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Lifetime and temporal occurrence of ectomycorrhizae on ponderosa pine (*Pinus ponderosa* Laws.) seedlings grown under varied atmospheric CO_2 and nitrogen levels*

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Abstract

Climate change (elevated atmospheric CO_2, and altered air temperatures, precipitation amounts and seasonal patterns) may affect ecosystem processes by altering carbon allocation in plants, and carbon flux from plants to soil. Mycorrhizal fungi, as carbon sinks, are among the first soil biota to receive carbon from plants, and thereby influence carbon release from plants to soil. One step in this carbon release is via fine root and mycorrhizal turnover. It is necessary to know the lifetime and temporal occurrence of roots and mycorrhizae to determine the capacity of the soil ecosystem to sequester carbon assimilated aboveground. In this study, ponderosa pine (*Pinus ponderosa* Laws) seedlings were grown under three levels of atmospheric CO_2 (ambient, 525 and 700 μmol CO_2 mol^{-1}) and three levels of annual nitrogen additions (0,100 and 200 kg N ha^{-1}) in open-top chambers. At a two-month frequency during 18 months, we observed ectomycorrhizal root tips observed using minirhizotron tubes and camera. The numbers of new mycorrhizal root tips, the numbers of tips that disappeared between two consecutive recording events, and the standing crop of tips at each event were determined. There were more mycorrhizal tips of all three types seen during the summer compared with other times of the year. When only the standing crop of mycorrhizal tips was considered, effects of the CO_2 and N addition treatments on carbon allocation to mycorrhizal tips was weakly evident. However, when the three types of tips were considered collectively, tips numbers flux of carbon through mycorrhizae was greatest in the: (1) high CO_2 treatment compared with the other CO_2 treatments, and (2) intermediate N addition treatment compared with the other N addition treatments. A survival analysis on the entire 18 month cohort of tips was done to calculate the median lifetime of the mycorrhizal root tips. Average median lifetime of the mycorrhizal tips was 139 days and was not affected by nitrogen and CO_2 treatments.

Introduction

The atmospheric/climatic factors associated with climate change, i.e., increased atmospheric CO_2 concentration, altered air temperature and precipitation (amounts and patterns), are hypothesized to affect plant, soil and ecosystem processes. Specifically, alterations in carbon (C) physiology due to climatic effects on assimilation and C allocation, likely will lead to changes in C flux from plants to soil (Curtis et al., 1994) and from soil to atmosphere (Vose et al., 1995). Mycorrhizae, as C sinks, play an important role in C allocation (Rygiewicz and Andersen, 1994), affect nutrient uptake and cycling (Harley and Smith, 1983), and influence regeneration of plants and thereby sus-

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tainability of ecosystems (Perry et al., 1989). Mycorrhizal fungi are among the first biota in soil to receive C from plants. Subsequent release of C as root exudates and by death of mycorrhizae and nonmycorrhizal hyphae may provide C to support enhanced soil foodweb activity.

Plants may respond to increased C assimilation under changed climates by producing larger root systems with different allometric relationships among root sizes (e.g., changed proportions of mycorrhizae, and nonmycorrhizal fine, intermediate and coarse roots). Carbon allocated belowground also may be used to support colonization by mycorrhizal fungi and development of hyphae in soil thus increasing plant growth. Rates of mycorrhizal colonization and extraradical mycelial development on Scots pine (*Pinus silvestris* L.) were greater under increased CO_2 where three times more mycorrhizal root clusters and twice more mass of extraradical mycelia were formed compared with the ambient CO_2 treatment (Ineichen et al., 1995). Elevated CO_2 may increase C allocation to fine roots of tree species (and perhaps root exudation), while total amount of C allocated to the root system remains unchanged. Norby et al. (1987) found increased allocation to fine roots and mycorrhizal colonization for shortleaf pine (*Pinus echinata* Mill) seedlings grown under elevated CO_2. O'Neill et al. (1987b) found increased numbers and proportions of ectomycorrhizal short root tips, and a more rapid colonization in white oak (*Quercus alba* L.) grown at elevated CO_2. However, O'Neill et al. (1987a) found similar mycorrhizal colonization levels between yellow-poplar (*Liriodendron tulipifera* L.) grown at ambient and elevated CO_2; greater root development and concomitantly greater mycorrhizal development did occur in the elevated CO_2 treatment. Elevated CO_2 may offset the temporarily reduced seedling growth rate sometimes found due to colonization by the mycobiont, for example, as found by O'Neill et al. (1987b) in *P. echinata* seedlings.

There appears to be an interaction among CO_2 concentrations, nutrient levels, environmental conditions, and mycorrhizal colonization (Lewis et al., 1994, 1996; Tingey et al., 1995). Depending upon the extent of hypothesized increases in water- and nutrient-use efficiency under elevated CO_2 (e.g., white oak (*Quercus alba* L.) Norby et al., 1986), less C may be supplied to support mycorrhizae because the host may meet its resource needs with less reliance on the symbiosis. In two studies (Norby et al., 1986; O'Neill et al., 1987b) a differential response of mycorrhizal fungal species to elevated CO_2 was found. *Cenococcum graniforme* colonization varied from abundant under ambient CO_2 to under-represented or absent under elevated CO_2 conditions. A higher proportion of nitrogen (N) occurred in fine roots of seedlings grown under atmospheric CO_2 enrichment than in control seedlings which was attributed to increased N retention resulting from increased C allocation to roots (Norby et al., 1986). They also found greater phosphorus uptake under elevated CO_2 which may be related to proliferation of fine roots and mycorrhizae under elevated CO_2. Results on responses of VAM plants to elevated CO_2 are also variable: no increase in colonization of grasses (Whitbeck, 1994); and variable colonization levels depending on temperature and water availability, and whether the plants were C3 or C4 (Monz et al., 1994).

Regardless of how mycorrhizae respond to elevated atmospheric CO_2 concentrations, estimates of their lifetime are needed to predict the capacity of the soil component of ecosystems to sequester C assimilated aboveground. To address this question, we followed the life history of ectomycorrhizae of ponderosa pine (*Pinus ponderosa* Laws) seedlings grown under elevated atmospheric CO_2 and varying levels of N addition.

Materials and methods

Experimental design

This work is part of a larger project on the effects of elevated atmospheric CO_2 and soil N on the growth of ponderosa pine seedlings conducted by the Desert Research Institute (DRI), University of Nevada System, at the USDA Forest Service Institute of Forest Genetics, Placerville, CA (Johnson et al., 1994).

In 1991 three seeds were sown in native soil at each of 21 locations in each open top chamber (8.4 m^2 footprint); the soil and its chemistry have been described previously (Johnson et al., 1994). Three, one-meter long, plastic minirhizotron tubes (5 cm i.d.) were installed in each chamber during the week of 17 August 1992 as described previously (Johnson et al., 1995). The tubes extended into the Bt (argillic) horizon and were at 45° from vertical. The tubes were placed along three of the four ordinal directions, halfway between the seedling of interest and its nearest neighbor. Seedlings were thinned to 21 per chamber resulting in approximately 2.5 seedlings m^{-2}. During the study additional seedlings were removed, but care was taken to leave the plants proximal to the minirhizotron tubes.

The treatments consisted of three levels of atmospheric CO_2 (ambient, 525 and 700 μmol CO_2 mol^{-1}) and three levels of N (0, 100 and 200 kg N ha^{-1}, added annually in the spring as $(NH_4)_2SO_4$). The middle treatment (i.e., 525 μmol CO_2 mol^{-1} and 100 kg N ha^{-1}) was omitted. The treatment structure was a 3×3 factorial with one treatment excluded (i.e., 3 CO_2 levels $\times$ 3 N levels = 9 treatments – the middle treatment = 8 treatments, see Johnson et al., 1994). There are three replicate chambers per treatment yielding 24 chambers in the experiment. The experimental design and chamber operation are more fully described in Ball et al. (1992). Seedlings were watered weekly and all received the same amounts of water (Johnson et al., 1994). Gravimetric soil water content (at 15 cm depth) generally varied from 31 to 35% with the exception of October 1992 when the value dropped to 19%. Mid-day soil temperatures (at 15 cm depth) varied sinusoidally ranging from 2.2 °C (December) to nearly 20 °C (August); yearly mean is approximately 14 °C (Tingey et al., 1995).

Ectomycorrhizae image collection

Images were collected on S-VHS tape using a minirhizotron camera (Bartz Technology Co., Santa Barbara, CA USA) modified with an indexing handle that locks the camera into a prescribed location. Images were collected along the uppermost surface of the tubes every other month from October 1992 through April 1994 (i.e., 10 recording events during the 18 month period) to assess mycorrhizae formation, development and death. The viewing image is approximately 1.76 cm^2 (1.1 cm along the tube length $\times$ 1.6 cm across the tube width). Forty-five frames were recorded in each tube at each recording event, for a total of 135 frames, or approximately 238 cm^2 soil, per chamber. The recorded frames comprise approximately 10% of the total surface area of each tube (see Johnson et al., 1995 for details). At each recording event, 3240 frames (approximately 5702 cm^2 soil) were recorded which totaled more than 32000 frames during the 18 month study period.

Extraction of data from video images

Video images were transcribed into numerical data using the 'ROOTS' PC-based interactive software program (Hendrick and Pregitzer, 1992) as modified by Johnson et al. (1995). Video images are displayed on a video monitor that magnifies the images (nearly 25 $\times$). Root tips were considered ectomycorrhizal if they were branched, less than 2 mm in diameter, luminescent (mantle), and had blunt, rounded tips. Individual mycorrhizal root tips were followed during the course of the 18 month study. At each recording event, the numbers of: (1) new tips that appeared, (2) remained in the image, and (3) that disappeared since the previous recording event, were determined.

Statistical analyses

Since the intermediate CO_2 and intermediate N treatment was omitted from the design, the eight remaining treatments were treated as 8 separate treatments rather than as a factorial arrangement with a missing combination. All analyses were carried out using the SAS® programming language (SAS Institute Inc., 1989).

An equation was developed to calculate, at each recording event, three measures of mycorrhizal tips (i.e., net number of new tips that appeared between recording events, net number of tips that disappeared between recording events, and the standing crop of tips). The equation is depicted by:

$$\mathrm{Net}_t = (\mathrm{Total\ New}_t + \mathrm{Net}_{t-1}) - \mathrm{Total\ Disappeared}_t \quad (1)$$

where:

Net_t = Standing Crop at recording event t

$\mathrm{Total\ New}_t$ = (Tips that were formed and disappeared between nrecording events t and $t-1$) + (Tips that were formed between recording events t and $t-1$, and then observed at recording event t)

Net_{t-1} = Standing Crop at recording event $t-1$

$\mathrm{Total\ Disappeared}_t$ = (Tips that were formed and disappeared between recording events t and $t-1$) + (Tips that were observed at $t-1$, and not observed at recording event t).

Note that the first component of the terms Total New_t and Total $\mathrm{Disappeared}_t$ is the same number, but used with opposite sign in Equation 1. Tips that were formed and disappeared between recording events t and t–1 were not observed or measured by us. However, their overall contribution to the model is zero. This results in unavoidable underestimates of the numbers for Total New_t and Total $\mathrm{Disappeared}_t$. However, we are assuming that the number of tips that were formed and disappeared between recording events t and t–1 have the same kinds of patterns for all treatments and

dates. Thus we are assuming that this underestimation does not affect our estimate of Net_t.

The square root of the number of mycorrhizal tips in each category was used in the analyses to satisfy constant variance assumptions. A repeated measures analysis (Multivariate ANOVA) was used to test for differences between treatments and recording events accounting for correlations between recording events (α = 0.10). One-way ANOVA was used to test for differences between treatments for a given recording event. Pairwise comparisons of treatment means were carried out using Fisher's Protected Least Significant Difference Test (α = 0.10, Steel and Torrie, 1980) to compare means from a single recording event, and 90% confidence intervals were calculated for treatment means.

In order to investigate the lifetimes of the mycorrhizal tips we used a nonparametric survival analysis (Lawless, 1982) to compare lifetimes in different treatments and to estimate the median lifetime and corresponding 90% confidence intervals. The analysis was done for the entire population of mycorrhizal tips observed during the 18 month study period and ignored any effects that would arise due to the first time a mycorrhizal tip was observed. That is, tips first observed near the end of the study period might be thought to have a different pattern of survival than tips first observed at the beginning of the study period. However, we did not set up this study to investigate these kinds of patterns and we lack adequate sample sizes for each cohort to do this.

Results

Temporal occurrence

Virtually all the root tips observed (99.9%) were ectomycorrhizal in symbiosis with *Thelephora terrestris*. Our colonization levels were considerably higher than the percent formation values (22% to 48%) found by R Walker (unpublished data) who also examined trees from this project. Note that our values are % mycorrhizal root tips (MRT, i.e., %MRT = number of mycorrhizal tips/total number of tips appearing in the chamber × 100). Walker calculated % formation (i.e., % of total root length colonized by the ectomycobiont) determined on root systems that were removed from the soil by partial excavation.

Number of new mycorrhizal tips

As defined in Equation 1, Total New_t has two components. One is the number of tips that were formed and disappeared between recording events t and t–1. The other is the number of tips that were formed between recording events t and t–1, and then observed at recording event t. There is no way to assess the first component unless the frequency of recording events is shorter than the lifecycle of the shortest-lived mycorrhizal tip. Therefore, our estimate of the total number of new tips observed at each recording event is an underestimate of the total production of new tips.

The numbers of new mycorrhizal tips appear to follow a pattern of increased counts during the summer of 1993 followed by a decline through to the end of the study period (Figure 1a). Greatest numbers of new tips were found at recording events between 6/93 and 10/93, depending on the treatment. The decline in numbers of new tips reached levels slightly above those found at the beginning of the study where none to very few new mycorrhizae were observed. The pattern in numbers of new mycorrhizal tips over time differed among treatments (time × treatment interaction p = 0.0001). Significant differences in tip numbers among treatments were found for 6/93 (p = 0.04), 10/93 (p = 0.09) and 12/93 (p = 0.001). On 6/93, the high CO_2 and intermediate N treatment had the greatest number of new mycorrhizal tips; generally the other treatments had similarly fewer tips. On both 10/93 and 12/93, numbers of new tips were greatest in the intermediate CO_2 and high N treatment; again, in general, new tip number in all other treatments were similarly lower in comparison.

When numbers of new mycorrhizal tips were pooled according to CO_2 treatment (i.e., N treatments ignored), the pattern of numbers of new tips over time was not different for different treatments (time × treatment interaction p = 0.15, Figure 2a). The time effect was significant (p = 0.005) indicating that the pattern of numbers of tips over time was different from a flat horizontal line. Differences among treatments were found on 6/93 (p = 0.08) and 12/93 (p = 0.09). On 6/93 there were more new mycorrhizal tips in the high CO_2 treatment compared with the intermediate and low treatments (which were similar to each other). While on 12/93, we found a trend of the greatest number of new tips in the intermediate CO_2 treatment.

When numbers of new tips were pooled according to N treatment (i.e., CO_2 treatments ignored), the pattern of numbers of new tips over time was differ-

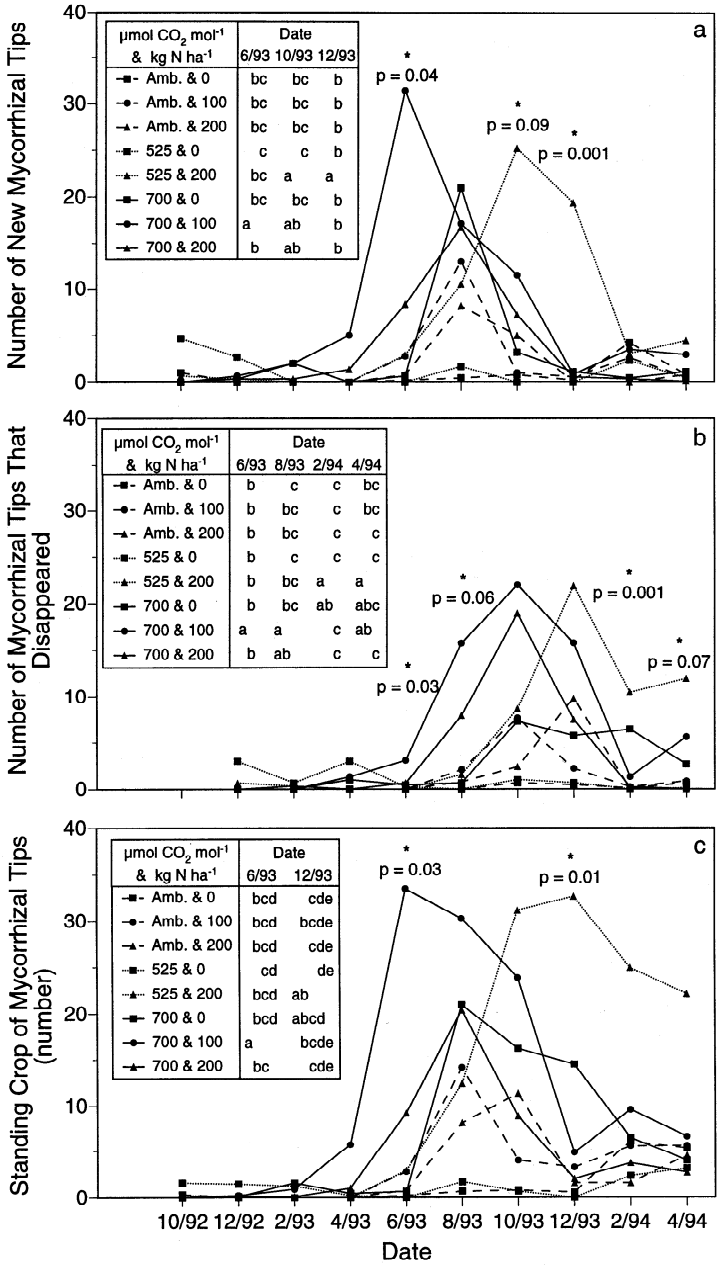

Figure 1. Numbers of mycorrhizal tips in the eight continuous CO_2 and annual N addition treatments observed using minirhizotron tubes in open-top chambers. Values presented are treatment means (3 tubes per chamber (a total of 238 cm^2 soil adjacent to the minirhizotrons were observed per chamber) and 3 chambers per treatment). Graphs represent, at each recording event, the (**a**) number of new mycorrhizal root tips observed (i.e., the tips that were not observed at the previous recording event), (**b**) number of mycorrhizal tips that disappeared since the previous recording event, and (**c**) standing crop of mycorrhizal tips. An asterisk signifies that a difference among treatments was found at the recording event indicated at the probability value (*p*) specified. Letters along side treatments indicate differences among treatments at the recording events where treatment effects were found; values followed by the same letter are not different ($\alpha = 0.10$). Note: the middle treatment (525 μmol CO_2, mol^{-1} and 100 kg N ha^{-1}) was not installed, and that the analyses for new tips (a) and tips that disappeared (b) were done using only dates 6/93 through 4/94 because there weren't enough tips before 6/93.

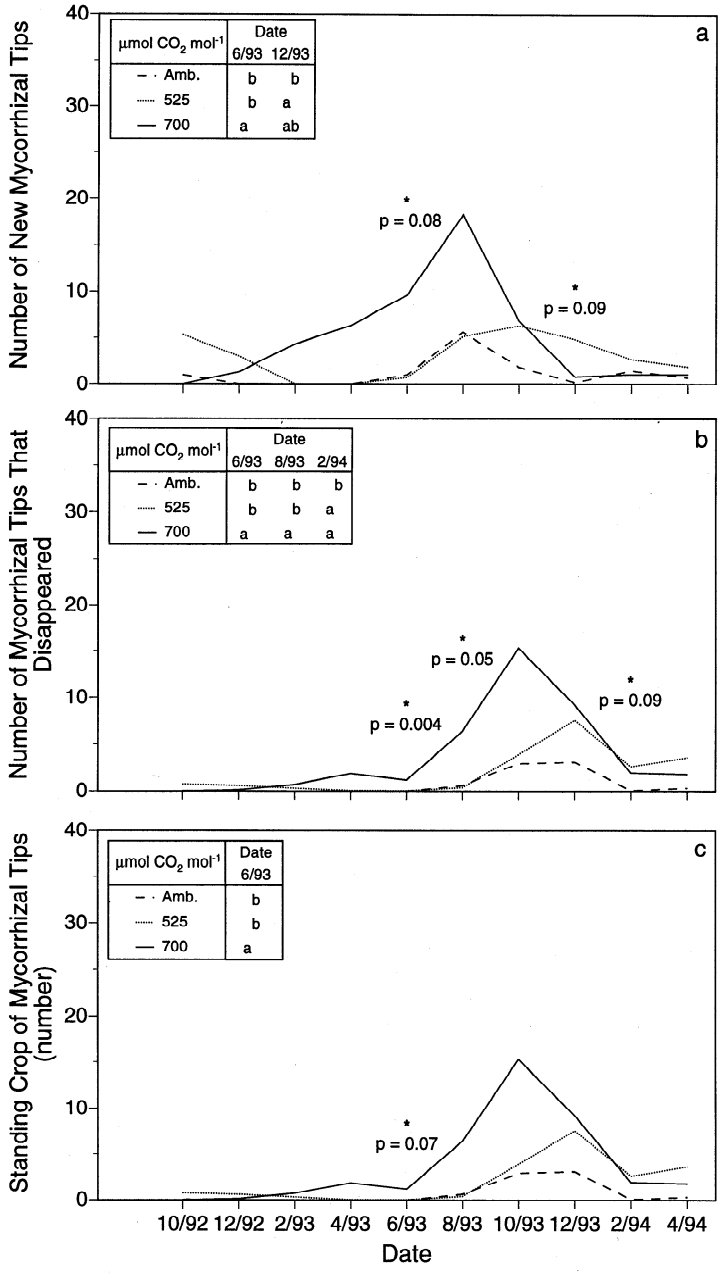

Figure 2. Numbers of mycorrhizal tips in open-top chambers for the three CO_2 treatments where the application of any N treatment to a chamber was ignored. All other conventions as in Figure 1.

ent among treatments (times × treatment interaction $p = 0.0004$, Figure 3a) indicating that the pattern of numbers of tips over time was different between N treatments. Differences among treatments were found for 6/93 ($p = 0.02$) and 10/93 ($p = 0.05$). We found a trend of the greatest number of new tips in the intermediate N treatment on 6/93. On 10/93, the trend was for the greatest number of new mycorrhizal tips to be found in the high N treatment.

Number of mycorrhizal tips that disappeared

As for the total number of new mycorrhizal tips, the total number of tips that disappeared is also an underestimate because there is no way to assess the number

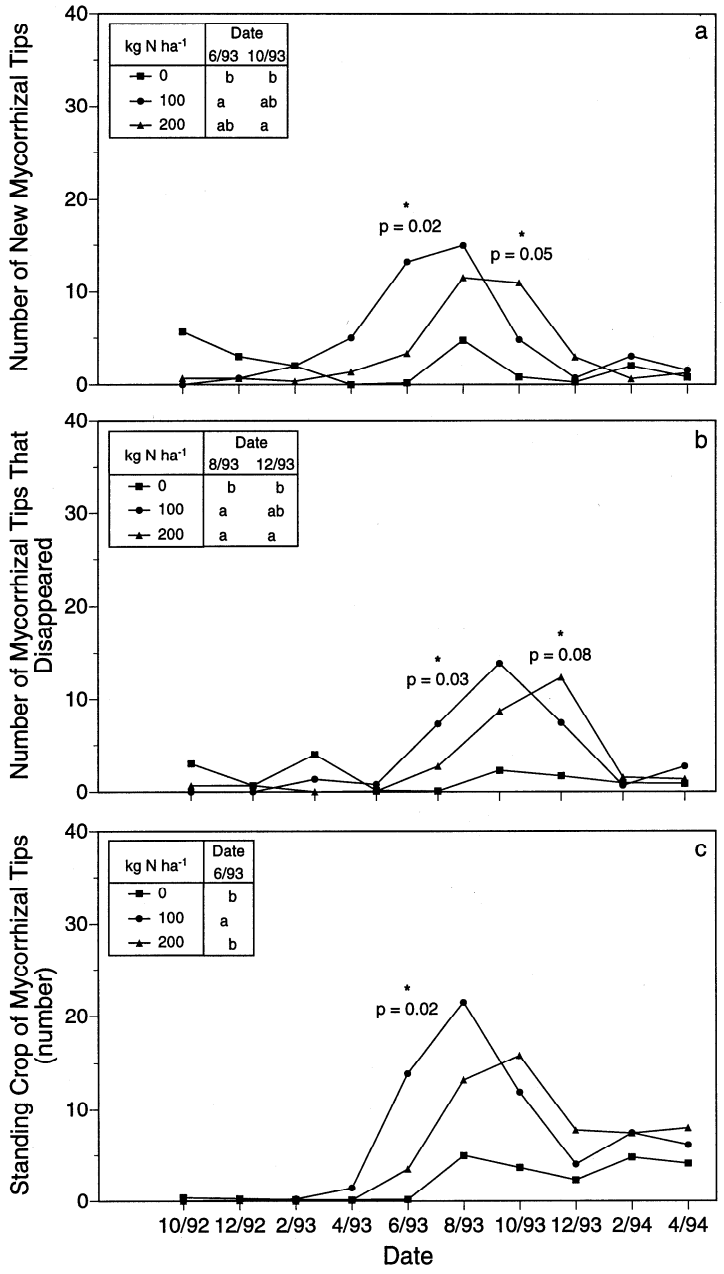

Figure 3. Numbers of mycorrhizal tips in open-top chambers for the three annual N addition treatments where the application of any CO_2 treatment to a chamber was ignored. All other conventions as in Figure 1.

of tips that were formed and disappeared between consecutive recording events, as noted above.

The numbers of mycorrhizal tips that disappeared also appear to follow a pattern of increased counts during the summer of 1993 followed by a decline through to the end of the study period (Figure 1b). However, for some treatments, the decline in numbers of tips that disappeared toward the end of the study did not reach low numbers similar to those found at the beginning of the study. The pattern in numbers of mycorrhizal tips that disappeared over time differed among treatments (time $\times$ treatment interaction $p = 0.0004$) indicating differences in the pattern over time between treatments. Numbers of mycorrhizal tips that disap-

peared peaked at recording events 10/93 and 12/93, slightly later than the recording events when the greatest numbers of new tips were observed. Significant differences in numbers of tips that disappeared among treatments were found for 6/93 (p = 0.03), 8/93 (p = 0.06), 2/94 (p = 0.001) and 4/94 (p = 0.07). On the first two dates, the high CO_2 and intermediate N treatment had the greatest number of mycorrhizal tips that disappeared; other treatments generally had similarly fewer tips. On the last two dates, there was a trend of the intermediate CO_2 and high N treatment to have the greatest numbers of tips that disappeared; all other treatments had similarly lower number of tips.

When numbers of tips that disappeared were pooled according to CO_2 treatment, the pattern over time was not different for different treatments (time × treatment interaction p = 0.71, Figure 2b). The time effect was significant (p = 0.01) indicating that the pattern of numbers of tips that disappeared over time was different from a flat horizontal line. Differences among treatments were detected on 6/93 (p = 0.04), 8/93 (p = 0.05) and 2/94 (p = 0.09). On 6/93 and 8/93, there were more mycorrhizal tips that disappeared in the high CO_2 treatment compared with the intermediate and low treatments. On 2/94, the high and intermediate CO_2 treatments had similar and higher tip numbers than were found in the ambient CO_2 treatment.

When tip numbers were pooled according to N treatment, the pattern of numbers of tips that disappeared over time was different for different treatments (time × treatment interaction p = 0.07, Figure 3b). Differences among treatments were found for 8/93 (p = 0.03) and 12/93 (p = 0.08). On both dates the high and intermediate N treatments had similar and higher number of mycorrhizal tips that disappeared compared with the no added N treatment.

Standing crop of mycorrhizal tips

The standing crop of mycorrhizal tips also appears to follow a pattern of increased counts during the summer of 1993 followed by a decline through to the end of the study period (Figure 1c). The decline in the standing crop of tips reached levels slightly above those found at the beginning of the study where none to very few mycorrhizae were observed. The pattern in the standing crop over time did not differ among treatments (time × treatment interaction p = 0.11). Generally, peak numbers of the standing crop of tips coincided in time with when maximum numbers of new tips were observed. Significant differences among treatments were found for 6/93 (p = 0.03) and 12/93 (p = 0.01). On 6/93, the high CO_2 and intermediate N treatment had the largest standing crop of tips. The standing crops in the other treatments were lower and similar compared among themselves. On 12/93, the standing crops of tips were not easily categorized according to treatment. In general, the largest standing crop of tips was in the intermediate CO_2 and high N addition, and the high CO_2 and intermediate N addition treatments, while the fewest tips were in the ambient CO_2 and no added N, and intermediate CO_2 and no added N treatments.

When the standing crop of tips numbers were pooled according to CO_2 treatment, the pattern of the standing crop of tips over time was not different for different treatments (time × treatment interaction p = 0.42, Figure 2c). The time effect was significant (p = 0.01) indicating that the pattern of standing crop over time was different from a flat horizontal line. On 6/93 differences were detected among treatments (p = 0.07); there were more mycorrhizal tips in the high CO_2 treatment compared with the intermediate and low treatments (which were similar to each other).

When standing crop numbers were pooled according to N treatment, the pattern over time was not different for different treatments (time × treatment interaction p = 0.64, Figure 3c). The time effect was significant (p = 0.01) indicating that the pattern of numbers of tips over time was different from a flat horizontal line. Differences among treatments were found only for 6/93 (p = 0.02); the largest standing crop of tips was found in the intermediate N treatment followed by lower but similar numbers in the no added N and high N addition treatments.

Lifetime

The average median lifetime for the mycorrhizal tips in the 18 month study period was 139 days, ranging from 123 to 185 days for the eight treatments. However, the confidence intervals overlapped among treatment median lifetimes so we did not find effects of the CO_2 or N addition treatments. Figure 4 exemplifies a temporal sequence spanning six months of mycorrhizal tip formation, development and disappearance that was used to estimate the median lifetime. By observing tips in the same image frame through time, one sees mycorrhizal tips with lifetimes of varying lengths; for example, compare tip M1 with M2.

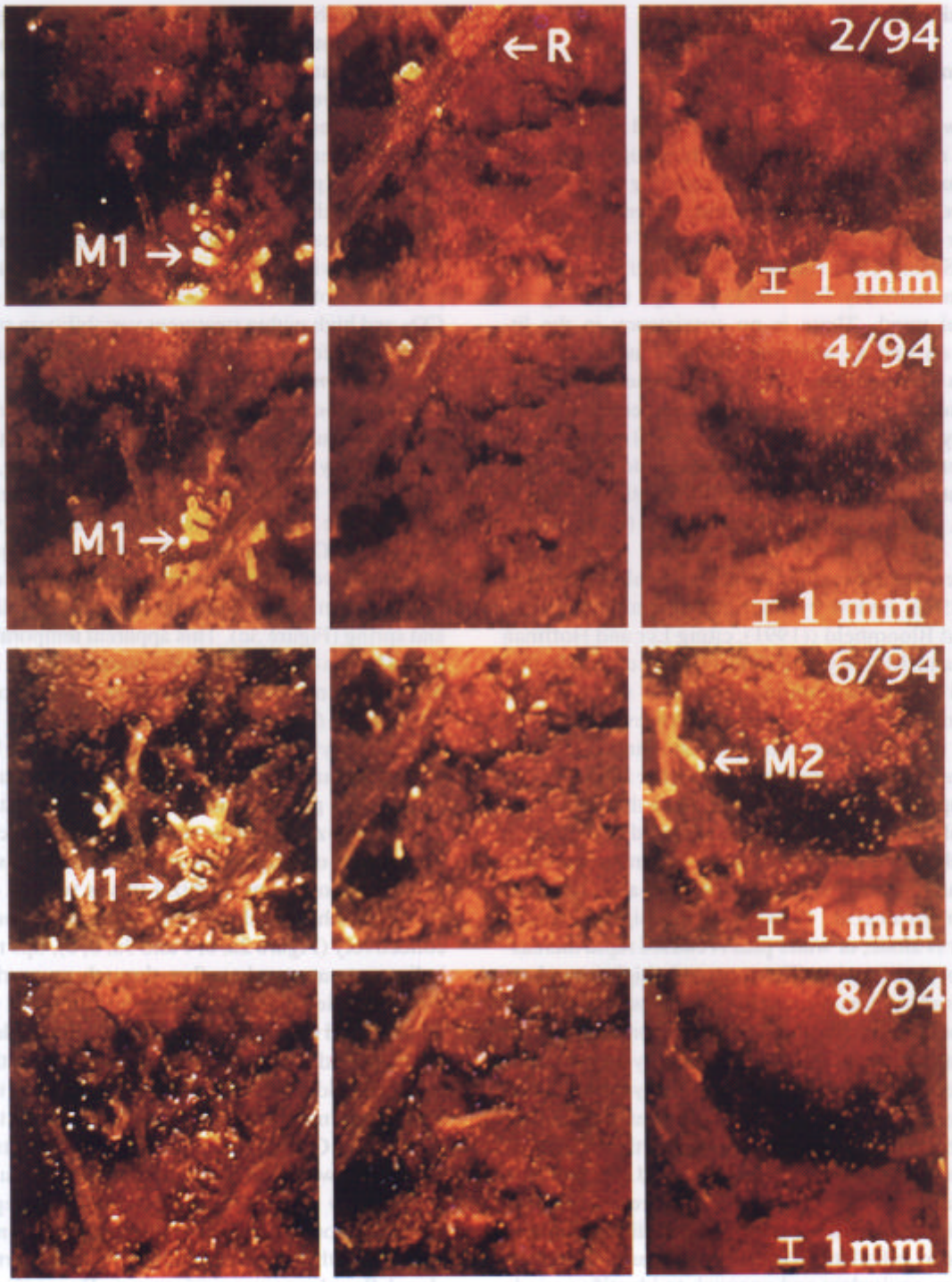

Figure 4. Temporal sequence (2/94, 4/94, 6/94 and 8/94) of minirhizotron camera images of the same mycorrhizal root tips, associated extraradical hyphae and nonmycorrhizal root segments of ponderosa pine seedlings grown in an open-top chamber. Each series of three exposures in the temporal sequence depicts the same 33 mm by 16 mm (each frame is 11 mm by 16 mm) of soil adjacent to the minirhizotron tube. M1 (left column) and M2 (right column) indicate mycorrhizal tips that have different lifetimes, and R (middle column) identifies a root segment. Note: colors recorded on video tape may not match actual colors of images.

Discussion

Temporal occurrence

There was a significant temporal trend in the numbers of all three types of mycorrhizal tips (new tips, those that disappeared, and the standing crop of tips) observed during the study period. There was a steep increase in numbers of all three types of tips observed during Spring 1993 followed by a relative decline in late Summer/early Fall 1993, perhaps signaling the temporal trend. There is no consistency in the literature on finding temporal fluctuations in fine root biomass and numbers. This lack of consensus is related to numerous factors including tree species, site productivity, aboveground tree phenological events, nutrient amendments, environmental conditions (drought, temperatures), and type of sampling method (i.e., soil corings, view windows, rhizotrons, etc.) (Edwards and Harris, 1977; Hendrick and Pregitzer, 1992; Keyes and Grier, 1981; Persson, 1978; Vogt et al., 1981; Vogt et al., 1982, review by Vogt and Bloomfield, 1991). Vogt and Bloomfield ((1991), citing Lyr and Hoffman (1967) and Mooney and Chu (1974)) conclude that reduced root growth during periods of shoot growth commonly have been observed. However, our data suggest that mycorrhizal tip formation increased during active shoot elongation when higher summer light and temperatures were prevalent. Note that water was not withheld during the summer, as would be the case in a natural field setting in California. Several researchers (Drew and Saker, 1975, 1978; Jackson and Caldwell, 1991; Pregitzer et al., 1993) found that higher levels of water or N alone, or water plus N caused rapid increases in root proliferation. The apparently higher numbers of mycorrhizal tips formed during the summer in this study indicate the plasticity in the response of mycorrhizae to changes in resource availability and environmental conditions. Disappearance of mycorrhizal tips was linked to tip formation in that the greatest numbers of tips that disappeared were found shortly after maximal numbers of tip formation were found. A larger data set (install more tubes, or record at a greater frequency) would be needed to perform more refined temporal analyses to calculate when formation and disappearance were equivalent and unequal, to identify periods of the greatest formation and turnover of mycorrhizal tips.

There were no persistent (i.e., for the 18 month study period) and consistent patterns in mycorrhizal tip numbers relative to the eight CO_2 and N treatments (Figure 1a, b, and c). For example, standing crop of tips in the intermediate CO_2 and high N treatment did not stand out from the other treatments at the earlier recording events, but then the treatment yielded some of the highest tip numbers during the latter half of the study period. The test for a significant temporal offset in tip production due to an interaction between CO_2 and N did not indicate such a lag compared with the other treatments, due primarily to a considerable amount of within treatment variability. Both a greater production of fine roots (nonmycorrhizal) at elevated CO_2 and high within treatment variability in root measures were found by Zak et al. (1993) working with *Populus grandidentata*.

More consistent patterns among treatments were evident when the tip data were analyzed for either only CO_2 or only N treatment effects. Generally, greater numbers of the standing crop of mycorrhizae were found at the high CO_2 treatment compared with the other two CO_2 treatments (Figure 2c). Nitrogen addition may have increased standing crop of tips during the summer months, but had little effect in the fall, winter and spring (Figure 3c). This apparent temporal pattern found here is different from the pattern for live fine root biomass found by Keyes and Grier (1981) working in Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) stands. They found higher relative fine root biomass during the summer in the low productivity stand, and no temporal pattern in the high productivity stand.

By analyzing only the standing crop of mycorrhizal tips, effects of CO_2 and N addition treatments on carbon allocation to mycorrhizal tips is weakly evident. However, when the three types of tips were considered collectively (Figure 2a, b, c and 3a, b, c), tips numbers indicate greater carbon flux through mycorrhizae in the: (1) pooled high CO_2 treatment compared with the other pooled CO_2 treatments, and (2) the pooled intermediate N addition treatment compared with the other pooled N addition treatments. Our data set is too variable to determine how nitrogen affects carbon throughput within a CO_2 treatment, or how CO_2 affects carbon throughput within an N addition treatment. Our results are partially supported by the literature. Pregitzer et al. (1995) found elevated atmospheric CO_2 increased rates of both fine root production and mortality of clonal *Populus* $\times$ *uramericana* cv. Eugenei, and that root mortality increased as soil nitrogen availability increased, regardless of CO_2 concentration. Working in the forest floor of even-aged, Douglas-fir stands of similar age (11 to 14 years), growing on sites of differing productivity classes, Vogt et al. (1983) found that

prior to and during crown closure there was no significant difference in mycorrhizal root biomass between productivity levels. However, after crown closure, the stands on the low productivity class sites had significantly higher mycorrhizal root biomass compared with the higher productivity stands.

Lifetime

The average median lifetime of mycorrhizal tips was approximately 3.5 months (139 days). There is no consistency in the literature concerning the lifetime of ectomycorrhizae. Through the 1960s, researchers considered whether ectomycorrhizae are ephemeral, annual structures; or longer-lived, persistent associations exhibiting variability in lifetimes relating to host and fungal species, seasonal patterns of development and maybe environmental constraints (reviews by Goss, 1960; Lobanow, 1960). Goss (1960) states that numerous researchers considered Masui's (1926) observation of renewed growth of mycorrhizae from structures formed the previous year as probably exceptional in conifers. However, Lobanow (1960) listed estimates of one year for root systems that were repeatedly excavated; two years in roots of young pine, oak and other forest tree species (Lobanow's own work), and two to four years after the tree trunk is harvested. Mikola and Laiho (1962) found spruce mycorrhizae lived more than one season, exhibiting renewed growth and branching in the second season. Harley (1969) repeatedly excavated and replaced the same cluster of ectomycorrhizae in a beech (*Fagus* sp.) forest and concluded that mycorrhizal colonization (1) was permanent (i.e., once a root system was colonized, some portion of the root system would remain colonized), (2) showed seasonal patterns in numbers of tips colonized, and (3) lasted for at least 9 months on tips along the same long root axis. Using a different type of technique (i.e., seasonal decrements of biomass in sequential soil cores), Vogt et al. (1982) determined that the mixed-species population of mycorrhizae in Pacific silver fir (*Abies amabilis* (Dougl.) Forbes) forests lived for 10 to 14 months. In these studies it is not explicitly indicated if the same mycorrhizal tip(s) were observed throughout the study. Orlov (1957, 1960) did follow the same mycorrhizal tips on spruce (*Picea excelsa* Link) during a five-year period by placing plastic sheets at the litter-soil interface above root clusters. Individual tips were observed by pulling back and replacing the litter at a frequency ranging from every 7 to 14 days. A microscope slide was embedded in the plastic sheet and a microscope was used to observe the tips. Most tips lived 2–3 years, with some living up to 4 years. Also, growth appeared to be dependent on the environment and not on plant-specific periodicity. In all studies cited above, the lifetimes of mycorrhizae are considerably longer than those found herein. Some estimates represent lifetimes of the longest-lived mycorrhizae while others (sequential cores) reflect net loss (formation - death) over time. Our values may not be directly comparable since we determined lifetimes by tracking the same mycorrhizae throughout their lives, and for the comparison with Orlov, the climate at our site is considerably warmer. Root longevity is increased at colder sites (Hendrick and Pregitzer, 1993).

Pregitzer et al. (1995) indicated that estimates of C input into soil due to mycorrhizae turnover are needed to determine how universal their results might be on nonmycorrhizal roots of *P.* × *euramericana* cv. Eugenei. Note, that species of *Populus* have been found to be either ectomycorrhizal or at times arbuscular-mycorrhizal (Harley and Harley, 1987; Trappe, 1962). Our mycorrhizal lifetime data indicate that the findings of Pregitzer et al. (1995) are not applicable to all types of root tips. While we found that the numbers of mycorrhizal tips that were formed and that disappeared increased in the pooled, high CO_2 treatment, their lifetime was unaffected by N and CO_2 treatments. Pregitzer et al. (1995) found shorter-lived nonmycorrhizal fine roots in the higher CO_2 treatments at both the low and high fertility treatments compared with respective lifetimes in the ambient CO_2 treatments. They also found nonmycorrhizal fine roots lived for shorter periods as N availability increased regardless of CO_2 levels.

Use of minirhizotrons

While the minirhizotron technique is being used increasingly to study fine roots, we are unaware of any published work using the technique to investigate mycorrhizae. We found the technique useful to monitor the formation and death of mycorrhizae. Unlike other methods that rely on excavation which can disrupt extraradical hyphae, the minirhizotron technique is a tool for making repeated, non-destructive observations in situ of the same mycorrhizae.

The minirhizotron technique does have shortcomings. For example, it is not possible to know when, between any two subsequent recording events, an individual tip may form or disappear. This results in at least two problems. First, the total production and

turnover of tips is underestimated (see descriptions above pertaining to Equation 1). Second, the accuracy of the estimate of the median lifetime is directly related to the recording frequency. As recording frequency increases, the accuracy of both of these estimates will increase. These problems are not unlike those presented when one uses the sequential coring method (Vogt and Bloomfield, 1991).

One way to manage overall workload and improve the utility of minirhizotron tubes, is to reduce the number of images recorded (tubes) while increasing recording frequency. However, this approach is not recommended. Nonmycorrhizal root tips (approximately 0.1% of all tips observed herein) and mycorrhizae appeared infrequently in this study. Using images recorded in this study, Tingey et al. (1995) found that less than 2% of the image frames contained mycorrhizae at each recording event between 10/92 and 10/93. Also, we found that mycorrhizae were not found in some tubes. As a result, in this study it was possible to do a survivorship analysis only if the population of mycorrhizal tips was considered as one cohort. We realize that lifetime of mycorrhizal tips may be different depending on the season in which they were formed. However, we had insufficient population sizes of tips for the individual cohorts appearing at each recording event to perform survivorship analyses on any other cohort. Thus, reducing the number of images collected, or subsequently examined, might severely reduce the power of the analyses performed.

When we designed this study our only estimates of mycorrhizal lifetimes were those presented in the literature using techniques other than minirhizotron tubes. Our results suggest that recording events take place at least monthly or sooner to conduct studies on the lifecycle of ectomycorrhizal root tips. Additionally, the number of minirhizotron tubes per treatment should be increased. Even though using minirhizotrons is labor intensive, it is a powerful tool to provide new insights into the phenology, morphology and demography of ectomycorrhizae.

Conclusion

Taken collectively, our results indicate that substantially more carbon enters the soil via mycorrhizae than previously thought. Virtually all of the root tips of the ponderosa pine seedings we observed were mycorrhizal. We calculated a median lifetime of these tips of approximately 3.5 months which indicates a lifecycle that is months to years less than previously found. The turnover and lifetime data should improve estimates of carbon throughput in soils made by modelers who incorporate a belowground component into their models.

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