Elevated atmospheric CO₂ concentration changes ectomycorrhizal morphotype assemblages in *Betula papyrifera*

D. L. GODBOLD¹,² and G. M. BERNTSON¹

¹ Department of Organismic and Evolutionary Biology, Harvard University, Biological Laboratories, 16 Divinity Avenue, Cambridge, MA 02138, USA
² Permanent address: Forstbotanisches Institut, Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

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Summary  Ectomycorrhizae are extremely diverse, with different species of fungi having very different physiologies and morphologies that, in turn, confer a range of different benefits to the host plant. To test the hypothesis that elevated CO₂ leads to changes in the assemblage of ectomycorrhizae associated with trees, we examined the number and frequency of ectomycorrhizal morphotypes colonizing roots of *Betula papyrifera* Marsh. saplings grown at an ambient or elevated (700 ppm) atmospheric CO₂ concentration for 24 weeks. Elevated CO₂ resulted in significant changes in the composition of the ectomycorrhizal assemblage toward morphotypes with a higher incidence of emanating hyphae and rhizomorphs. We conclude that *B. papyrifera* saplings will be able to support a more costly mycorrhization in future elevated-CO₂ atmospheres.

Keywords: carbon dioxide, ectomycorrhizae, hyphae, mycorrhizal morphotype, rhizomorph.

Introduction

The concentration of atmospheric CO₂ has been steadily rising since pre-industrial times (Boden et al. 1994). Increasing concentrations of atmospheric CO₂ have significant impacts on the earth’s climate system because it is a radiatively active gas (Schneider 1989), and may also lead to changes in terrestrial ecosystem structure and function through the direct effects of elevated CO₂ on plant physiology, development, and growth (Bazzaz 1990). The ability of plants to respond to rising atmospheric CO₂ concentrations with increased growth depends on their ability to acquire soil nutrients and water (Norby 1994, Berntson and Bazzaz 1996).

The ability of plants to acquire soil resources in an elevated-CO₂ environment may be mediated by symbiotic associations with mycorrhizal fungi. Several recent studies have shown that elevated-CO₂ can lead to significant increases in the extent of mycorrhizal colonization (extent of association, on a percent root length, short root, or root tip basis), and these effects appear to be greater for ectomycorrhizal associations than for endomycorrhizal associations (O’Neill 1994, Ineichen et al. 1995). Thus, alteration in the degree of mycorrhizal colonization may be an important component of belowground plant responses to elevated CO₂. O’Neill et al. (1987) obtained preliminary evidence that elevated CO₂ can decrease the relative occurrence of *Cenococcum graniforme* Fr. on *Quercus alba* L.; however, few studies have documented how ectomycorrhizal assemblages in trees are affected by elevated CO₂.

Ectomycorrhizal fungi are diverse, and many different species can colonize a single tree (Fleming 1983, Taylor and Alexander 1989, Allen et al. 1995). Differences among ectomycorrhizal species in their functional morphology (e.g., total hyphal density produced, Rousseau et al. 1994), ability to improve host plant mineral nutrient status (Bougher et al. 1990), drought tolerance (Guelh et al. 1992) and resistance to heavy metal toxicity (Colpaert and Assche 1992, Marschner 1994) have been shown. These differences in physiology and morphology of ectomycorrhizal species demonstrate the need to understand the effects of elevated CO₂ on mycorrhizal symbioses and function. To gain insight into possible changes in species composition of ectomycorrhizal fungi in response to increasing atmospheric CO₂ concentrations, we quantified the degree of colonization of all morphologically distinct types of ectomycorrhizae on roots of saplings of *Betula papyrifera* Marsh. grown for 24 weeks at an ambient or elevated (700 ppm) atmospheric CO₂ concentration.

Materials and methods

Saplings of paper birch (*Betula papyrifera*), which were 10–15 cm high, and had 4–6 fully expanded leaves, were collected from Harvard Forest NSF LTER site (Petersham, MA). The saplings were excavated in intact soil cores and transferred to 7.6-dm³ containers filled with a 10/10/1 (v/v) mixture of forest soil, sphagnum moss peat and silica sand. The forest soil, which was collected from an adjacent site at Harvard Forest, was a mixture of the humic layer and approximately 0–3 cm of the mineral soil. The soil was sieved through an 8-mm screen to remove roots and large stones.

Three days after the saplings had been collected, they were transferred to climate-controlled greenhouses and grown in an ambient or elevated atmospheric CO₂ concentration for 24 weeks. Each CO₂ chamber was replicated three times with three plant replicates in each chamber giving a 3 × 3 × 2 treatment design. Throughout the 24-week treatment period, a 12-h photoperiod was maintained with supplementary lighting (high intensity discharge mercury va-
por lamps; 350–450 µmol m⁻² s⁻¹), the average day/night temperature was 26/21 °C, and the CO₂ concentrations were maintained at about 375 ppm (ambient CO₂ treatment) or 700 ppm (elevated CO₂ treatment).

To obtain a representative sample of root tips, the soil–root ball was carefully removed from the pot, and all roots collected from a previously defined 7 × 8 cm area of the pot. A soil core was then cut through the center of the soil–root balls and all roots collected within the core. From these roots, two random subsamples were taken and carefully washed. The roots of each subsample were stored on moist filter paper in petri dishes at 4 °C until analyzed. On average, 250 (standard error = 26) individual root tips were examined from each of the two subsamples taken per plant.

Mycorrhizal root tips were categorized by morphotype on the basis of morphology, color, characteristics of the surface of the hyphal mantle, and planar views of different mantle layers by the methods described by Agerer (1992). Initial categorization and counting of root tips was carried out with the aid of a binocular dissecting microscope at a magnification of 10–30×. Analyses of the mantle structure and plan view of the mantle layers were carried out with the aid of a compound microscope and photomicrographs.

To summarize the data quantitatively, we first employed a nested, repeated measures ANOVA (fixed effects = CO₂, Block, mycorrhizal morphotype nested in individual plant). However, residuals were highly heteroscedastic because of the high variation in frequency among different morphotypes. We then used the G-test to quantify overall CO₂ effects, homogeneity of replicate frequency counts (individual plants), and differences in the mean observed frequency of the different morphotypes between the two CO₂ treatments. We used a replicated goodness of fit test employing the G-test to assess overall CO₂ effects and homogeneity of replicates within each CO₂ treatment (Sokal and Rohlf 1981). For each CO₂ treatment, we used the average frequency observed in the other CO₂ concentration as the null frequency for this G-test. For testing differences in the average observed frequency of each morphotype between the two CO₂ concentrations, we carried out separate G-tests for each morphotype with an assumed null frequency of 1/1 between CO₂ concentrations. A significant G-statistic for this test rejects the hypothesis that the observed frequency of a given morphotype was equivalent between the two CO₂ concentrations.

Overall, the heterogeneity in observed morphotype frequencies among replicates in both CO₂ treatments constituted about 60% of the total G-statistic (Table 2). The remaining lack of fit was caused by the CO₂ treatments. Although the degree of heterogeneity among replicate plant samples was high and statistically significant, the overall effect of CO₂ on the frequencies of the different morphotypes was also highly statistically significant (P < 0.01). The difference in observed ectomycorrhizal morphotype frequencies caused by CO₂ treatment was largely the result of significant changes in frequency of the four most commonly observed morphotypes among the 13 root tip morphotypes identified in the ambient CO₂ treatment (P < 0.01; 12 mycorrhizal morphotypes plus one nonmycorrhizal root tip; Figure 1). Of the four most commonly occurring morphotypes, two showed significant increases in frequency in response to elevated CO₂, whereas the other two decreased in frequency.

The fifth most common morphotype (Cenococcum) showed no change in frequency with increase in atmospheric CO₂ concentration, which is in contrast to the findings of O’Neill et al. (1987) who observed a qualitative decrease in frequency of Cenococcum with elevated CO₂. Significant changes in frequency were also observed in some of the less common morphotypes. For example, the chocolate-brown morphotype, which was the eighth most common in the ambient CO₂ treatment, was significantly more common in the elevated CO₂ treatment than in the ambient CO₂ treatment, whereas the cistidia morphotype was present in the ambient CO₂ treatment, but almost entirely absent in the elevated CO₂ treatment.

Exposure to elevated CO₂ caused an increase in colonization by morphotypes producing abundant emanating hyphae and a high frequency of rhizomorphs (woolly and chocolate-brown). This increase occurred at the expense of the black tip morphotype, which had a smooth mantle, few emanating hyphae and no rhizomorphs. The number of nonmycorrhizal root tips decreased in response to elevated CO₂. Although no direct measurements of mantle thickness were made, planar views of the mantle showed that the smooth elongated black tip morphotype had fewer mantle layers than the woolly morphotype. In the smooth elongated morphotype, cortical cells could be observed through the hyphal mantle. This suggests that the elevated CO₂ treatment increased the frequency of those morphotypes with a thick mantle. Because of the low transmission of light through the chocolate-brown morphotype, only the mantle surface could be observed.

Although we know little about the physiological function of individual species of ectomycorrhizae, it is evident that there are large differences among species in their physiological effects on the host plant. This has been shown for nutrient acquisition (Bending and Read 1995), drought tolerance (Guehl et al. 1992) and resistance of trees to heavy metal toxicity (Colpaert and Assche 1992, Marschner 1994). Central to the benefits conferred by ectomycorrhizae is the production of an extensive extramatrical mycelium either to explore the surrounding soil or to bind toxic elements. However, production of the extramatrical mycelium puts a high C demand on the host plant. In Pinus sylvestris L., a negative correlation
between plant growth and fungal biomass has been shown (Colpaert et al. 1992). Similarly changes in ectomycorrhizal assemblage from early- to late-stage fungi may be related to carbohydrate cost (Deacon and Fleming 1992). Late-stage fungi may have a higher carbohydrate cost than pioneer fungi.

Marx et al. (1977) reported that the degree of colonization with late-stage fungi was correlated with sugar concentrations in roots. We found that elevated CO$_2$ resulted in significant changes in the composition of the ectomycorrhizal assemblage toward morphotypes with a high production of hyphae and rhizomorphs. Because we cannot identify the morphotypes to genus or species, it is not possible to determine whether ectomycorrhizal succession is being driven by elevated CO$_2$, and hence by the assimilate supply to the roots. However, we demonstrated that, under conditions of increased atmospheric CO$_2$ concentration, birch saplings can support a more costly mycorrhization, which may have positive benefits for nutrient and water acquisition. This may be a critical factor in understanding tree response to predicted elevated atmospheric CO$_2$ concentrations.

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Figure 1. Average frequency (%) of occurrence of 12 identified morphotypes and nonmycorrhizal root tips in ambient CO$_2$ (open bars) and elevated CO$_2$ (solid bars). Error bars are a single standard error of the mean. Morphotypes are arranged in order according to the difference between the observed frequency in elevated CO$_2$ (above the line) and in ambient CO$_2$ (below the line).

References

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References