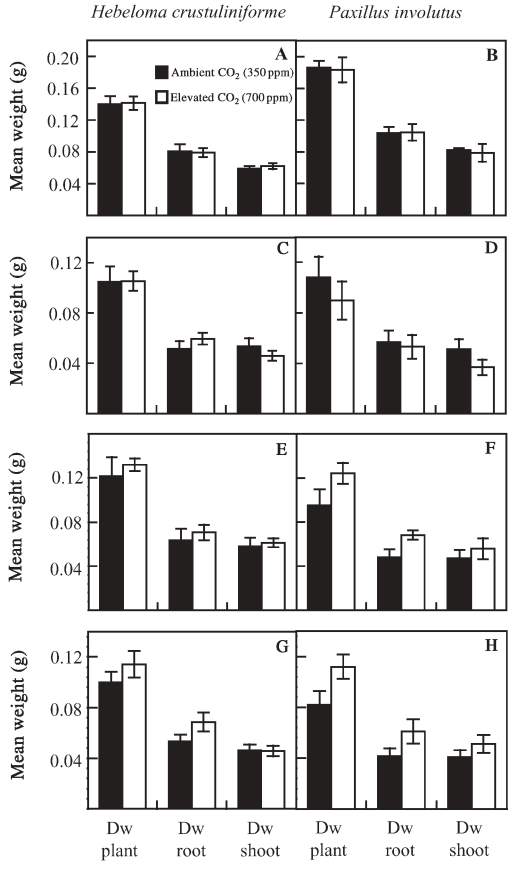

Fig. 4A–H Plant biomass in an experiment investigating mycelial spread from ECM Scots pine seedlings and root colonisation of uninoculated seedlings under CO_2-enrichment. Mean dry weights ±standard error for *H. crustuliniforme* or *P. involutus* seedlings grown under ambient (350 ppm) and elevated (700 ppm) CO_2 levels. **A**, **B** Inoculated seedling d_0; **C**, **D** seedling d_{45} (45 mm from inoculated seedling); **E**, **F** seedling d_{90} (90 mm from inoculated seedling); and **G**, **H** seedling d_{135} (135 mm from inoculated seedling). *H. crustuliniforme*: *n*=8, *P. involutus*: *n*=5 for ambient treatment, *n*=6 for elevated treatment

involutus-inoculated seedlings. However, *P. involutus* seedlings d_{90} and d_{135} had some ECM root tips in the elevated CO_2 treatment but none in the ambient CO_2 treatment (Fig. 3b). *Paxillus involutus*-inoculated seedlings did not show any consistent pattern of fungal biomass distribution in the growth substrate (Fig. 3f).

No significant treatment differences were found for plant biomass (Fig. 4) with few exceptions. Both *H. crustuliniforme* and *P. involutus* seedling d_{45} had significantly higher root/shoot ratios ($P<0.05$), when grown at elevated CO_2 levels. No significant interactions were found between CO_2 treatment and distance to inoculated seedling. Nitrogen concentrations and total N amounts in plant shoots were significantly higher for *H. crustuliniforme* and *P. involutus* inoculated plants grown at ambient CO_2 levels compared with those grown at elevated CO_2 levels (Table 1). Carbon concentration of the plants did not differ significantly between treatments for either fungal species. For bait seedlings no significant differences were found in C or N content.

Discussion

Fungal mycelia respond to changes in both C and nutrient availability. Changes in ECM community structure due to atmospheric CO_2-enrichment have been observed in the field (Rey and Jarvis 1997; Fransson et al. 2001). This could be explained by differential responses by ECM fungi to increased C-availability arising from elevated CO_2. In the present study we investigated one possible mechanism behind observed shifts in community structure: under increased C availability some ECM fungi may increase their inoculum potential by increasing the production of extraradical mycelium. We found that the amount of fungal mycelium produced by *H. crustuliniforme* under elevated CO_2 levels was up to three times higher than in ambient CO_2 levels, suggesting that the fungus was able to produce more mycelium as a consequence of increased C availability. This agrees with earlier findings of increased mycelial growth under elevated CO_2 levels for seedlings inoculated with *Pisolithus arhizus*, *Paxillus involutus* or *Suillus bovinus* (Ineichen et al. 1995; Rouhier and Read 1998a). The mycelial biomass of *H. crustuliniforme* corresponded to 0.5–1.2% of the total plant biomass, or ca. 50% of the total fungal biomass, figures similar to earlier reports.

Table 1 Nitrogen and carbon content of seedlings inoculated with either *Hebeloma crustuliniforme* or *Paxillus involutus* grown at either ambient (350 ppm) or elevated (700 ppm) levels of CO_2

	Nitrogen		Carbon	
	Concentration (%)	Total amount (mg)	Concentration (%)	Total amount (mg)
H. crustuliniforme				
Ambient CO_2	0.90±0.06	0.52±0.03	49.3±0.8	28.9±1.3
Elevated CO_2	0.67±0.04	0.41±0.04	47.2±1.0	29.1±1.8
P	0.007***	0.034*	n.s.[a]	n.s.
P. involutus				
Ambient CO_2	0.86±0.04	0.70±0.04	49.3±0.2	40.6±1.0
Elevated CO_2	0.64±0.02	0.50±0.07	49.4±0.2	38.6±4.6
P	0.001***	0.033*	n.s.	n.s.

* $P<0.05$, *** $P<0.001$
[a] Not significant

There were, however, no significant differences in the present study in plant biomass between the treatments. This could be due to nutrient limitation of plant growth, or the short duration of the experiment. If the plants are limited by water and/or nutrients they may allocate more C to the fungus in order to meet their increasing nutrient demand. For example, Klironomos et al. (1997) found that the extraradical mycelium of AM fungi associated with *Populus tremuloides* increased under elevated CO_2 levels only when the N availability was low. In the present experiment the significant effects on mycelial production but lack of a significant plant biomass response suggests that an increased supply of current assimilates may have been allocated directly to fungal biomass without affecting root biomass.

In the mycelial spread experiment, no significant differences were found for plant biomass, and few for fungal biomass. We found a higher number of ECM roots of *H. crustuliniforme*-inoculated seedlings in the elevated CO_2 treatment compared to the ambient CO_2 treatment, and all seedlings of both *H. crustuliniforme* and *P. involutus* tended to have more ECM roots under CO_2-enrichment (not significant). This may indicate a potential for ECM fungi not only to produce more extraradical mycelium under elevated CO_2 regimes, but also to increase the rate of spread of that mycelium. However, the results in the present study do not support the hypotheses about spread of mycelia and colonisation of roots.

One consequence of tree response to elevated levels of CO_2 is an increase in nutrient demand, which will follow if the tree increases its photosynthetic rate and biomass production. If the ECM fungi growing on the tree roots produce more mycelium under CO_2 enrichment, nutrient acquisition by the fungus may increase. However, a possible increase in fungal biomass production may not necessarily result in increased transfer of N to the host plant, since responses of plants to elevated CO_2 may be influenced by physiological differences between the ECM species colonising the roots. In a study by Gorisson and Kuyper (2000), where Scots pine seedlings inoculated in vitro with either *Laccaria bicolor* or *S. bovinus* were grown under ambient or elevated CO_2 levels, the plant biomass response depended on the ECM species. In seedlings inoculated with *S. bovinus*, the extra C assimilated under CO_2 enrichment did not increase shoot biomass, but did increase ECM root biomass. In seedlings inoculated with *L. bicolor*, no increases in plant biomass or mycelial production were found, but respiration by the fungus increased. Finally, the *L. bicolor*-inoculated plants had lower N concentrations than plants inoculated with *S. bovinus*, suggesting that *L. bicolor* was less effective in transferring N to the host plant. In our study there was no increase in plant N associated with the significant increase in the number of ECM root tips colonised by *H. crustuliniforme* under elevated CO_2. The concentrations of N in the seedlings in elevated CO_2 treatment were low, and similar to those reported by Gorisson and Kuyper (2000). The mean N concentration in plants exposed to elevated CO_2 was significantly lower than in plants grown under ambient levels. However, no significant effect was seen on plant biomass. A possible explanation for this may be sequestration of N in the fungal mycelium. Retention of N in this manner has been shown in *Scleroderma citrinum* by Colpaert et al. (1996).

As pointed out by Cairney and Meharg (1999), we still do not know to what extent apparent differences in the response of mycorrhizal fungi to a CO_2-enriched atmosphere relate to differences in soil condition, nutrient availability or host-specific differences in C availability to the mycorrhizal roots. On the other hand, microbial activity and nutrient availability in the soil are likely determinants of the forest CO_2 response.

Finally, the increased C availability to the root system and fungi may give individual mycorrhizal root tips larger amounts of C. This has not been examined so far. However, there seems to be a general increase in the below-ground biomass, in the size of root systems and in the number of root tips. Individual root tips may thus not experience any increase in the amount of C, but rather there may be an overall increase in the number of roots. Higher C uptake by the plant may also increase C cycling in the system (Rouhier et al. 1994), possibly as a result of the rapid turnover of the mycelial compartment (Fitter et al. 2000).

In conclusion, we have shown that the production of extraradical mycelium in *H. crustuliniforme* may increase under elevated levels of atmospheric CO_2. However, this increase in production was not reflected in a significant increase in spread of mycelium and root colonisation in the experimental set-up used here. Significantly less N was found in plants exposed to elevated CO_2, possibly explained by sequestration of N in the fungal mycelium. Further studies of how CO_2-enrichment may affect ECM fungi through alterations of inoculum potential and competitive balance between species are necessary, as are studies of C-use efficiency in different mycorrhizal species to evaluate possible effects at the community level.

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