New Phytol. (1997), **137**, 433–440

Growth and mycorrhizal colonization of three North American tree species under elevated atmospheric CO_2

By DOUGLAS L. GODBOLD*, GLENN M. BERNTSON and F. A. BAZZAZ

Department of Organismic and Evolutionary Biology, Biological laboratories, Harvard University, 16 Divinity Ave, Cambridge, MA 02138, USA

(*Received 21 November 1996; accepted 4 June 1997*)

SUMMARY

We investigated the effect of elevated CO_2 on the growth and mycorrhizal colonization of three tree species native to north-eastern American forests (*Betula papyrifera* Marsh., *Pinus strobus* L. and *Tsuga canadensis* L. Carr). Saplings of the tree species were collected from Harvard Forest, Massachusetts, and grown in forest soil under ambient (*c.* 375 ppm) and elevated (700 ppm) atmospheric CO_2 concentrations for 27–35 wk.

In all three species there was a trend to increasing whole-plant, total-root and fine-root biomass in elevated CO_2, and a significant increase in the degree of ectomycorrhizal colonization in *B. papyrifera* and *P. strobus*, but not in *T. canadensis*. However, in *T. canadensis* the degree of colonization with arbuscular mycorrhizas increased significantly. In both the ambient and elevated environments, on the roots of *B. papyrifera* and *P. strobus* 12 distinct ectomycorrhizal morphotypes were identified. Distinct changes in the ectomycorrhizal morphotype assemblage of *B. papyrifera* were observed under CO_2 enrichment. This change resulted in an increase in the frequency of ectomycorrhizas with a higher incidence of emanating hyphae and rhizomorphs, and resulted in a higher density of fungal hyphae in a root exclusion chamber.

Key words: *Betula papyrifera*, *Pinus strobus*, *Tsuga canadensis*, ectomycorrhizas, elevated CO_2.

INTRODUCTION

The concentration of atmospheric CO_2 is rising at a rate greater than at any previous time in the earth's history, and is expected to double within the next century. In general, plants respond to rising atmospheric CO_2 levels with increased growth, which in part also depends upon their ability to acquire limiting soil nutrients and water (Norby, 1994; Berntson, 1996). Most elevated CO_2 studies have documented an increase in net belowground growth (reviewed in Rogers, Runion & Krupa, 1994). In addition to increased belowground biomass production, elevated CO_2 can lead to a number of other changes in the growth of plant root systems which might confer increased ability to acquire nutrients. These changes include alteration in the dynamics of root growth (Berntson & Woodward, 1992; Pregitzer *et al.*, 1995; Berntson, 1996; Berntson & Bazzaz, 1996), deployment within the soil (Berntson & Woodward, 1992; Prior *et al.*, 1994), kinetics of nutrient uptake (Bassirirad *et al.*, 1996), root system architecture (Berntson & Woodward, 1992), and symbiotic relationships of mycorrhizal fungi (reviewed in O'Neill, 1994). Owing to the higher rates of growth in elevated CO_2, mycorrhizas might be especially important in meeting the increased nutrient demand of the host plants.

The benefits of mycorrhizas for improving the nutrient status of trees are largely related to the hyphal net of extramatrical mycelia in the soil, providing a large surface area for the uptake of nutrients (Rousseau, Sylvia & Fox, 1994) and possibly the utilization of organically bound mineral nutrients through the production of extracellular enzymes (Maijala, Ragerstadt & Raudaskoski, 1991; Bending & Read, 1995*b*). However, production of the extramatrical mycelium might make a high C demand on the host plant (Rygiewicz & Anderson, 1994). The amount of extramatrical mycelium produced by an ectomycorrhizal fungus is species-specific, thus different species might impose a

* To whom correspondence should be addressed at: Forstbotanisches Institut, Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany.
E-mail: dgodbol@gwdg.de

different cost on the tree. Differences between ectomycorrhizal fungal species in their ability to improve the mineral nutrient status (Bougher, Grove & Malajczuk, 1990; Rousseau *et al.*, 1994), drought tolerance (Guehl, Garbaye & Wartinger, 1992) and resistance of trees to heavy metal toxicity (Colpaert & van Assche, 1993; Marschner, 1994) have been shown, and might be related to the amount of extramatrical mycelium produced (Cain, 1994).

In the majority of studies to date, elevated atmospheric CO_2 has resulted in a significant increase in the degree of colonization by ectomycorrhizas on their host trees (O'Neill, 1994; Ineichen, Wiemken & Wiemken, 1995; Berntson, 1996). However, in some studies with artificial inoculation of a single mycobiont, no increase in the degree of colonization was found (Lewis, Thomas & Strain, 1994). In *Quercus alba* colonized by indigenous ectomycorrhizas under elevated CO_2, not only did the percentage of mycorrhizal root tips increase, but also a change in the frequency of one mycorrhizal species was observed (O'Neill, Luxmoore & Norby, 1987). Under elevated CO_2 the percentage colonization of the easily identifiable species *Cenococcum graniforme* decreased. Changes in mycorrhizal species assemblage have also been shown for other factors including drought (Kåren *et al.*, 1996) and N deposition (Rapp & Jentschke, 1994).

In contrast to ectomycorrhizas, where increase in the degree of colonization under elevated CO_2 is common, in arbuscular mycorrhizas, changes in the degree of infection under elevated CO_2 are more variable (Monz *et al.*, 1994; O'Neill, 1994). In *Liriodendron tulipifera* grown under elevated CO_2, no effect on the degree of infection with arbuscular mycorrhizas was found (O'Neill, O'Neill & Norby, 1991; O'Neill, 1994).

In this work, the effect of elevated CO_2 on the growth and mycorrhizal colonization of three tree species native to north-eastern American forests has been investigated. Of the species selected, two, paper birch (*Betula papyrifera* Marsh., a deciduous tree) and Eastern white pine (*Pinus strobus* L., a conifer), are ectomycorrhizal species, and one, Eastern hemlock (*Tsuga canadensis* (L.) Carr., a conifer), forms both ectomycorrhizas and arbuscular mycorrhizas. For the ectomycorrhizas, not only has the degree of colonization been estimated, but an attempt has been made to determine the effects of elevated CO_2 on the morphotype assemblage. The goal of this paper is to document the degree and type of mycorrhizal response to rising CO_2 in each of the three species, along with overall patterns of tree growth and biomass allocation.

MATERIALS AND METHODS

Cultivation of plants

Saplings of paper birch (*Betula papyrifera* marsh.), Eastern white pine (*Pinus strobus* L.) and Eastern hemlock (*Tsuga canadensis* (L.) Carr.) were collected in July 1995 from Harvard Forest, Petersham, Massachusetts. *B. papyrifera* saplings were 10–15 cm high, and had four to six fully expanded leaves. Saplings of *P. strobus* had two sets of needles, and saplings of *T. canadensis* had three to four, respectively. The saplings were excavated in intact soil cores and transferred to 7·6-l containers filled with a 10:10:1 mixture of forest soil, sphagnum moss peat and silica sand. In the top third of the growth container a 416 cm^3 ($8 \times 8 \times 6{\cdot}5$ cm) root-exclusion chamber was incorporated, one face of which was a 35 μm nylon mesh (Zurich Bolting Co., Switzerland). The forest soil (a mixture of the humic layer and *c.* 0–3 cm of the mineral soil) was collected from a site adjacent to where saplings were collected at Harvard Forest. The soil was sieved through an 8-mm screen to remove all large roots and stones.

Three days after collection the saplings were transferred to climate-controlled glasshouses maintained with either ambient (about 375 ppm) and elevated (700 ppm) atmospheric CO_2 concentrations. As controls for hyphal length estimates (see below) pots without plants were also placed in the glasshouse. Three glasshouses were maintained at ambient and three at elevated atmospheric CO_2 concentrations. Each glasshouse had three replicate plants per species (and three pots without plants) for a total of nine replicate plants per species per CO_2 level. Throughout the treatment period, photoperiod was maintained at 12/12 h (day/night length) through supplementary lighting (HID mercury vapour lamps); minimum PAR was 250–300 $\mu mol\ m^{-2}\ s^{-1}$, and average day/night temperatures were 26/21 °C. The pots were watered once a day to field capacity. The plants were maintained under these conditions for 25 (*B. papyrifera*), 35 (*T. canadensis*) or 37 (*P. strobus*) wk. *B. papyrifera* has harvested earlier than the two conifers owing to its much higher growth rate.

Biomass

After collection of root tip samples (see below) the roots were separated from the stem, washed, and divided into woody and fine roots. The leaves, stem and roots were dried at 70 °C and weighed.

Root tips and ectomycorrhizal morphotypes

Because of its large root system, roots of *B. papyrifera* were collected from two locations in each pot to obtain a representative sample of root tips for mycorrhizal assessments. First, after the root ex-

clusion chamber had been removed, all the roots on the surface of the 8×8 cm area of the contact zone between the nylon mesh side of the root exclusion chamber and the soil of the growth container were collected. After this, the plant in the intact soil monolith was removed from the pot, a horizontal slice (10-cm thick) was cut through the centre of the monolith, and all the roots within this slice were collected. For *T. canadensis* and *P. strobus*, the complete root system was eased from the soil with minimal disturbance. A random subsample of the collected roots was taken and carefully washed. The difference in sampling methods for *B. papyrifera* and the two conifers was necessary because the extremely small diameter (100–200 μm) and high density of *B. papyrifera* roots made it impossible to remove the bulk of roots from the soil without breaking a large fraction of the root tips. The larger diameter (> 400 μm) and lower root density of *T. canadensis* and *P. strobus* roots made complete extraction from the soil possible. The roots were stored on moistened filter paper in Petri dishes at 4 °C until analysis. On average 250 (range = 67–616) individual *B. papyrifera* root tips were examined from each of the two subsamples taken from each plant. Of *T. canadensis* and *P. strobus*, an average of 276 (range = 168–440) and 532 (range = 392–602) roots tips were examined, respectively.

The mycorrhizal root tips were categorized into different morphotypes on the basis of morphology, colour, characteristics of the surface of the hyphal mantle, and planar views of different mantle layers, using methods described by Agerer (1992*a*, *b*). Initial categorization and counting of root tips were carried out using a binocular dissecting microscope at a $\times$10–30 magnification. Analysis of the mantle structure and planar views of the mantle layers was made with a compound microscope and photographed on Ectochrome® T160 film at a $\times$100 magnification.

Arbuscular mycorrhizas

Roots of *T. canadensis* were fixed in 50% ethanol, and cleared and stained with trypan blue according to the method of Koske & Gemma (1989). The roots were examined at $\times$200 and the counts of vesicles and arbuscules converted, with a gridline eyepiece, to percentage root length colonized (Brundrett Melville & Peterson, 1994).

Determination of extramatrical hyphal length

Two g of fresh soil were suspended in 90 cm^3 of deionized water, and blended for 30 s. The suspension was passed through a 250 μm screen; 15 cm^3 of the suspension was passed through a cellulose membrane (0·45 μm pore size), which was flooded with acid glycerol trypan blue solution for 1 h, rinsed with deionized water and vacuum-filtered. The membrane was allowed to dry, and a 17×17 mm piece was placed on a microscope slide wetted with acidic glycerol, then covered with a coverglass. The hyphae were observed at $\times$200 magnification through an eyepiece that had a 15×15 lined grid. The total length of hyphae was estimated using a gridline-intersect method (Brundrett *et al.*, 1994). To estimate mycorrhizal fungi hyphal density, the hyphal density in pots without plants was determined and subtracted as a background from that of the pots with plants (Sylvia, 1992).

Statistical analysis

Owing to heteroscedasticity, parametric means for statistically comparing ectomycorrhizal morphotype frequencies could not be reliably used. Instead, a replicated 'goodness of fit' test was used (separately for each tree species) employing the *G*-test to characterize overall CO_2 effects and homogeneity of replicates (individual plants) within each CO_2 level (Sokal & Rohlf, 1981). For each CO_2 level, the average frequency observed in the other CO_2 level was used as the null frequency for the *G*-test. For testing differences in the average observed frequency of each morphotype between the CO_2 levels, separate *G*-tests for each morphotype with an assumed null frequency of 1:1 between the CO_2 levels were carried out. *P*-values were adjusted with a Bonferroni correction to avoid type-II errors (Rice, 1989). A significant *G*-statistic for this test rejects the hypothesis that the observed frequency of a given morphotype was equivalent between the two CO_2 levels.

All plant measurements (e.g. biomass, number of root tips, percentage of mycorrhizal root tips shoot:root biomass ratio) were analysed by a three-way ANOVA, the three factors being species, CO_2 level and block. Carbon dioxide was tested with the overall error term as the denominator in the *F*-test because individual CO_2 glasshouses were not a priori paired by CO_2 levels. To improve compliance with assumptions of homoscedasticity and normality, some variables were log or square-root transformed before analysis.

RESULTS

All three species grew well under both ambient and elevated CO_2. Even the slower-growing *T. canadensis* increased in size approx. threefold over the treatment period. In all three species there was a trend to increasing whole-plant biomass with elevated CO_2, but this was not statistically significant (Table 1). There was also a trend towards increased root biomass for all three species in elevated CO_2, but it was significantly different only in *B. papyrifera*. When total root biomass was partitioned into woody

Table 1. *Summary of CO_2 effects on* Betula papyrifera, Pinus strobus *and* Tsuga canadensis

Species	Biomass (g)					Root tips		EM		AM	Hyphal length (mm g^{-1} soil)	
	Whole plant	Total roots	Woody roots	Fine roots	Shoot:root ratio	Number per plant	Number per g^{-1} d. wt	Number per plant	%	%	Roots present†	Roots absent‡
Betula papyrifera												
A	34·8	16·5	9·9	6·5	1·13	27012	4·46	23379	84·8	—	388	739
	(3·9)	(1·4)	(0·9)	(0·6)	(0·06)	(3643)	(0·73)	(3757)	(3·8)		(140)	(147)
E	38·8	19·6	11·2	8·4	1·00	42008	5·88	40767	95·7	—	438	1216
	(2·0)	(1·2)	(0·7)	(0·8)	(0·06)	(8182)	(1·49)	(8255)	(1·6)		(147)	(187)
P	n.s.	*	n.s.	†	n.s.	*	n.s.	**	n.s.		n.s.	†
β	1·11	1·19	1·13	1·29	0·88	1·56	1·32	1·74	1·13		1·13	1·65
Pinus strobus												
A	10·3	2·5	0·4	2·1	3·44	4873	2·34	3136	63·7	—	705	528
	(0·8)	(0·3)	(0·1)	(0·3)	(0·31)	(610)	(0·10)	(440)	(4·6)		(144)	(103)
E	13·5	3·9	0·7	3·3	2·70	7260	2·23	6593	88·2	—	929	732
	(4·8)	(0·6)	(0·1)	(0·5)	(0·25)	(1260)	(0·23)	(121)	(3·2)		(203)	(177)
P	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	***		n.s.	n.s.
β	1·32	1·56	1·72	1·57	0·78	1·49	0·95	2·10	1·38		1·32	1·39
Tsuga canadensis												
A	4·8	2·9	0·3	2·5	0·73	8514	3·52	1690	20·1	18·0	419	243
	(0·4)	(0·3)	(0·1)	(0·3)	(0·03)	(873)	(0·27)	(301)	(3·2)	(2·4)	(174)	(61)
E	6·5	4·3	0·5	3·9	0·52	11775	3·35	1611	14·1	26·4	438	440
	(1·2)	(0·8)	(0·1)	(0·7)	(0·03)	(1575)	(0·37)	(654)	(5·2)	(3·6)	(138)	(174)
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
β	1·35	1·48	1·36	1·56	0·71	1·38	0·95	0·95	0·70	1·47	1·05	1·10

n.s. $P > 0{\cdot}10$, † $P < 0{\cdot}10$, * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

† Soil samples collected from main root pot.

‡ Soil samples collected from root exclusion chamber.

A, mean values for ambient CO_2 levels; E, mean values for elevated CO_2 level (standard errors in parentheses); β, ratio of elevated over ambient CO_2 means. *P*, probability (from ANOVA) that ambient CO_2 level means = elevated CO_2 level means. EM, ectomycorrhizas, absolute number of colonized root tips, % colonized. AM, arbuscular mycorrhizas, percentage of total root length containing vesicles and/or arbuscles.

Table 2. *Results of 3-way ANOVA for all one-value per plant measures*

		Biomass (g)					Root tips		EM		Hyphal length (mm g^{-1} soil)	
	df	Whole plant	Total roots	Woody roots	Fine roots	Shoot:root ratio	Number per plant	Number g^{-1} d. wt	Number per plant	%	Roots present†	Roots absent‡
Species (S)	2	***	***	***	***	***	***	***	***	***	n.s.	***
CO_2 (C)	1	*	**	n.s.	**	**	*	n.s.	n.s.	**	n.s.	*
S × C	2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.
Block	2	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	n.s.	**	n.s.
Error	46											

† Soil samples collected from main root pot.
‡ Soil samples collected from root exclusion chamber.
*** $P < 0{\cdot}001$, ** $P < 0{\cdot}01$, * $P < 0{\cdot}05$, n.s. $P > 0{\cdot}05$.

and fine roots, the greatest increase in woody roots under elevated CO_2 was found in *P. strobus* (72 %). In *B. papyrifera*, woody root biomass increased marginally (13 %) under elevated CO_2. Although the magnitude of increase was greater for *T. canadensis* (36 %) it was also not significant. Fine root biomass increased in all three speices, the increase was greatest in *P. strobus* (57 %) and *T. canadensis* (56 %). The relative enrichment in CO_2 of the shoot:root ratio decreased in the order *B. papyrifera* < *P. strobus* < *T. canadensis*. A decrease in the shoot:root ratio was statistically significant only in the case of *Pinus strobus*, but no statistically significant species × CO_2 interactions were found for any biomass parameters (Table 2).

The total number of root tips per plant increased with CO_2 enrichment for all species. The increase was greatest in *B. papyrifera* (56 %) and smallest in *T. canadensis* (38 %) (Table 1). In *B. papyrifera* the increase in the number of root tips per plant was accompanied by a 32 % increase in the number of root tips per unit root mass. In *P. strobus* and *T. canadensis* the number of roots tips per unit root mass did not increase under elevated CO_2.

In ambient CO_2 there were large differences in the degree of ectomycorrhizal colonization between the three species (Table 1, Fig. 1). In *B. papyrifera* over 84 % of the root tips were ectomycorrhizal, whereas in *P. strobus* only 63 % and *T. canadensis* only 20 % of the root tips of were ectomycorrhizal. Under elevated CO_2 a significant increase in the degree of ectomycorrhizal colonization was shown in *B. papyrifera* and *P. strobus*, but not in *T. canadensis* (highly significant species × CO_2 interaction). In *B. papyrifera* the increase in degree of mycorrhizal colonization was such that almost all root tips were ectomycorrhizal. However, the greatest increase in the degree of mycorrhizal colonization with ectomycorrhizas was shown in *P. strobus* (110 %). In *T. canadensis* the degree of mycorrhizal colonisation with ectomycorrhizal fungi was unchanged; however, a statistically significant increase in the degree of colonization (47 %) with arbuscular mycorrhizas under elevated CO_2 was observed.

Growth under elevated CO_2 concentration not only led to an increase in the degree of ectomycorrhizal colonization in *B. papyrifera* and *P. strobus*, but also resulted in changes in the ectomycorrhizal morphotype assemblages (Fig. 1). On roots of *B. papyrifera*, 12 distinct ectomycorrhizal morphotypes were identified in both ambient and elevated CO_2. Under elevated CO_2 there was a significant increase in the frequency of four of the morphotypes and a significant decrease in frequency of two others. The greatest increase in frequency was shown in morphotype B1, which was white and had an extensive plectenchymatous hyphal mantle of irregularly arranged hyphae with emanating hyphae and rhizomorphs, closely matching descriptions of *Sclero-*

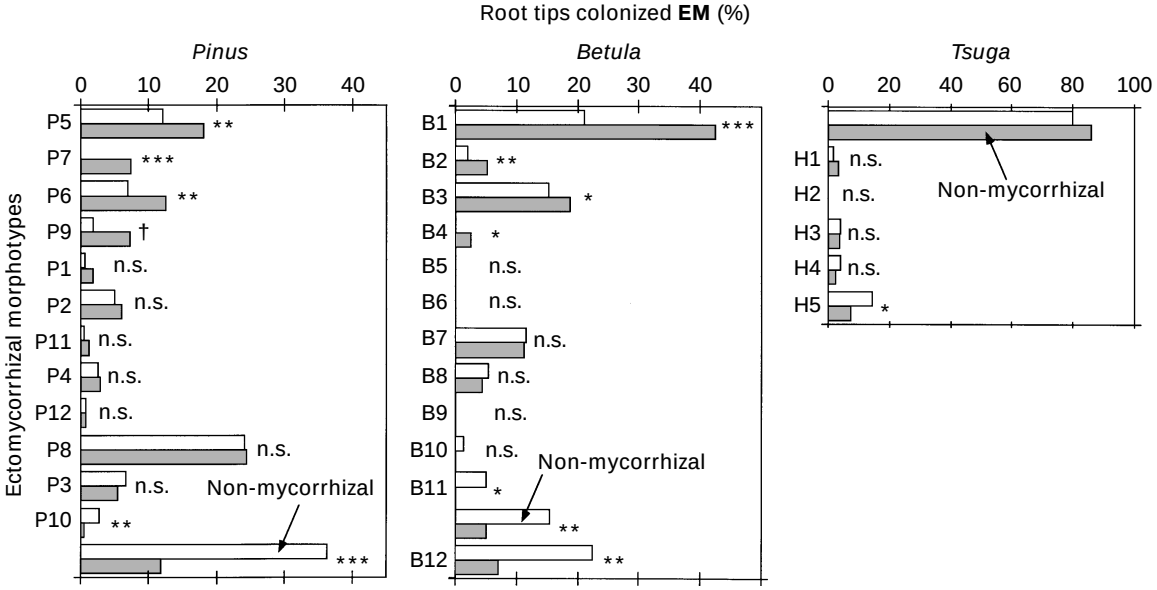

Figure 1. Mean relative frequency (%) of ectomycorrhizal morphotypes and non-mycorrhizal root tips in *Pinus strobus*, *Betula papyrifera* and *Tsuga canadensis* saplings growing under ambient (□) or elevated (■) CO_2. The indices next to each bar are the P values from the individual morphotype G-tests (n.s. $P > 0{\cdot}10$, † $P < 0{\cdot}10$, * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$).

derma citrinum (Agerer, 1992*b*). The morphotype which showed the second largest increase in elevated CO_2 (B2) was a dark brown mycorrhiza with many emanating hyphae and black rhizomorphs. Of the two other morphotypes (B3 and B4), which also increased in frequency, morphotype B3 was a black mycorrhiza colonizing the apical third of long (4–5 mm) lateral roots, and was the third most common morphotype. Morphotype B4 was a silver-brown monopodial-pinnate mycorrhiza and was only found on three plants. Of the two morphotypes which significantly decreased in frequency under elevated CO_2, morphotype B11 was rare, occurring on a third of the plants, and B12 was quite common. The rare (B11) morphotype had prominent white cystidia on a green plectenchymatous mantle, and was almost eliminated under elevated CO_2. The greatest decrease in frequency was found in the second most common morphotype (B12), which had a thin white/brown hyaline plectenchymatous mantle with no emanating hyphae or rhizomorphs.

In *P. strobus* there was also a significant increase in frequency of three morphotypes and a significant decrease in frequency of one morphotype under elevated CO_2. All three morphotypes which increased under elevated CO_2 increased in overall frequency between 6–8 %. Morphotype P7, a dichotomous mycorrhiza with loose white hyphae over an orange mantle was only found under elevated CO_2. The hyphae formed an irregularly arranged plectenchymatous mantle. The diameter of the hyphae in the mantle varied considerably, the largest being 2–3 times that of the smallest. Morphotype P5 was dichotomous, commonly had up to four ramifications at the branching points and an ochre, smooth plectenchymatous mantle with net-like hyphae. The deeper layer of the mantle was a pseudoparenchymatous mantle of lobed cells. Morphotype P6 was a dichotomously branched, smooth white mycorrhiza. The surface of the mantle was a plectenchymatous-pseudoparenchymatous transition type mantle, the deeper layer which a pseudoparenchymatous mantle of angular cells. The morphotype which decreased in frequency in elevated CO_2 (P10), was a thin black mycorrhiza which appeared to be growing over older, brown, highly ramified mycorrhizas.

On roots of *T. canadensis* five ectomycorrhizal morphotypes could be distinguished, and there was a significant change in the frequency of only one morphotype (H1 decreased) under elevated CO_2. Morphotype H1 was mostly unramified, black and carbonizing with black emanating hyphae.

The hyphal length density in the soil compartments is shown in Table 1. The values presented are after the subtraction of the background value (see 'Materials and Methods'). In *B. papyrifera* was 4–23 % of the total length, in *P. strobus* 4–28 % and in *T. canadensis* 12–50 %. Elevated CO_2 led to an overall increase in the hyphal length density within the root exclusion chambers (Table 1). However, when examining the response of individual species was examined, only *B. papyrifera* showed a clear statistical trend of increasing hyphal length. Hyphal length density was not significantly increased in the bulk soil for any of the species.

DISCUSSION

Large changes in biomass production, allocation, and patterns of mycorrhizal symbioses were observed for all three species under CO_2 enrichment.

All showed a small increase in whole-plant biomass, though larger increases were observed in root systems. This change in allocation resulted in trends toward reduced shoot:root ratios in all speices. There has been considerable debate about the validity of determinations of shoot:root ratios as a measure of changing allocation under elevated CO_2 (Stulen & den Hertog, 1993; Norby, 1994). In preliminary estimates of fine root turnover in these species, we found that root turnover in *B. papyrifera* was far greater than in *P. strobus* or *T. canadensis* (Godbold & Berntson, unpublished; Berntson & Bazzaz 1996, 1997). This suggests that if shoot:root ratios were derived from gross biomass production, these ratios would be reduced more in *B. papyrifera* than in the other species.

Although the smallest degree of enhancement in fine root biomass was seen in *B. papyrifera*, the number of root tips per unit d. wt increased markedly under CO_2 enrichment. This suggests an increase in branching of *B. papyrifera* fine roots under CO_2 enrichment. This was not seen in either *P. strobus* or *T. canadensis*. However, in all three species, growth under elevated CO_2 increased the total number of root tips. Thus, in *B. papyrifera* the increase in the number of root tips is a consequence of greater root growth and an increase in branching, whereas in *P. strobus* and *T. canadensis* the increase is due to increased root growth alone. These changes in root morphology and/or size might have important implications for the ability to acquire nutrients from the soil. In general, increased size and, possibly, branching, in *B. papyrifera*, are likely to lead to an increase in the total amount of soil explored by plant-root systems and thus in their potential to acquire nutrients from the soil (Berntson, 1994).

Considerable differences in patterns of ectomycorrhizal colonization were observed between the three tree species. An increase in the degree of ectomycorrhizal colonization has been shown in a number of tree species (reviewed in O'Neill *et al.*, 1991; O'Neill, 1994), but this response is not universal. In *Pinus sylvestris* grown in natural soil (Pérez-Soba *et al.*, 1995), and in *Pinus taeda* inoculated with *Pisolithus tinctorius* (Lewis *et al.*, 1994) no increases were observed. The changes in degree of ectomycorrhizal colonization might be driven by changes in root carbohydrates in both ectomycorrhizal (Marx, Hatch & Mendicino, 1977) and arbuscular mycorrhizal fungi (Douds & Schenk, 1990). However, Lewis *et al.* (1994) found an increase in soluble sugars in roots of pine without a rise in percentage of short roots colonized. All three species investigated in the present work increased biomass allocation to roots. However, the ectomycorrhizal response differed considerably between species. In *T. canadensis* the lack of a significant increase in ectomycorrhizal fungi was probably not driven by a lack of carbohydrates, since the percentage of root length colonized by arbuscular mycorrhizas increased significantly.

In *B. papyrifera* and *P. strobus* not only did the total fraction of root tips colonized by ectomycorrhizas increase, but the assemblage of morphotypes colonizing the roots changed. In *B. papyrifera* the morphotype which showed the single largest increase had an extremely thick mantle and a large number of emanating hyphae and rhizomorphs. The increase in this morphotype was at the expense of a morphotype with a thinner mantle, few emanating hyphae, and no rhizomorphs. An increase in hyphal density was observed in the root exclusion chamber in *B. papyrifera*. This 'local' increase in hyphae could result from the high density of morphotype B1 mycorrhizas which formed on the mesh–soil interface of plants grown at elevated CO_2. Although little is known about nutrient acquisition by individual species of ectomycorrhizas, it is becoming increasingly clear that there are differences in the physiological performance (Bending & Read, 1995*a*) and hyphal production (Rousseau *et al.*, 1994) of different species.

In *P. strobus* no single morphotype increased so greatly in frequency as in *B. papyrifera*. The changes in the ectomycorrhizal species assemblage in the almost completely ectomycorrhizal *B. papyrifera* suggests that there might have been competition between morphotypes for available non-mycorrhizal root tips. From this work it cannot be determined whether the changes in assemblage are regulated by the plants or the fungi. We suggest that the increased total allocation of C belowground by plants in elevated CO_2 environments might be a controlling factor in the response of different ectomycorrhizas to elevated CO_2.

The morphological typing used here is not sufficient to identify the mycorrhizas to species. Several studies have shown that a morphotype might be composed of several species (Egli *et al.*, 1993; Erland, 1995). Thus, the analysis of mycorrhizal morphotype assemblages presented needs to be taken as a preliminary analysis. In general, analyses of morphotypes tends to under-represent actual species diversity (Egli *et al.*, 1993). If this error is consistent between both CO_2 levels we examined, then the results strongly indicate that elevated CO_2 is likely to be an important factor in influencing the species assemblage of ectomycorrhizas and thus, possibly, terrestrial ecosystem nutrient-cycling and productivity.

ACKNOWLEDGEMENTS

Alison Li, Colin Godbold, and Jasmin Godbold provided valuable assistance in harvesting the plants. We thank Georg Jentschke for reading the manuscript, and Christine Rapp for advice in typing the morphotypes. For D. L. G.,

this research was supported by a Bullard Fellowship from the Harvard Forest. For G.M.B., this research was performed under appointment to the Graduate Fellowships for Global Change Program administered by ORISE from the U.S. DOE, Office of Health and Environmental Research, Atmospheric and Climate Research Division.

REFERENCES

Agerer R. 1992*a*. Characterization of ectomycorrhiza. *Methods in Microbiology* **23**: 25–73.

Agerer R. 1992*b*. *Colour atlas of ectomycorrhizas*. Schwäbisch Gmünd, Germany: Einhom-Verlag.

Bassirirad H, Thomas RB, Reynolds JF, Strain BR. 1996. Differential responses of root uptake kinetics of NH_4^+ and NO_3^- to enriched atmospheric CO_2 concentration in field-grown loblolly pine. *Plant, Cell and Environment* **19**: 367–371.

Bending GD, Read DJ. 1995*a*. The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behaviour and translocation of nutrients from exploited litter. *New Phytologist* **130**: 401–409.

Bending GD, Read DJ. 1995*b*. The structure and function of the vegetative mycelium of ectomycorrhizal plants. VI. Activities of nutrient mobilizing enzymes in birth litter colonized by *Paxillus involutus* (Fr.) Fr. *New Phytologist* **130**: 411–417.

Berntson GM. 1994. Modelling root architecture: are there trade-offs between efficiency and potential of resource acquisition? *New Phytologist* **127**: 483–493.

Berntson GM. 1996. *Root growth and nitrogen cycling in temperate deciduous forests in an elevated* CO_2 *world*. Ph.D. thesis, Harvard University, USA.

Berntson GM, Bazzaz FA. 1996. The influence of elevated CO_2 on the allometry of root production and root loss in *Acer rubrum* and *Betula papyerifera*. *American Journal of Botany* **83**: 608–616.

Berntson GM, Bazzaz FA. 1997. The influence of elevated CO_2 on the magnitude and seasonal dynamics of root production and root loss in *Betula papyrifera*. *Plant and Soil* **190**: 211–216.

Berntson GM, Woodward FI. 1992. The root system architecture and development of *Senecio vulgaris* in elevated carbon dioxide and drought. *Functional Ecology* **6**: 324–333.

Bougher NL, Grove TS, Malajczuk N. 1990. Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor* F. Muell.) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. *New Phytologist* **114**: 77–85.

Brundrett M, Melville L, Peterson L. (eds) 1994. *Practical methods in mycorrhiza research*. Canada: Sidney.

Cain ML. 1994. Consequences of foraging in clonal plan species. *Ecology* **75**: 933–944.

Colpaert JV, van Assche JA. 1993. The effect of cadmium on ectomycorrhizal *Pinus sylvestris* L. *New Phytologist* **123**: 325–333.

Douds DD, Schenk NC. 1990. Relationship of colonization and sporulation by VA mycorrhizal fungi to plant nutrient and carbohydrate contents. *New Phytologist* **116**: 621–627.

Egli S, Amiet R, Zollinger M, Schneider B. 1993. Characterization of *Picea abies* (L.) Karst. ectomycorrhizas: discrepancy between classification according to macroscopic versus microscopic features. *Trees* **7**: 123–129.

Erland S. 1995. Abundance of *Tylospora fibrillosa* ectomycorrhizas in south Swedish spruce forest measured by RFLP analysis of the PCR-amplified rDNA ITS region. *Mycological Research* **99**: 1425–1428.

Guehl JM, Garbaye J, Wartinger A. 1992. The effects of ectomycorrhizal status on plant-water relations and sensitivity of leaf-gas exchange to soil drought in Douglas fir (*Pseudotsuga menziesii*) seedlings. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ, eds. *Mycorrhizas in Ecosystems*. Wallingford, UK: CAB International, 323–332.

Ineichen K, Wiemken V, Wiemken A. 1995. Shoots, roots and ectomycorrhiza formation of pine seedlings at elevated atmospheric carbon dioxide. *Plant, Cell and Environment* **18**: 703–707.

Kåren O, Högberg N, Dahlberg A, Grip K, Nylund JE. 1996. Influence of drought on ectomycorrhizal species composition-morphotype versus PCr identification. In: Azcon-Aguilar C, Barea JM eds. *Mycorrhizas in Integrated Systems from Gene to Plant Development*. Brussels: EC, 43–46.

Koske RE, Gemma JN. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92**: 486–488.

Lewis JD, Thomas RB, Strain BR. 1994. Effect of elevated CO_2 on mycorrhizal colonization of loblolly pine (*Pinus taeda* L.) seedlings. *Plant and Soil* **165**: 81–88.

Maijala P, Ragerstadt KV, Raudaskoski M. 1991. Detection of extracellular cellulolytic and proteolytic activity in ectomycorrhizal fungi and *Heterobasidion annosum* (Fr.) Bref. *New Phytologist* **117**: 643–648.

Marschner P. 1994. *Einfluß der Mykorrhizierung auf die Aufnahme von Blei bei Fichtenkeimlingen*. Ph.D. thesis, Universität Göttingen, Germany.

Marx D, Hatch AB, Mendicino JF. 1977. High fertility decreases sucrose content and susceptibility of loblolly pine roots to ectomycorrhizal infection by *Pisolithus tinctorius*. *Canadian Journal of Botany* **55**: 1569–1574.

Monz CA, Hunt HW, Reeves FB, Elliott ET. 1994. The response of mycorrhizal colonization to elevated CO_2 and climate change in *Pascopyrum smithii* and *Bouteloua gracilis*. *Plant and Soil* **165**: 75–80.

Norby RJ. 1994. Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. *Plant and Soil* **165**: 9–20.

O'Neill EG. 1994. Responses of soil biota to elevated atmospheric carbon dioxide. *Plant and Soil* **165**: 55–65.

O'Neill EG, Luxmoore RJ, Norby RJ. 1987. Increases in mycorrhiral colonisation and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched CO_2 atmosphere. *Canadian Journal of Forestry Research* **17**: 878–883.

O'Neill EG, O'Neill RV, Norby RJ. 1991. Hiearchy theory as a guide to mycorrhizal research on large-scale problems. *Environmental Pollution* **73**: 271–284.

Pérez-Soba M, Dueck TA, Puppi G, Kuiper PJC. 1995. Interactions of elevated CO_2, NH_3 and O_3 on mycorrhizal infection, gas exchange and N metabolism in saplings of Scots pine. *Plant and Soil* **176**: 107–116.

Pregitzer KS, Zak DR, Curtis PS, Kubiske ME, Teeri JA, Vogel CS. 1995. Atmospheric CO_2, soil nitrogen and turnover of fine roots. *New Phytologist* **129**: 579–585.

Prior SA, Rogers HH, Runion GB, Hendrey GR. 1994. Free-air CO_2 enrichment of cotton: vertical and lateral root distribution patterns. *Plant and Soil* **165**: 33–44.

Rapp C, Jentschke G. 1994. Acid deposition and ectomycorrhizal symbiosis: field investigations and causal relationships. In: Godbold DL, Hüttermann A, eds. *Effects of Acid Rain on Forest Processes*. New York: Wiley, 183–230.

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.

Rogers HH, Runion GB, Krupa SV. 1994. Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**: 155–189.

Rousseau JVD, Sylvia DM, Fox AJ. 1994. Contribution of ectomycorrhiza to the potential nutrient-absorbing surface of pine. *New Phytologist* **128**: 639–644.

Rygiewicz PT, Anderson CP. 1994. Mycorhizae alter quality and quantity of carbon allocated belowground. *Nature* **369**: 55–58.

Sokal RR, Rohlf FJ. 1981. *Biometry. 2nd edn*. New York: W.H. Freeman.

Stulen I, den Hertog J. 1993. Root growth and functioning under atmospheric CO_2 enrichment. *Vegetatio* **104/105**: 99–115.

Sylvia DM. 1992. Quantification of external hyphae of vesicular–arbuscular mycorrhizal fungi. *Methods in Microbiology* **24**: 53–65.