

Effects of elevated CO₂ and nitrogen on the synchrony of shoot and root growth in ponderosa pine

DAVID T. TINGEY,¹ MARK G. JOHNSON,² DONALD L. PHILLIPS,¹ DALE W. JOHNSON³ and J. TIMOTHY BALL³

¹ U.S. Environmental Protection Agency, Corvallis, OR 97333, USA

² ManTech Environmental Technology, Inc., Corvallis, OR 97333, USA

³ Desert Research Institute, Reno, NV 89506, USA

Received October 25, 1995

Summary We monitored effects of elevated CO₂ and N fertilization on shoot and fine root growth of *Pinus ponderosa* Dougl. ex P. Laws. and C. Laws. grown in native soil in open-top field-exposure chambers at Placerville, CA, over a 2-year period. The experimental design was a replicated 3 × 3 factorial with the center treatment missing; plants were exposed to ambient (~365 μmol mol⁻¹) air or ambient air plus either 175 or 350 μmol mol⁻¹ CO₂ in combination with one of three rates of N addition (0, 100 or 200 kg ha⁻¹ year⁻¹). All CO₂ by N interactions were nonsignificant. Both the CO₂ and N treatments increased plant height, stem diameter and leaf area index (LAI). Elevated CO₂ increased fine root area density and the occurrence of mycorrhizae, whereas N fertilization increased coarse root area density but had no effect on fine root area density. Spring flushes of shoot height and diameter growth were initiated concurrently with the increase in new root area density but height and diameter growth reached their maxima before that of fine roots. The temporal patterns of root and shoot growth were not altered by providing additional CO₂ or N. Greatest root loss occurred in the summer, immediately following the period of greatest new fine root growth. Elevated N initially reduced the fine root area density/LAI ratio independently of CO₂ treatment, indicating that the relationship between fine roots and needles was not changed by CO₂ exposure.

Keywords: leaf area index, minirhizotron, N fertilization, open-top chambers, *Pinus ponderosa*, root–shoot balance.

Introduction

With increasing atmospheric CO₂ concentrations and associated climate change, the potential for direct effects of elevated CO₂ on forest trees is large. Numerous studies on leaves, stems, and branches have shown that elevated CO₂ increases carbon assimilation and plant biomass (e.g., Körner 1993, Rogers et al. 1994, Norby 1994). However, few studies have focused on the impacts of elevated CO₂ on root processes or determined if

elevated CO₂ alters root–shoot relationships or the timing of root or shoot growth.

Recent research suggests that an important plant response to elevated CO₂ is enhanced belowground carbon allocation (Curtis et al. 1990, Norby 1994) leading to increased resource acquisition (Lekkerkerk et al. 1990, Berntson and Woodward 1992, Norby 1994) as a result of CO₂-induced increases in (1) root density, (2) rate of root elongation, (3) root exudation, and (4) mycorrhizal colonization (Berntson and Woodward 1992). Proportionately more carbon was allocated to fine roots of *Pinus echinata* Mill. seedlings grown in elevated CO₂ than in ambient air (Norby et al. 1987) and fine root biomass of *Liriodendron tulipifera* L. increased in elevated CO₂ (Norby et al. 1992). In *Pinus echinata* Mill. and *Quercus alba* L., elevated CO₂ increased root growth more than shoot growth, suggesting a greater allocation of carbohydrates to roots (O'Neill et al. 1987).

A large portion of carbon assimilated by plants is allocated to fine root production (Fogel 1983). In many forests, fine root turnover can add more carbon and return more nutrients to the soil than litter fall (Hendrick and Pregitzer 1992, 1993). Addition of nitrogen to a mixed hardwood forest caused a rapid increase in new fine root production (Pregitzer et al. 1993). Similarly, nitrogen addition increased fine root extension in *Populus × euramericana*, but fine root biomass was unchanged (Pregitzer et al. 1995).

Plant response to CO₂ may be controlled by N availability, the nutrient most commonly limiting forest growth in the northern hemisphere (Johnson 1992). Eamus and Jarvis (1989) suggested that nutrient stress could eliminate the potential growth stimulation caused by elevated CO₂. Bazzaz (1990) reported that the stimulatory effect of elevated CO₂ disappeared under N or phosphorus limitation. However, Zak et al. (1993) proposed that, even under nutrient limiting conditions, CO₂ enrichment could increase belowground carbon allocation by increasing root exudation into the soil which, in turn, would result in increased organic matter mineralization and N availability. Based on the observation that growth of *Quercus*

alba seedlings in low nutrient soil was enhanced 85% by elevated CO₂, with the greatest growth enhancement occurring in the root systems, Norby et al. (1986) suggested that the occurrence of a growth response to CO₂ enrichment under conditions of low nutrient availability could be the result of increased P supply as a result of larger root systems or increased mycorrhizal associations.

Mycorrhizal associations enhance plant mineral nutrition (Allen 1991). The influence of elevated CO₂ and N on mycorrhizal infection is of interest because mycorrhizal infection is influenced by the carbohydrate status of the roots and the soil N concentration (Nylund 1988). O'Neill et al. (1987) observed increased mycorrhizal densities in *Pinus echinata* and *Quercus alba* in response to elevated CO₂ treatment, whereas Walker et al. (1995) found no enhancement of mycorrhizal infection in *P. ponderosa* exposed to elevated CO₂. This lack of response may have been associated with the nutrient content of the soil because increasing the concentration of nitrogenous compounds in the soil has been found to decrease the frequency of mycorrhizae (Harley and Smith 1983). Wallander and Nylund (1991) observed that high root carbohydrate concentrations could partially compensate for the effect of N on mycorrhizal development.

The objectives of the current study were to determine if elevated CO₂ and N fertilization changed (1) shoot and root growth, including mycorrhizal occurrence on *Pinus ponderosa* Dougl. ex P. Laws. and C. Laws. growing in native soil, (2) the functional relationship between needle and root growth, and (3) the temporal pattern of shoot and fine root growth.

Materials and methods

Experimental design and soil characteristics

The study is part of a long-term investigation of the effects of elevated CO₂ and N fertilization on ponderosa pine (*Pinus ponderosa*) being conducted by the Desert Research Institute (DRI), University of Nevada System, at the US Forest Service Institute of Forest Genetics (840 m; 38°44' N; 120°45' W) near Placerville, CA (Johnson et al. 1994, Tingey et al. 1995). The soil is an Aiken clay loam (Xeric Haplohumult derived from andesite) with a bulk density of 1.2 g cm⁻³ and a pH of approximately 6.0 (H₂O) throughout the profile (Johnson et al. 1995). Details of the experimental design, open-top chambers, and soil physical and chemical characteristics have been described by Johnson et al. (1994).

The experimental design is a replicated (three replicate 3 × 3 factorial) with three CO₂ concentrations (ambient air [~365 μmol mol⁻¹]; ambient air + 175 μmol mol⁻¹ [~535 μmol mol⁻¹]; and ambient air + 350 μmol mol⁻¹ [~720 μmol mol⁻¹]) and three rates of N addition (0, 100 and 200 kg ha⁻¹). However, the 100 kg ha⁻¹ N treatment at ambient + 175 μmol mol⁻¹ CO₂ was omitted from the experimental design because of financial constraints (Johnson et al. 1994). Ammonium sulfate was hand broadcast each March, except in 1994 when it was applied in April. Chambers received equal amounts of water each week.

In spring 1991, three ponderosa pine seeds were planted in each of 21 locations distributed throughout each of 24 hexago-

nal open-top chambers (3.6 m diameter) (Johnson et al. 1994). To prevent crowding and provide root samples, three trees were harvested from each chamber in October 1991 and 1993, and four trees were harvested in October 1992 and an additional 4–6 trees were cut at ground level in July 1993. Trees proximal to the minirhizotron tubes were not disturbed or harvested during the study.

Environmental conditions were recorded 1.7 m above-ground with a Campbell 012 meteorological station located approximately 10 m to the west of the site. Maximum and minimum air temperatures were measured daily and summarized as weekly means (Figure 1a); missing data were inferred from another monitoring site in the Placerville area. Soil temperature at a depth of 15 cm was measured periodically with a thermocouple inserted permanently in the soil (Figure 1b), and soil water (Figure 1b) was measured with gypsum block sensors and the data converted to volumetric water content based on water release curves.

Aboveground growth

Plant height (ground level to terminal bud) and stem diameter (at ground level) were measured every 1 to 3 months from May 1992 through December 1994. As part of the whole-plant harvest in October 1993, specific leaf area was determined for a subset of needles and plant leaf area was computed from the

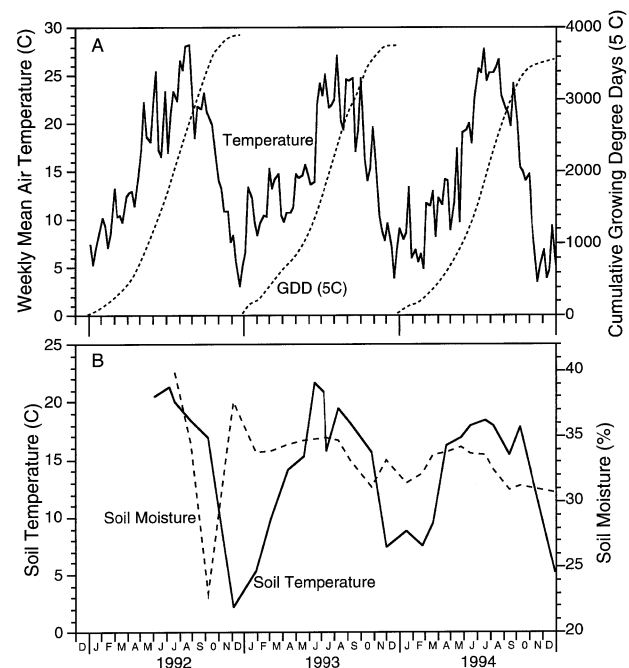


Figure 1. Air temperature, growing degree days, soil temperatures and soil water content over a 3-year period at the Placerville, CA research site. Air temperature (A) is presented as the weekly mean temperature. Growing degree days (5 °C base) are shown at weekly intervals (A). Air temperature was measured with a thermistor. Soil temperature (B) and water content (B) are the means of 24 chamber values for each sampling period. Soil temperature and water content data were measured at a depth of 15 cm.

product of specific leaf area and needle dry weight. Based on data from 60 plants (comprising all treatments and chambers) from the October 1993 harvest, we derived an equation [Leaf area = 25.736 Height × Diameter^{1.5}; $R^2 = 0.989$] relating stem diameter and plant height to plant leaf area. The equation was used to estimate leaf area for each plant in each chamber when stem diameter and height were measured. Leaf areas of the individual plants were summed and divided by the chamber ground area to estimate leaf area index (LAI). A single leaf area regression was used for all treatments because the R^2 for the pooled data indicated that 99% of the variance in leaf area was accounted for by the independent variable. Separate regressions for each treatment resulted in slope estimates that differed by no more than 8% from the pooled slope estimate.

Root image collection and data extraction

During August 1992, three minirhizotron tubes (1 m long with an inside diameter of 5 cm) were installed in each chamber at 45° from the vertical with the bottom of the tubes extending into the Bt (argillic) horizon (46 cm deep) as described by Tingey et al. (1995). Images were recorded every other month on the uppermost surface of the minirhizotron tubes with a minirhizotron camera system. Forty-five frames were recorded in each tube for a total of 135 images per open-top chamber per sampling event. Based on tube length and video camera field of view (1.1 cm high by 1.6 cm wide), a continuous strip of soil, 49.5 cm long and 1.6 cm wide and representing about 10% of the total minirhizotron tube surface area, was sampled in each minirhizotron tube.

Individual images were digitized by means of the "ROOTS" software program (Hendrick and Pregitzer 1992, Tingey et al. 1995). Root area densities were determined by multiplying the length and width of each individual root segment, summing these areas, and dividing by the total area of the video images in a minirhizotron to give the proportion of area covered by roots. Fine roots were ≤ 2 mm in diameter and coarse roots were > 2 mm in diameter. All roots were "new" when first observed hence new root area density was the area of roots that had appeared since the previous sampling. Roots that disappeared between samplings were classified as "lost;" lost root area density was calculated based on the root areas from the previous sampling. The occurrence of mycorrhizal fungi was inferred by the presence of monopodial, bifurcated or highly branched root tips. *Thelephora terrestris* Fr. was the predominant mycorrhizal fungus (R. Walker, personal communication). Mycorrhizal occurrence was defined as the proportion of video images containing mycorrhizal structures.

Statistical analysis

Because chambers were the experimental units to which treatments were applied, chamber means were used in all analyses. Statistical analyses for CO₂ and N treatment effects were performed for the aboveground variables of plant height, stem diameter, and LAI and the belowground variables of root area density and mycorrhizal occurrence. Log transformations were used for the aboveground and root area density variables, and

arcsin transformations were applied to the mycorrhizal occurrence data to normalize their distributions (Snedecor and Cochran 1989).

Data were analyzed by repeated measure ANOVAs, with tests for effects of CO₂ treatment (three concentrations), N treatment (three rates), sampling date (13 dates for belowground variables, 19 for aboveground variables), and their interactions. Time, CO₂ and N were all treated as fixed effects. Because of interactions between time and CO₂ or time and N treatments or both, further ANOVAs were performed for each time period. Because CO₂ and N were quantitative variables with equally spaced treatments, the ANOVAs were response surface analyses that examined for linear and quadratic responses to CO₂ and N treatments, and their interactions (Mize and Schultz 1985, Snedecor and Cochran 1989). The missing 100 kg ha⁻¹ N treatment at ambient + 175 μmol mol⁻¹ CO₂ treatment necessitated dropping the 100 kg ha⁻¹ N and ambient + 175 μmol mol⁻¹ CO₂ concentrations from the orthogonal polynomial contrasts for quadratic responses and interactions. Thus, the CO₂ response curves were based on the low (0) and high (200) N addition rates, and the N response curves were based on the low (ambient) and high (ambient + 350) CO₂ concentrations. Means for treatments and time periods presented below have been back-transformed and reported in the original units.

Results

For all growth responses, there were significant ($P < 0.001$) time × CO₂ and time × N interactions but no significant CO₂ × N interactions.

Aboveground responses

Until July 1993, there were no significant differences in plant height among CO₂ treatments (Figure 2a). However, after July 1993, plants in the elevated CO₂ (A+350) treatment were significantly taller than plants in the ambient (A) treatment and this difference persisted throughout the remainder of the study. Plants in the A+350 treatment were always tallest and plants in the A+175 treatment tended to be similar in height to plants in the A treatment. Initially, the elevated N treatments had a greater influence on plant height than the elevated CO₂ treatments. There was a significant linear increase in plant height with increasing N over the entire 3-year period (Figure 3a).

Stem diameter was more responsive to CO₂ treatment than plant height (Figure 2b). By May 1992, mean stem diameter of plants in the A+350 treatment was significantly larger than that of plants in the A treatment and this difference persisted for the remainder of the study. Mean stem diameter of plants in the A+175 treatment was intermediate between that of plants in the A+350 and A treatments. Stem diameters of plants in the N200 treatment were always larger than those of plants in the N0 treatment (Figure 3b), whereas stem diameters of N100-treated plants were of an intermediate size. The relative difference in stem diameters of plants in the high (N200) and low (N0) treatments remained constant over the study.

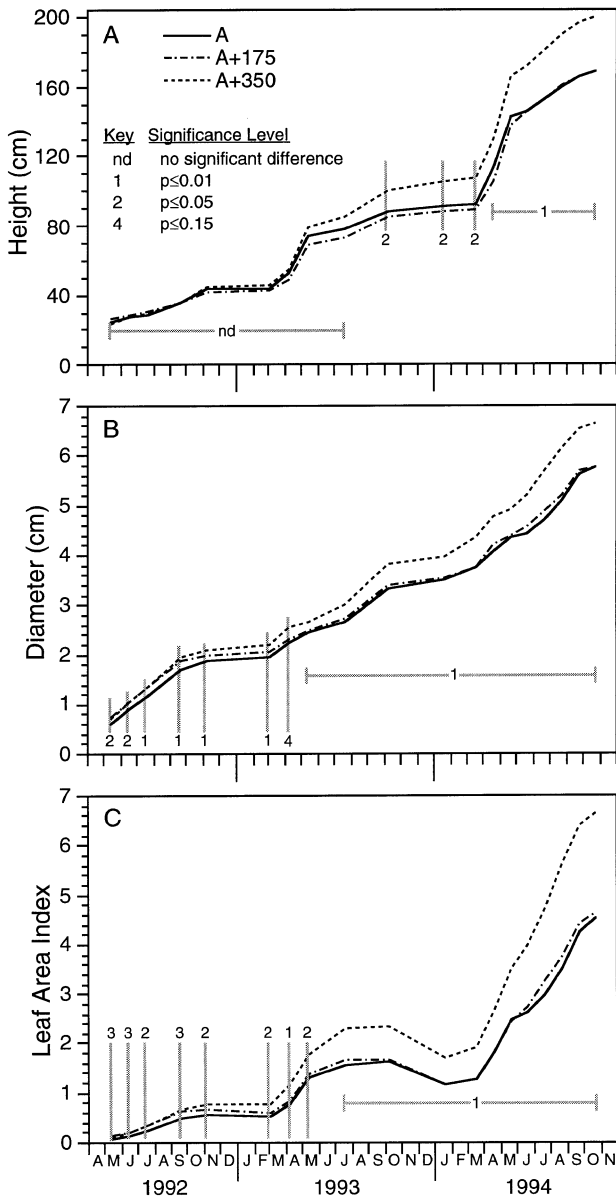


Figure 2. Effects of CO₂ treatment on height, stem diameter and LAI. The CO₂ treatments were: A = ambient air, A+175 = ambient air + 175 μmol mol⁻¹, A+350 = ambient air + 350 μmol mol⁻¹. Data for the CO₂ treatments were averaged across the low (N0) and high (N200) N treatments to account for the unbalanced experimental design. Means are based on six open-top chambers per treatment. The lines show the sample dates when there were significant linear treatment effects; the numbers and associated key show the significance levels of the tests.

Leaf area index increased significantly during the study (Figures 2c and 3c). Temporary declines in LAI were the consequence of periodic plant harvests to reduce aboveground crowding, rather than an indication of needle loss. The A+350 treatment significantly increased LAI, whereas plants in the A+175 treatment had intermediate values of LAI. Similarly, the N200 treatment significantly increased LAI (Figure 3c) compared to the N0 treatment. The LAI of N100-treated plants

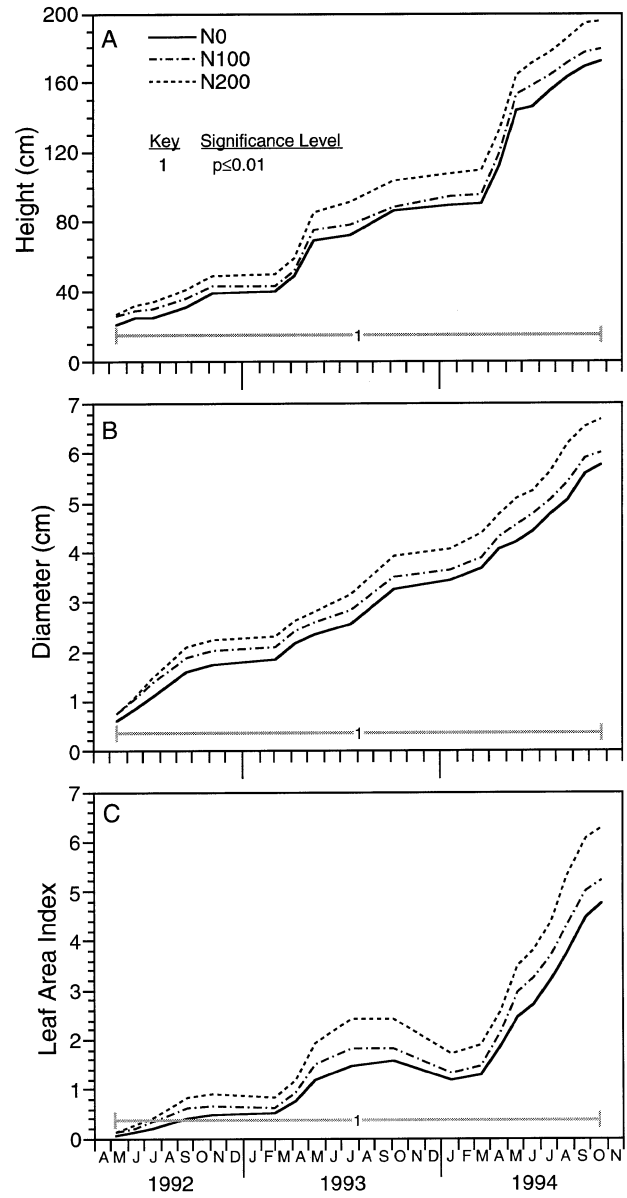


Figure 3. Effects of N fertilization on height, stem diameter and LAI. The N treatments were: N0 = no N added, N100 = 100 kg N ha⁻¹, N200 = 200 kg N ha⁻¹. Data for the N treatment were averaged across the low (A) and high (A+350) CO₂ treatments to account for the unbalanced experimental design. Means were based on six open-top chambers per treatment. The lines show the sample dates when there were significant linear treatment effects; the numbers and associated key show the significance levels of the tests.

was intermediate between that of the N200- and N0-treated plants.

Belowground responses

Variation in belowground responses was much larger than in aboveground responses. The pooled among-chambers within-treatment coefficients of variation (CVs) of plant height and stem diameter were 3 and 10%, respectively, whereas the

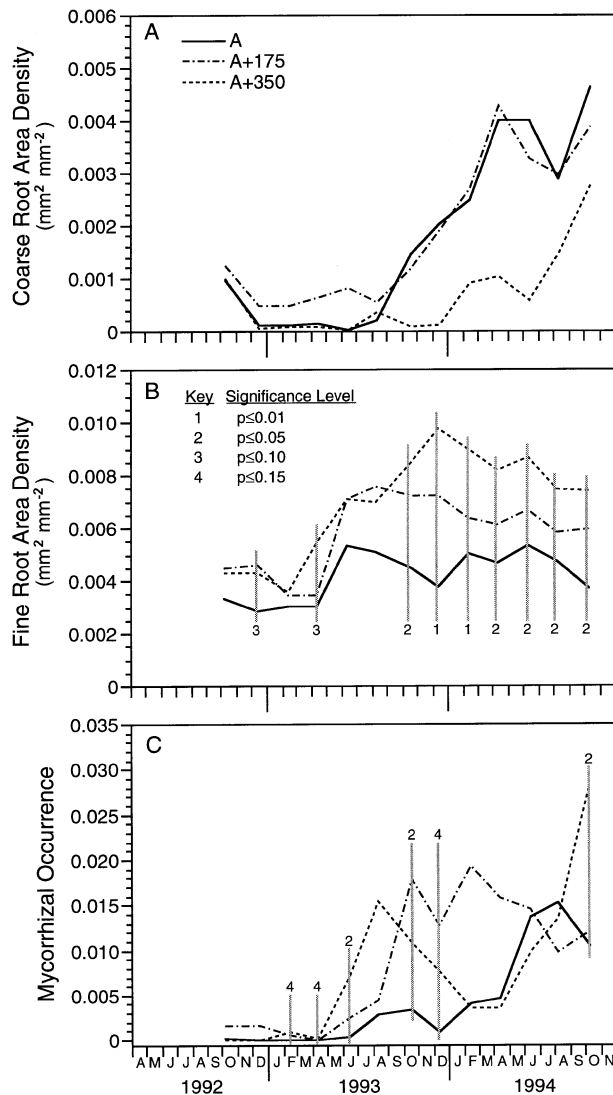


Figure 4. Effects of CO₂ treatment on coarse roots, fine roots and mycorrhizal occurrence. The CO₂ treatments were: A = ambient air, A+175 = ambient air + 175 $\mu\text{mol mol}^{-1}$, A+350 = ambient air + 350 $\mu\text{mol mol}^{-1}$. Data for the CO₂ treatments were averaged across the low (N0) and high (N200) N treatments to account for the unbalanced experimental design. Means are based on six open-top chambers per treatment. The vertical lines show the sample dates when there were significant linear treatment effects; the numbers and associated key show the significance levels of the tests.

pooled CV for fine root area density was 31% and the pooled CVs for coarse root area density and mycorrhizal occurrence were greater than 100%. The CVs associated with coarse root area density and mycorrhizal colonization are high partly because only a small number of measurements were made. For example, coarse roots accounted for only 5% of all the root segments observed in the study; however, their root area density was approximately half of that of the fine roots during the latter portion of the study (Figures 4 and 5).

Very few coarse roots were observed until the fall of 1993; subsequently their occurrence increased in both the CO₂ and N

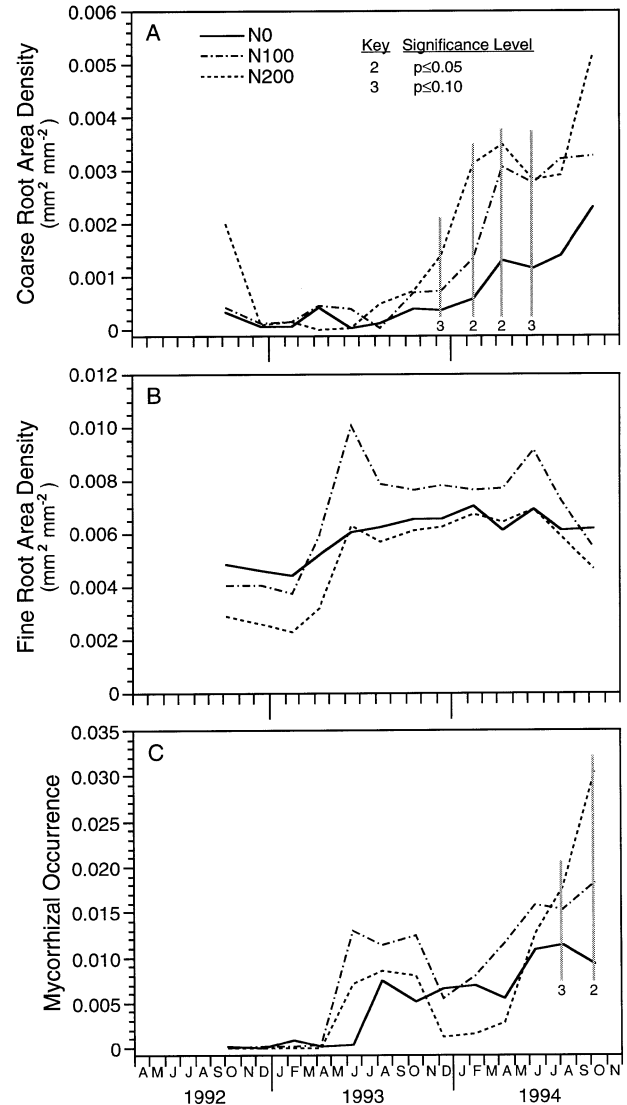


Figure 5. Effects of N fertilization on coarse roots, fine roots and mycorrhizal occurrence. The N treatments were: N0 = no N added, N100 = 100 kg N ha⁻¹, N200 = 200 kg N ha⁻¹. Data for the N treatments were averaged across the low (A) and high (A+350) CO₂ treatments to account for the unbalanced experimental design; means are based on six open-top chambers per treatment. The vertical lines show the sample dates when there were significant linear treatment effects; the numbers and associated key show the significance levels of the tests.

treatments (Figures 4a and 5a). Elevated CO₂ delayed coarse root formation but the trend was not statistically significant. In contrast, the elevated N (N200) treatment significantly increased coarse root area density from December 1993 through October 1994. The coarse root area density of plants in the N100 treatment was intermediate between that of N0- and N200-treated plants.

In both the CO₂ and N treatments, fine root area densities were low during the fall of 1992 and early spring of 1993 and then they approximately doubled (Figures 4b and 5b). After June 1993, fine root area densities remained essentially con-

stant except in the A+350 treatment where fine root area density increased significantly from October 1993 until the end of the study. Generally, fine root area density of plants in the A+175 treatment was intermediate between that of plants in the A+350 and the A treatments. The N treatments had no significant effect on fine root area density at any time during the study.

The A+350 treatment significantly increased mycorrhizal occurrence from February 1993 through December 1993 (August 1993 $P = 0.18$) (Figure 4c), whereas mycorrhizal occurrence in the A+175 treatment remained significantly above the other two CO₂ treatments from December 1993 through April 1994. There were no significant effects of N on mycorrhizal occurrence until fall 1994 when it increased in the N200 treatment (Figure 5c).

Temporal changes in growth

To establish temporal growth patterns, cumulative height and stem diameter data (Figures 2 and 3) were used to derive monthly changes in height and stem diameter. Because there were no differences in the timing of growth events between CO₂ and N treatments, only data for the CO₂ treatment are shown (Figure 6). Although there were cumulative differences in plant height and stem diameter (Figures 2 and 3), the monthly changes among treatments were small (Figure 6a and

6b). Height growth started in April and reached its maximum during May. The rapid spring growth was followed by two smaller increases (flushes) in height in 1993 and one additional flush in 1994. Stem diameter increased concurrently with the increase in stem height growth but its maximum growth rate peaked before stem height growth reached its maximum. In both years and in all treatments, stem diameter growth displayed a second growth peak between August and October.

There were differences in the magnitudes (35 to 40% increase in the elevated CO₂ treatments) of the incremental changes in new root area density, but there were no differences in the timing of root growth events among CO₂ or N treatments. Consequently only root data for the CO₂ treatment are shown. New fine root growth (Figure 6c) showed a single peak each year, in contrast to the multiple pulses of stem height and diameter increase (cf. Figures 6a and 6b). New fine root production was lowest during February, it started to increase by April and reached a maximum in June and then declined during the remainder of the summer and early fall. New fine root area density in the elevated CO₂ treatments displayed a more gradual late-summer decline than in the ambient treatment. The large increase in new root area density occurred concurrently with the largest increase in stem height and the initial increase in stem diameter, whereas the decline in new fine root production occurred while there were additional pe-

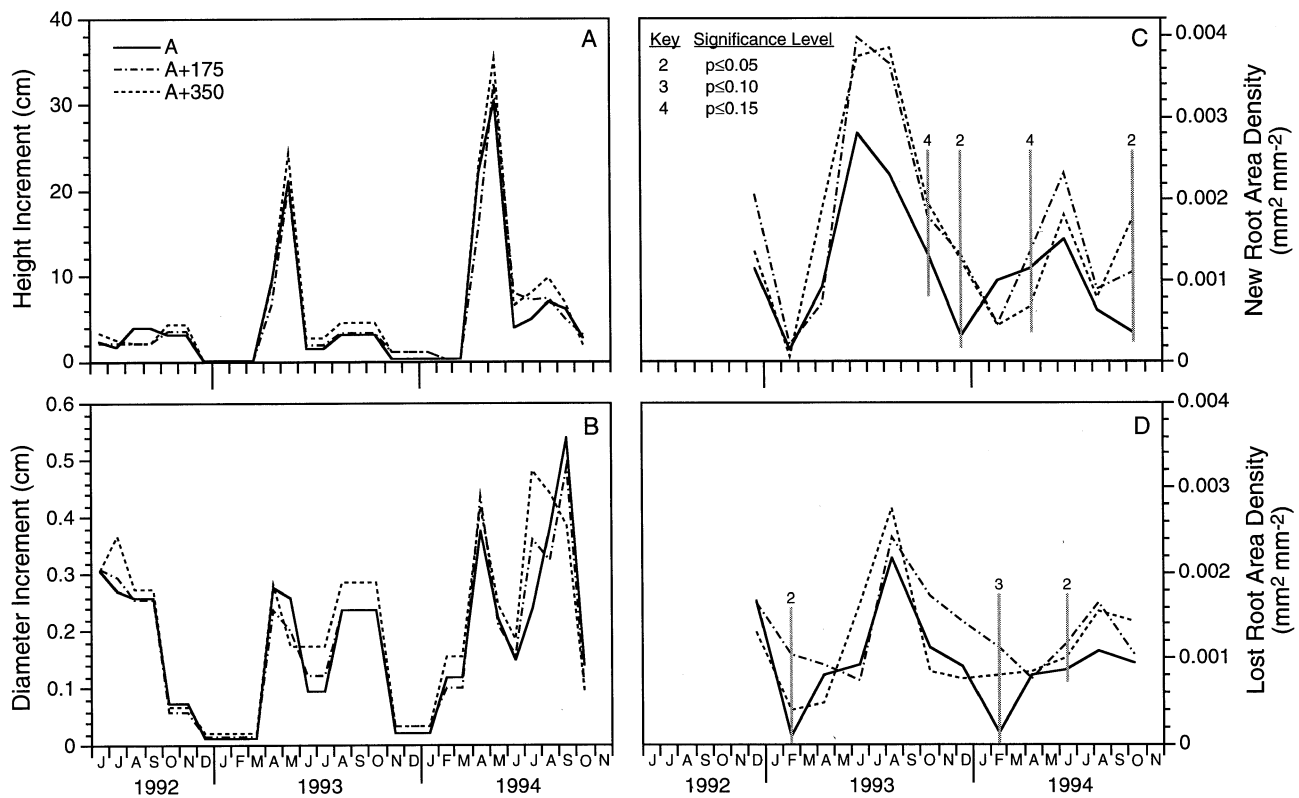


Figure 6. Influence of elevated CO₂ on developmental timing in shoot and roots. The CO₂ treatments were: A = ambient air, A+175 = ambient air + 175 $\mu\text{mol mol}^{-1}$, A+350 = ambient air + 350 $\mu\text{mol mol}^{-1}$. Data for the CO₂ treatments were averaged across the low (N0) and high (N200) N treatments to account for the unbalanced experimental design; means are based on six open-top chambers per treatment. The vertical lines show the sample dates when there were significant linear treatment effects; the numbers and associated key show the significance levels of the tests.

riods of increase in stem height and diameter. Elevated CO₂ tended to increase new fine root length during the summer and early fall of each year, but the increase was only significant on a few measurement days because of high variability (CV = 85%).

The seasonal variation in rate of fine root area lost (Figure 6d) was small compared with that of new fine root area production. Maximum root loss occurred in August of each year. It followed within 2 months of the period of maximum new root formation and occurred concurrently with the second period of stem height and diameter growth. Neither the CO₂ nor N treatments altered seasonal root mortality patterns.

Association between shoots and roots

To determine if the CO₂ or N treatments altered the relationship between shoots and fine roots, we compared needle and root development using LAI as an index of needle area and fine root area density as an analog index of rooting (Figure 7). In the CO₂ treatments, as LAI increased from approximately 0.5 to 4.5–6.5 from October 1992 through October 1994, the ratio

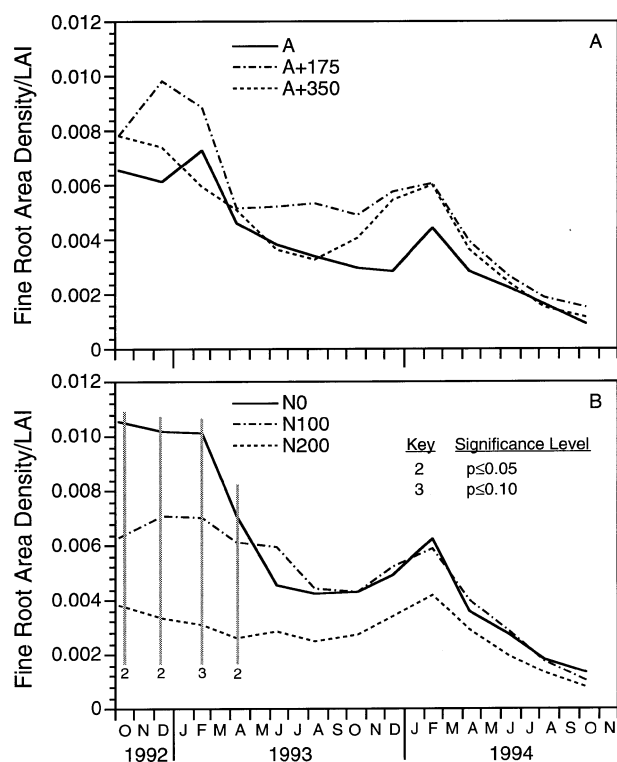


Figure 7. Impact of elevated CO₂ and N on the ratio of fine root length to LAI. LAI was derived from the leaf areas of individual trees. Leaf area was estimated for each sampling using an equation relating plant height and stem diameter (cm) to plant leaf area (cm²) which was derived from measurements on 60 plants harvested in October 1993. Data for the CO₂ treatments were averaged across the low (N0) and high (N200) N treatments and the data for the N treatments were averaged across the low (A) and high (A = 350) CO₂ treatments to account for the unbalanced experimental design; means are based on six open-top chambers per treatment. The vertical lines show the sample dates when there were significant linear treatment effects; the numbers and associated key show the significance levels of the tests.

of fine root area density to LAI decreased approximately four- to five-fold (Figure 7a). However, there was no significant CO₂ effect on the ratio, indicating a similar relationship between nutrient and water absorbing surfaces and photosynthetic surfaces among CO₂ treatments during the study. In contrast, the elevated N treatments significantly reduced the fine root area density/LAI ratio over the first 8 months of the study (Figure 7b), indicating that the amount of fine root area density needed to support a given amount of LAI was reduced by the N treatment. However, by the end of the study the ratio was similar among treatments.

Discussion

Root growth and mycorrhizal occurrence

Elevated CO₂ increases fine root biomass (e.g., Körner and Arnone 1992, Körner 1993, Norby 1994, Rogers et al. 1994). Berntson and Woodward (1992) reported that elevated CO₂ had no effect on root density but increased root branching and length. Elevated CO₂ increased fine root biomass more than two-fold during a multi-year study of *Citrus aurantium* L. (Idso and Kimball 1992). The fine roots extended further from the trunk and penetrated deeper into the soil than fine roots from trees grown in ambient CO₂. Fine root extension and biomass were stimulated by elevated CO₂ in *Populus grandidentata* Michx. (Curtis et al. 1994). In *Pinus taeda* L., elevated CO₂ increased the length of second-order laterals, whereas specific root length of first-order laterals declined (Larigauderie et al. 1994). In *P. ponderosa*, fine root area density initially increased one- to two-fold in response to elevated CO₂ (Figure 4b), suggesting that plants in elevated CO₂ extracted water and nutrients from a larger soil volume than plants in the ambient treatment. However, despite exploring a larger soil volume, the fine root area density of plants in the elevated CO₂ treatment did not continue to increase throughout the study even though shoot growth continued (Figures 2 and 3).

Nitrogen fertilization had no effect on fine root area density (Figure 5b). This result contrasts with those of Pregitzer et al. (1993) who reported that, in a northern hardwood forest, N fertilization stimulated fine root production and, although there was no increase in root diameter, root elongation, lateral branching and root longevity increased. The effects of N fertilization on root processes may be species-specific because Griffin et al. (1995) found that *P. ponderosa* seedlings were less responsive to changes in N fertilization and CO₂ resources than *Pinus taeda* seedlings.

Mycorrhizae are important in plant nutrient acquisition. The host plant provides the fungus with a carbon supply and the fungus enhances nutrient uptake for the plant–fungus complex (Harley and Smith 1983, Allen 1991). Enhanced mycorrhizal production is an efficient means of acquiring nutrients because the production and maintenance of mycorrhizae and the associated hyphal network requires less energy than the production of new plant roots (Marshall and Perry 1987, Fahey 1992). Assuming equal respiration rates for roots and hyphae, Harley (1989) concluded that roots require 100 times more carbon

than hyphae to exploit equal soil surface areas. O'Neill et al. (1987) hypothesized that elevated CO₂ stimulates mycorrhizal production, providing a mechanism for increasing nutrient acquisition. Similarly, Ineichen et al. (1995) found that elevated CO₂ resulted in a three-fold increase in the formation of mycorrhizal clusters on *P. sylvestris* L. seedlings and doubled the size of the extraradical mycelium.

In our study, elevated CO₂ stimulated an earlier increase in mycorrhizal occurrence (Figure 4c), permitting greater nutrient uptake at an earlier stage of plant development compared with the ambient treatment. Nitrogen fertilization only influenced mycorrhizal occurrence late in the study (Figure 5c), possibly because plant nutrient demand outstripped root uptake capacity as a result of continued plant growth. We conclude that the increases in fine root production and mycorrhizal occurrence in response to CO₂ enrichment indicate that additional photosynthate was allocated to production of fine roots and mycorrhizae to meet expanding aboveground resource needs resulting from CO₂-stimulated growth of needles and branches.

Synchrony of fine root and shoot growth

Various seasonal patterns of root and shoot growth synchrony have been observed in trees. Persson (1978) found no seasonal changes in fine root formation in a *P. sylvestris* L. stand in Central Sweden. In *Cedrus atlantica* (Endl.) G. Manetti ex Carrière, Kaushal et al. (1989) found that root growth was initiated before shoot growth, but that root growth slowed during the initial period of stem elongation; they found, however, that when *Cedrus* was grown in 800 μmol mol⁻¹ CO₂, root growth rate did not decrease during the initial period of stem elongation, suggesting that resource limitation influenced synchrony. Root elongation follows the initial period of stem elongation in *Pinus nigra* Arnold (Kaushal et al. 1989), whereas Ford and Deans (1977) reported that, in *Picea sitchensis* (Bong.) Carr., fine root growth occurred throughout the period of spring shoot elongation. In contrast, Deans (1979) reported that fine root growth of *Picea sitchensis* increased with increasing soil temperature during the spring, but slowed during the major period of shoot extension, and then accelerated as shoot extension slowed.

In all CO₂ and N fertilization treatments applied in the present study, shoot growth of *P. ponderosa* began concurrently with root growth, although root growth reached its maximum later than shoot growth (Figure 6). The temporal patterns of root and shoot growth were not altered by providing additional C or N resources.

Seasonal periodicity in fine root growth has been associated with changes in soil water content. For example, root growth of *Picea sitchensis* stopped during the summer when soil water content dropped below a critical level and began again following periods of precipitation that rewetted the soil (Deans 1979). Similarly, root growth in *Quercus* continued until soil water was depleted during summer and started again following fall rains and continued until the soil became too cold (Reich et al. 1980). Because soil water remained high (> 30%) throughout our study (Figure 1b), it is unlikely that the sea-

sonal changes in new fine root development were associated with changes in soil water content.

Root elongation, number of laterals and root dry weight all increased with increasing soil temperature. New fine root area density (Figure 6c) was low when soil temperature (Figure 1b) was low and increased as temperature increased; a flush of new roots occurred when soil temperature was high (i.e., 15 to 22 °C at 15 cm). The larger flush of roots in 1993 than 1994 is also likely to have been the consequence of high soil temperatures in 1993. We conclude, therefore, that soil temperature is a key factor influencing seasonal changes in new fine root area density. Soil temperature had a greater effect on *P. ponderosa* root growth than air temperature (Larson 1967). *Pinus ponderosa* root growth was slow at soil temperatures below 10 °C, whereas maximum root growth occurred at 20 °C and declined substantially at soil temperatures greater than 25 °C (Lopushinsky and Max 1990).

Functional balance of root and shoots

The functional balance hypothesis (Brouwer 1962, 1983) implies that roots and shoots continually adjust their relationships as resources change. Consequently, we followed the recommendation of Körner (1994) and used a three-compartment (needles, woody tissue and fine roots) model rather than root/shoot ratio to describe carbon allocation and functional relationships in plants. We used the fine root area density/LAI ratio to evaluate changes in resource absorbing tissues.

In *P. ponderosa*, N fertilization initially decreased the fine root/LAI ratio (Figure 7b), however, this effect decreased as the plants grew; possibly because the relative abundance of N to plant size decreased. Pregitzer et al. (1995) also observed that N fertilization decreased the fine root/leaf ratio.

In a multi-year study with *Liriodendron tulipifera* L., Norby et al. (1992) found that whole-plant carbon storage was unaffected by elevated CO₂, but leaf area was reduced and fine root (< 7 mm) production enhanced, indicating that more fine root area supports a given leaf area under conditions of elevated CO₂. Norby et al. proposed that the change in allocation represented a substitution between potential carbon assimilation and water and nutrient acquisition. Similarly, Körner (1994) reported that, in a complex ecosystem containing 15 tropical plant species, elevated CO₂ increased fine root mass but had no effect on leaf mass leading to an increase in the fine root/leaf mass ratio. In contrast, Pregitzer et al. (1995) found that elevated CO₂ had no effect on the fine root/leaf mass ratio. Similarly, we found that fine root area density (Figure 4b) and LAI (Figure 2c) of *P. ponderosa* increased with increasing CO₂; however, the root area density/LAI ratio was similar among CO₂ treatments indicating that the relationship between roots and needles was not changed by CO₂ exposure. The reasons for the differences among studies is unclear but may reflect species-specific responses to CO₂.

In conclusion, both CO₂ and N treatments increased shoot growth, and fine root growth increased with increasing CO₂ concentrations. Nevertheless, the temporal patterns of root and shoot growth were not altered by providing additional C or N resources. Over time, the fine root area density/LAI ratio of

P. ponderosa was similar among CO₂ treatments, indicating that fine root growth increased in relation to shoot growth rather than being a specific response to elevated CO₂ concentration. In contrast, N fertilization initially decreased the fine root area density/LAI ratio indicating a specific N effect on root growth. However, this effect did not persist and subsequently fine root growth increased in relation to increased shoot growth.

Acknowledgments

The assistance of the Desert Research Institute, Reno, Nevada staff in operating and maintaining the experimental site at Placerville, CA and the assistance of R.F. Walker of the University of Nevada, Reno with the identification of the mycorrhizae are appreciated. Tino Boerner, Glenn Jarrell, Steve Holman, Toni Hoyman, Dave Olszyk, Frank Senner, Ron Waschmann and Claudia Wise assisted in the collection of video images. The financial assistance of Southern California Edison Company and the U.S. Environmental Protection Agency in conducting this research is gratefully acknowledged. A portion of the research described in this article has been supported by the U. S. Environmental Protection Agency in part through Contract 68-C4-0019 to ManTech Environmental Research Services Corporation. The research described has been subjected to the Agency's peer and administrative review, and has been approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

References

- Allen, M.F. 1991. The ecology of mycorrhizae. Cambridge University Press, Cambridge, England, 184 p.
- Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO₂ levels. *Annu. Rev. Ecol. Syst.* 21:167–196.
- Bertson, G.M. and F.I. Woodward. 1992. The root system architecture and development of *Senecio vulgaris* in elevated CO₂ and drought. *Funct. Ecol.* 6:324–333.
- Brouwer, R. 1962. Nutritive influences on the distribution of dry matter in the plant. *Neth. J. Agric. Sci.* 10:399–408.
- Brouwer, R. 1983. Functional equilibrium: sense or nonsense? *Neth. J. Agric. Sci.* 31:335–348.
- Curtis, P.S., L.M. Baldum, B.G. Drake and D.F. Whigman. 1990. Elevated atmospheric CO₂ effects on belowground processes in C₃ and C₄ estuarine marsh communities. *Ecology* 71:2001–2006.
- Curtis, P.S., D.R. Zak, K.S. Pregitzer and J.A. Terri. 1994. Above- and belowground response of *Populus grandidentata* to elevated atmospheric CO₂ and soil N availability. *Plant Soil* 165:45–51.
- Deans, J.D. 1979. Fluctuations of the soil environment and fine root growth in a young Sitka spruce plantation. *Plant Soil* 52:195–208.
- Eamus, D. and P.G. Jarvis. 1989. The direct effects of increase in the global atmospheric CO₂ concentration on natural and commercial temperate trees and forests. *Adv. Ecol. Res.* 19:1–55.
- Fahey, T.J. 1992. Mycorrhizae and forest ecosystems. *Mycorrhiza* 1:83–89.
- Fogel, R. 1983. Root turnover and productivity of coniferous forests. *Plant Soil* 71:75–85.
- Ford, E.D. and J.D. Deans. 1977. Growth of a sitka spruce plantation: Spatial distribution and seasonal fluctuations of lengths, weights and carbohydrate concentrations of fine roots. *Plant Soil* 47:463–485.
- Griffin, K.L., W.E. Winner and B.R. Strain. 1995. Growth and dry matter partitioning in loblolly and ponderosa pine seedlings in response to carbon and nitrogen availability. *New Phytol.* 129:547–556.
- Harley, J.L. 1989. The significance of mycorrhizae. *Mycol. Res.* 92:129–134.
- Harley, J.L. and S.E. Smith. 1983. Mycorrhizal symbiosis, Academic Press, New York, 483 p.
- Hendrick, R.L. and K.S. Pregitzer. 1992. The demography of fine roots in a northern hardwood forest. *Ecology* 73:1094–1104.
- Hendrick, R.L. and K.S. Pregitzer. 1993. The dynamics of fine root length, biomass, and nitrogen content in two northern hardwood ecosystems. *Can. J. For. Res.* 23:2507–2520.
- Idso, S.B. and B.A. Kimball. 1992. Seasonal fine-root biomass development of sour orange trees grown in atmospheres of ambient and elevated CO₂ concentration. *Plant Cell Environ.* 15:337–341.
- Ineichen, K., V. Wiemken and A. Wiemken. 1995. Shoots, roots, and ectomycorrhiza of pine seedlings at elevated atmospheric carbon dioxide. *Plant Cell Environ.* 18:703–707.
- Johnson, D.W. 1992. Nitrogen retention in forest soils. *J. Environ. Qual.* 21:1–12.
- Johnson, D.W., D. Geisinger, R. Walker, J. Newman, J. Vose, K. Elliot and T. Ball. 1994. Soil pCO₂, soil respiration, and root activity in CO₂-fumigated and nitrogen-fertilized ponderosa pine. *Plant Soil* 165:129–138.
- Kaushal, P., J.M. Guehl and G. Aussenac. 1989. Differential growth response to atmospheric carbon dioxide enrichment in seedlings of *Cedrus atlantica* and *Pinus nigra* ssp. *Laricio* var. *Corsicana*. *Can. J. For. Res.* 19:1351–1358.
- Körner, C. 1993. CO₂ Fertilization. *In* *Vegetation Dynamics and Global Change*, Ed. A.M. Solomon and H.H. Shugart. Chapman Hall, New York, pp 53–70.
- Körner, C. 1994. Biomass fractionation in plants: a recommendation of definitions based on plant function. *In* *A Whole Plant Perspective on Carbon-Nitrogen Interactions*. Eds. J. Roy and E. Garnier. SPB Academic Publishing, The Hague, The Netherlands, pp 173–185.
- Körner, C. and J.A. Arnone III. 1992. Responses to elevated carbon dioxide in artificial tropical ecosystems. *Science* 257:1672–1675.
- Larigauderie, A., J.F. Reynolds and B.R. Strain. 1994. Root response to CO₂ enrichment and nitrogen supply in loblolly pine. *Plant Soil* 165:21–32.
- Larson, M.M. 1967. Effect of temperature on initial development of ponderosa pine seedlings from three sources. *For. Sci.* 13:286–294.
- Lekkerkerk, L.J.A., S.C. Van De Geijn and J.A. Van Veen. 1990. Effects of elevated atmospheric CO₂-levels on the carbon economy of a soil planted with wheat. *In* *Soils and the Greenhouse Effect*. Ed. A.F. Bouwman. John Wiley and Sons Ltd., New York, pp 423–429.
- Lopushinsky, W. and T.A. Max. 1990. Effect of soil temperature on root and shoot growth and on budburst timing in conifer seedling transplants. *New For.* 4:107–124.
- Marshall, J.D. and D.A. Perry. 1987. Basal and maintenance respiration of mycorrhizal and nonmycorrhizal root systems of conifers. *Can. J. For. Res.* 17:872–977.
- Mize, C.W. and R.C. Schultz. 1985. Comparing treatment means correctly and appropriately. *Can. J. For. Res.* 15:1142–1148.
- Norby, R.J. 1994. Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. *Plant Soil* 165:9–20.
- Norby, R.J., C.A. Gunderson, S.D. Wullschleger, E.G. O'Neill and M.K. McCracken. 1992. Productivity and compensatory responses of yellow-poplar trees in elevated CO₂. *Nature* 357:322–324.
- Norby, R.J., E.G. O'Neill and R.J. Luxmoore. 1986. Effects of atmospheric CO₂ enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil. *Plant Physiol.* 82:83–89.

- Norby, R.J., E.G. O'Neill, W.G. Hood and R.J. Luxmoore. 1987. Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO₂ enrichment. *Tree Physiol.* 3:203–210.
- Nylund, J.-E. 1988. The regulation of mycorrhiza formation—Carbohydrate and hormone theories reviewed. *Scand. J. For. Res.* 3:465–479.
- O'Neill, E.G., R.J. Luxmoore and R.J. Norby. 1987. Increase in mycorrhizal colonization and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched CO₂ environment. *Can. J. For. Res.* 17:878–883.
- Persson, H. 1978. Root dynamics in a young Scots pine stand in Central Sweden. *Oikos* 30:508–519.
- Pregitzer, K.S., R.L. Hendrick and R. Fogel. 1993. The demography of fine roots in response to patches of water and nitrogen. *New Phytol.* 125:575–580.
- Pregitzer, K.S., D.R. Zak, P.S. Curtis, M.E. Kubiske, J.A. Teeri and C.S. Vogel. 1995. Atmospheric CO₂, soil nitrogen and turnover of fine roots. *New Phytol.* 129:579–585.
- Reich, P.B., R.O. Teskey, P.S. Johnson and T.M. Hinckley. 1980. Periodic root and shoot growth in oak. *For. Sci.* 26:590–598.
- Rogers, H.H., G.B. Runion and S.V. Krupa. 1994. Plant response to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environ. Pollut.* 83:155–189.
- Snedecor, G.W. and W.G. Cochran 1989. *Statistical methods*, 8th Edn. Iowa State University Press, Ames, Iowa, 593 p.
- Tingey, D.T., M.G. Johnson, D.L. Phillips and M.J. Storm. 1995. Effects of elevated CO₂ and nitrogen on ponderosa fine roots and associated fungal components. *J. Biogeog.* 22:281–287.
- Walker, R.F., D.R. Geisinger, D.W. Johnson and J.T. Ball. 1995. Interactive effects of atmospheric CO₂ enrichment and soil N on growth and ectomycorrhizal colonization of ponderosa pine seedlings. *For. Sci.* 41:491–500.
- Wallander, H. and J.-E. Nylund. 1991. Effects of excess nitrogen on carbohydrate concentration and mycorrhizal development on *Pinus sylvestris* L. *New Phytol.* 119:405–411.
- Zak, D.R., K.S. Pregitzer, P.S. Curtis, J.A. Teeri, R. Fogel and D.L. Randlett. 1993. Elevated atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant Soil* 151:105–117.