

Mechanisms of Phosphorus Acquisition for Ponderosa Pine Seedlings under High CO₂ and Temperature

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To test the hypothesis that elevated atmospheric CO₂ and elevated temperature, simulating current and predicted future growing season conditions, act antagonistically on phosphorus acquisition of ponderosa pine, seedlings were grown in controlled-environment chambers in a two temperature (25/10 °C and 30/15 °C) × two CO₂ (350 and 700 μl⁻¹) experimental design. Mycorrhizal seedlings were watered daily with a nutrient solution with P added in organic form as inositol hexaphosphate (64 ppm P). Thus seedlings were challenged to use active forms of P acquisition. Elevated CO₂ increased the relative growth rate by approx. 5% which resulted in an approx. 33% increase in biomass after 4 months. There was no main effect of temperature on growth. Increased growth under elevated CO₂ and temperature was supported by increases in specific absorption rate and the specific utilization rate of P. The contribution of mycorrhizae to P uptake may have been greater under simulated future conditions, as elevated CO₂ increased the number of mycorrhizal roots. There was no main effect of temperature on root phosphatase activity, but elevated CO₂ caused a decrease in activity. The inverse pattern of root phosphatase activity and mycorrhizal infection across treatments suggests a physiological coordination between these avenues of P acquisition. The concentration of oxalate in the soil increased under elevated CO₂ and decreased under elevated temperature. This small molecular weight acid solubilizes inorganic P making it available for uptake. Increased mycorrhizal infection and exudation of oxalate increased P uptake in ponderosa pine seedlings under elevated CO₂, and there was no net negative effect of increased temperature. The increased carbon status of pine under elevated CO₂ may facilitate uptake of limiting P in native ecosystems.

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INTRODUCTION

The response of trees to simulated anthropogenic increases in atmospheric CO₂ is highly variable, depending to a large extent on mineral nutrition supplied during the experiment. As an average of experiments to date, the biomass of coniferous species is increased by 38% when grown under twice current CO₂ concentrations (approx. 700 μl⁻¹) (Ceulemans and Mousseau, 1994), an atmospheric concentration expected in 50–100 years (Houghton, Jenkins and Ephraums, 1990). Similarly, the growth of hardwoods is increased by 63%. Fertilizer was supplied freely in most of these experiments and the responses to CO₂ are greatly reduced when plants are confronted with nutrient limitations. For example, the 33 to > 50% increases in growth of *Pinus taeda* under twice-ambient CO₂ concentrations is reduced to < 10% when grown with limiting N or P (Strain and Thomas, 1992; Griffin, Thomas and Strain, 1993; Tissue, Thomas and Strain, 1993), and Norby *et al.* (1992) observed no increase in above-ground production of *Liriodendron tulipifera* when grown for several years in elevated CO₂ in a nutrient-deficient forest soil. Whether increased production of woody plants will be realized in the

field will depend on their ability to extract limiting nutrients. This ability may be compromised, to some extent, by expected increases in temperature which may increase the respiratory demand for carbon that might otherwise be invested in nutrient procurement (DeLucia, Callaway and Schlesinger, 1993).

Because of the heavy metabolic demands of P acquisition, we expect uptake of this limiting nutrient to be responsive to future climate conditions. Rates of weathering of P-containing primary minerals are slow and availability of this element is controlled in forest ecosystems primarily by mineralization of organic P (Schlesinger, 1991). Continued root growth and fine root turnover are necessary to overcome the low availability of P in most forest soils and the central role of mycorrhizae in P uptake is well established (Chapin, 1980; Harley and Smith, 1983; Allen, 1991). Moreover, both mycorrhizal and non-mycorrhizal roots produce phosphatases (bound and extracellular) which hydrolyse ester linkages between P and C, liberating inorganic P (Schlesinger, 1991; Duff, Sarath and Plaxton, 1994). Increased uptake of P by mycorrhizae or increased production of phosphatases may support sustained plant growth at elevated atmospheric CO₂.

Another important mechanism increasing P availability and uptake is root exudation of low molecular weight

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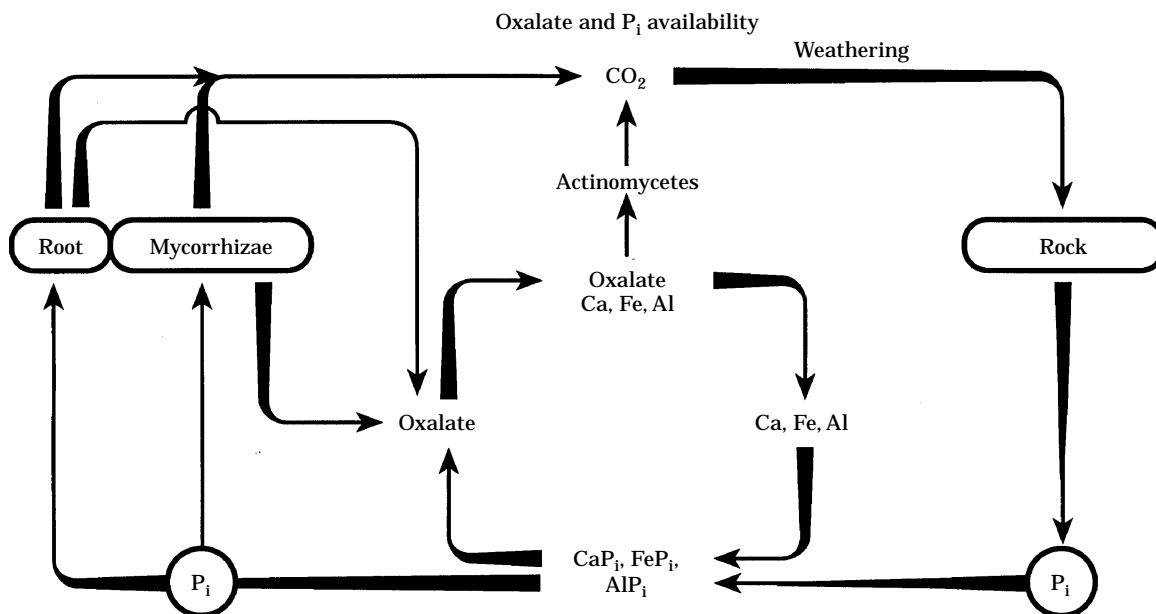


FIG. 1. Conceptual model for the production and action of oxalate on availability of inorganic P (P_i) in the soil. Oxalate released by roots or fungal hyphae binds cations covalently linked to P thereby liberating P_i for uptake. Actinomycetes consume oxalate as an energy source releasing CO_2 which participates in rock weathering. Drawn from data provided by Jurinak *et al.* 1986 and Allen, 1991.

organic compounds (Rovira, 1969). These compounds, released in greatest concentration by fine white roots (Norton, Smith and Firestone, 1989; Norton and Firestone, 1991) support the growth and activity of P-mineralizing microbes in the rhizosphere. Organic acids released from roots and associated mycorrhizae, such as oxalate, also directly increase availability of inorganic P (P_i) (Jurinak *et al.*, 1986; Fox and Commerford, 1992).

Once liberated by mineralization or weathering, P_i is rapidly bound to Ca^{++} in most forest soils producing insoluble, and thus unavailable, calcium phosphates (Fig. 1). Oxalate released by roots or mycorrhizae chelates Ca, Al, and Fe thereby liberating P_i for uptake (Graustein, Cromack and Sollins, 1977). Oxalate salts are then consumed by microbes, producing CO_2 that contributes to weathering of P-containing minerals. These mechanisms of P acquisition (support of mycorrhizae, exudation of oxalates, and production of phosphatases) are presumably energetically demanding and are thus limited by the carbon budget of the plant.

We used ponderosa pine seedlings under controlled growth conditions as a model system to examine the potential interaction of elevated CO_2 and temperature on P uptake. Growth of ponderosa pine in glasshouse experiments (Grulke, Horn and Roberts, 1993; Callaway *et al.*, 1994; Johnson, Ball and Walker, 1995) and in open-top chambers in the field (Green and Wright, 1977; T. Ball, unpubl. res.) is stimulated by elevated atmospheric CO_2 . Most of the response is, however, confined to the initial few weeks of the treatments (DeLucia *et al.*, 1993; Callaway *et al.*, 1994). We hypothesize that elevated atmospheric CO_2 and elevated temperature, simulating future climate conditions, act antagonistically on P acquisition and growth of ponderosa pine. To test our hypothesis we grew ponderosa pine seedlings with abundant water and a complete inorganic

fertilizer except for P. Phosphorus was added in organic form as inositol hexaphosphate (phytate); thus, seedlings were challenged to employ active mechanisms of P acquisition under the treatment conditions.

MATERIALS AND METHODS

Growth conditions and experimental design

Ponderosa pine seedlings were grown in a factorial design (two CO_2 \times two temperatures \times two mycorrhizal infections) in 1.8 l PVC tubes (35 cm deep) filled with sterilized sand. Seeds (half-sib family) were collected from an open-grown tree on the east slope of the Sierra Nevada near Reno, Nevada, and four sown per pot. Soon after germination seedlings were thinned to one per pot. After 1 month pots were randomly sorted into ten groups of 24 pots each and transferred into the experimental treatments in four growth chambers in the Duke University Phytotron. One group was harvested at this time and the remaining groups grew for 4 months under the treatment conditions.

The 'mycorrhizal' plants were inoculated with approximately 1 g per pot of *Pisolithus tinctorius* culture from MYCORR TECH Inc. (Pittsburg, PA, USA) at the time plants were moved into the CO_2 \times temperature treatments. The non-mycorrhizal plants were treated with 1 g per pot of autoclaved culture. Soon after inoculation roots were checked for mycorrhizae. Because mycorrhizae of similar morphology to the inoculated seedlings were also found on the non-inoculated seedlings, though at a reduced level of infection, this treatment was omitted from the ANOVA used to examine CO_2 and temperature effects.

Current models predict that a doubling of atmospheric CO_2 will drive a 2–6 °C increase in mean annual temperature in the western United States (Mitchell *et al.*, 1991). Based on

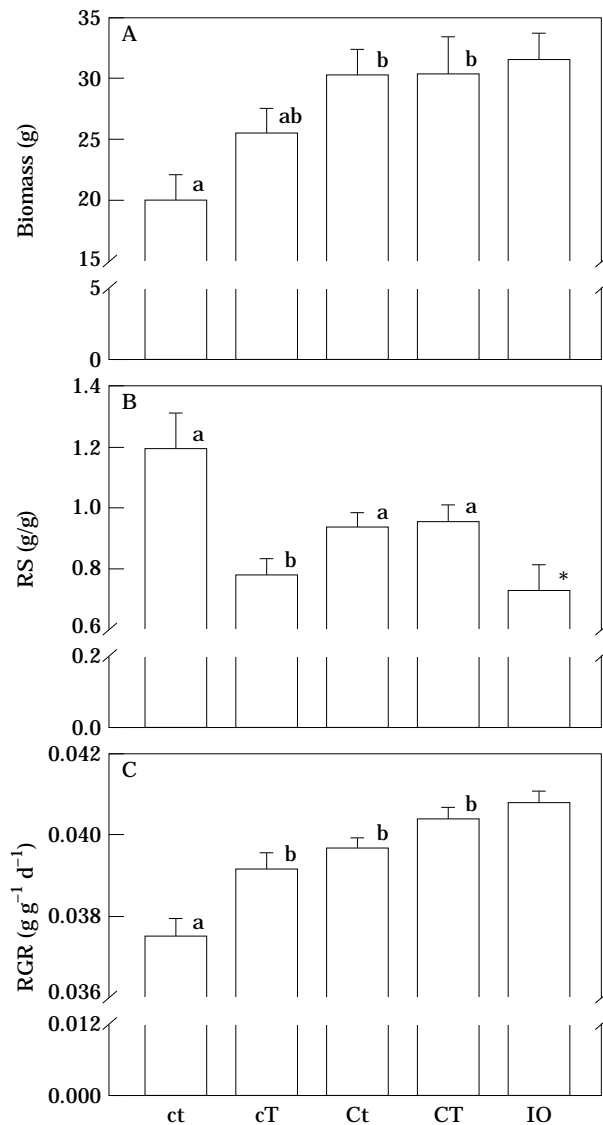


FIG. 2. The influence of atmospheric CO₂ concentration and growth temperature on final biomass (A), root/shoot ratio (R/S) (B) and relative growth rate (RGR) (C) of ponderosa pine seedlings. Plants were grown in a 2 × 2 design at high (T, 30 °C) or low (t, 25 °C) temperature and high (C, 700 μl l⁻¹) or low (c, 350 μl l⁻¹) atmospheric CO₂ concentration. Plants grown at high CO₂ and high temperature but fertilized with inorganic phosphate are designated by 'IO'. There was a significant ($P < 0.05$) CO₂ effect in (A), temperature and CO₂ × temperature interaction in (B) and CO₂ and CO₂ × temperature interaction in (C). The asterisk indicates a significant difference between the IO and CT treatments. Bars with different superscripts are significantly different. Error bars represent s.e. and $n = 24$.

this range and current climate conditions for the eastern Sierra we selected the following treatment conditions: low CO₂/low temperature (350 μl l⁻¹ and 25/10 °C); low CO₂/high temperature (30/15 °C); high CO₂/low temperature (700 μl l⁻¹); and high CO₂/high temperature. The difference between the first and the last combination represent current and predicted growing season air temperatures for the northwestern Great Basin in the next 50–100 years. The high and low CO₂ treatments and the high and low temperature

treatments are designated by 'C' and 'c' and 'T' and 't', respectively. Soil temperatures at 10 cm depth in the pots were typically 0.5–1.5 °C greater than air temperatures and did not vary significantly below this depth. Changes in soil temperature lagged behind the step-change in air temperature by as much as 3 h.

Fluorescent tubes and supplemental incandescent bulbs in the growth chambers provided an irradiance of 650 μmol m⁻² s⁻¹ (PAR) at mid-shoot height over a 14 h photoperiod. The total daily irradiance was therefore approx. 33 mol m⁻² or more than 1/2 full sun measured close to the summer solstice. The environmental conditions in the chambers were monitored continuously. Plants were rotated through each chamber daily, and the treatments and plants were rotated between chambers weekly.

Pots were watered twice daily until they drained freely. A 15th-strength modified Hoagland's solution (*sensu* Downs and Hellmers, 1975) with P added as inositol hexaphosphate (0.34 M or 64 ppm P) was applied each morning and distilled water was applied each afternoon. A subset of the plants in the high CO₂/high temperature treatment received inorganic P (64 ppm) applied in the nutrient solution instead of inositol hexaphosphate. Thus, all plants were well watered but received either organic or inorganic P.

Biomass, tissue nutrients, and growth analysis

Plants from the first and second harvests were divided into their component parts and oven dried (70 °C) to constant mass in a forced-convection oven. After weighing, tissues were ground to 20 mesh in a Wiley Mill for determination of N and P. The concentrations of these nutrients were measured colorimetrically with an auto-analyser (TRAACS 800, Bran-Luebbe, Buffalo Grove, IL, USA) following acid digestion (Lowther, 1980). Relative growth rate (RGR), specific utilization rate for N and P (SUR), and specific absorption rate (SAR) of these nutrients were calculated according to Hunt (1990). The mean SUR over the growth interval is defined as:

$$\text{SUR} = [(W_2 - W_1)/(t_2 - t_1)] \times [(\log_e M_2 - \log_e M_1)/(M_2 - M_1)]$$

and the mean SAR over the growth interval is defined as:

$$\text{SAR} = [(M_2 - M_1)/(t_2 - t_1)] \times [(\log_e R_2 - \log_e R_1)/(R_2 - R_1)].$$

The subscripts indicate data from harvest 1 or 2 and 't' represents the time interval (days). The variables W, M, and R represent total plant dry mass, total nutrient content (N or P), and root dry mass, respectively.

Mycorrhizal infection

Mycorrhizal infection was assessed under an incident-light microscope on 12 plants randomly selected from each treatment. The percent infection (infection %), relative cover of mycorrhizae (cover %), density of mycorrhizal branch roots (root density), and the relative surface area of mycorrhizal branch roots (root area) were measured on five randomly selected lateral roots (originating directly from

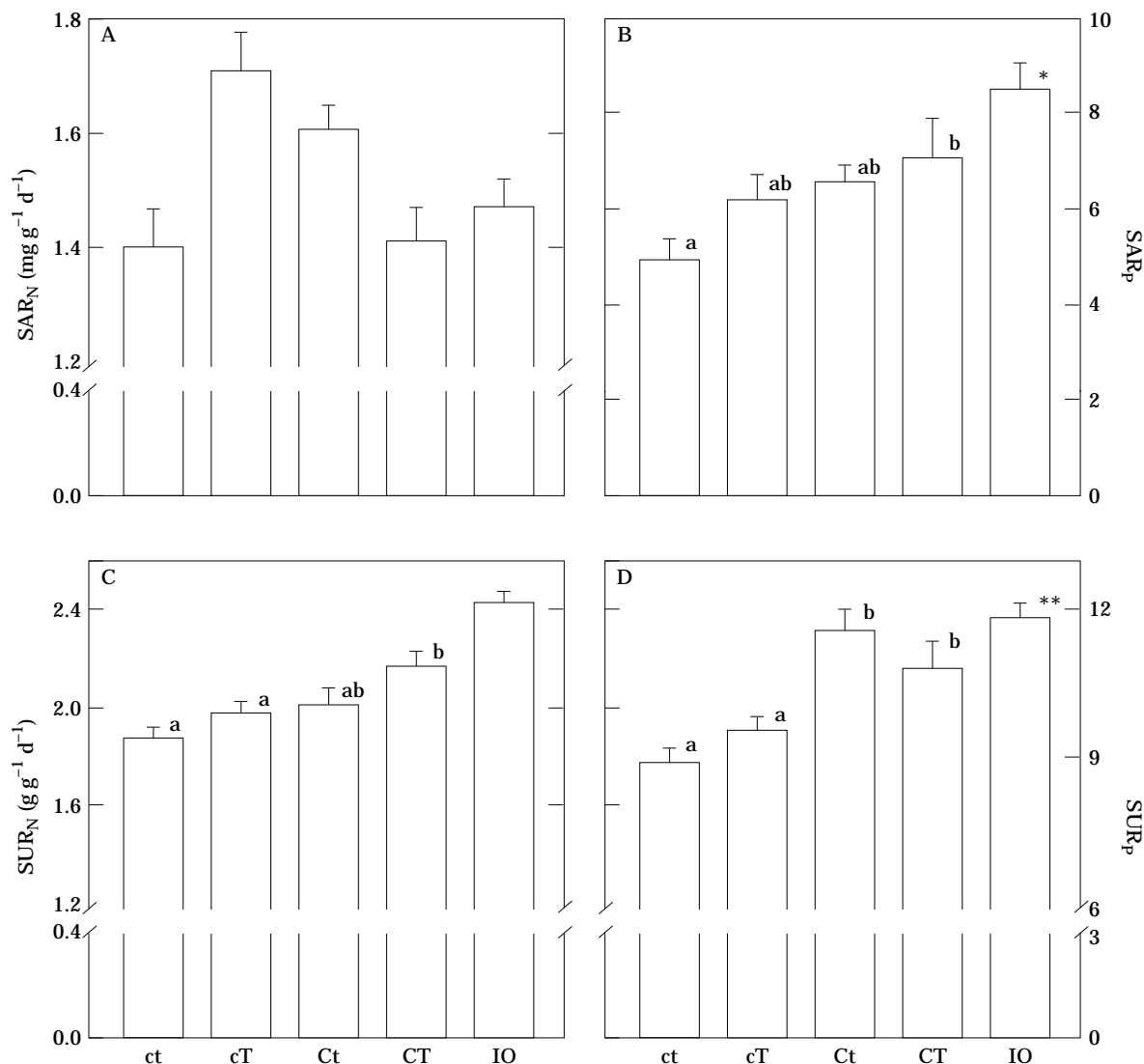


FIG. 3. The effect of atmospheric CO₂ concentration and growth temperature on the specific absorption rate (SAR_N, SAR_P) and specific utilization rate (SUR_N, SUR_P) for nitrogen and phosphorus. For treatments and symbols see Fig. 2. There was a significant ($P < 0.05$) effect of CO₂ and temperature in (B) and (D) and CO₂ only in (C). An asterisk or double asterisks indicate(s) a significant difference between the IO and CT treatments at the $P < 0.5$ or $P < 0.1$ levels, respectively. Bars with different superscripts are significantly different. Error bars represent s.e. and $n = 24$.

the primary root) per plant. Percent infection was the proportion of all infected branch roots [(branch roots originating from the laterals/total number of branch roots) × 100]. Relative cover provides a measure of the density of hyphae and was determined by counting the number of hyphal intersections with a transect placed along each branch root. An ocular micrometer was used to place a 20-division transect along each branch root and relative mycorrhizal cover was calculated as the number of hyphal intersections / total sample points (20) × 100. The architecture of root systems was quantified as root density and root area. Root density was the total number of mycorrhizal branch roots per length of lateral root, and root area was the one-sided surface area (cm²) of branch roots per length of lateral root. Root area was measured by placing a dot grid over photographs of the root systems and counting the number of points covered by branch roots.

Phosphatase activity and soil oxalates

Root and rhizosphere phosphatase activity (phosphomonoesterase) and rhizosphere oxalate concentration were measured on 12 randomly-selected plants from each treatment. At the final harvest plants were gently removed from their pots with the soil column intact. After removal of the bulk soil, plants were shaken over a tray to remove rhizosphere soil which was frozen for subsequent analysis of rhizosphere phosphatase activity and oxalate content.

The phosphatase activity of excised white root tips (expressed on a dry mass and area basis) and the intact root system were measured as in Kroehler and Linkins (1989). Roots were incubated in a buffered solution (pH 5.0) with p-nitrophenyl phosphate (3.8 mM) as a substrate, and the enzymatic release of p-nitrophenol was measured at 410 nm in a spectrophotometer (Lambda 38, Perkin Elmer).

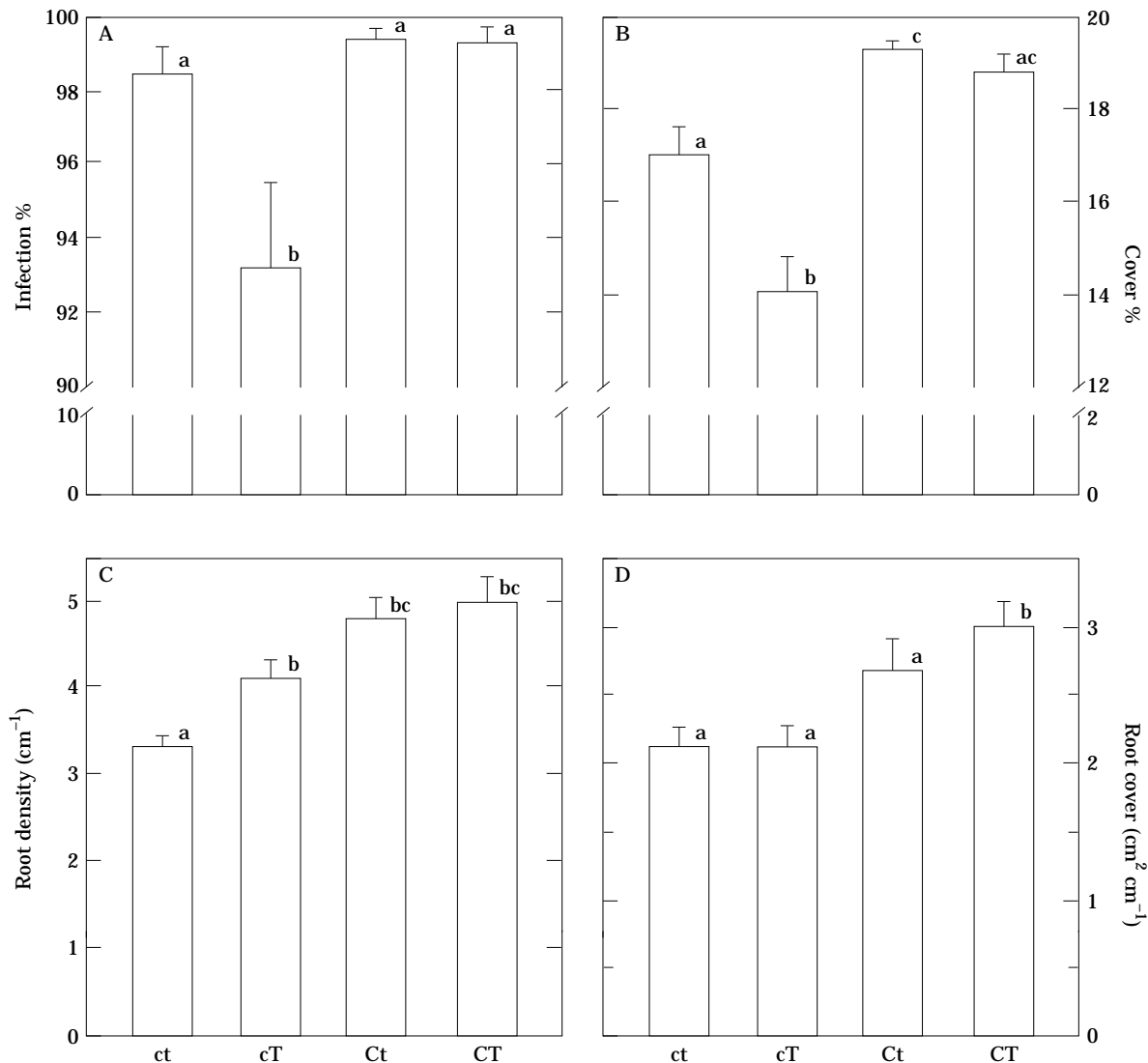


FIG. 4. The effect of atmospheric CO₂ concentration and growth temperature on mycorrhizal infection and root architecture of ponderosa pine seedlings. The percent infection (infection %) is the mean percent of all root tips that were infected. The mycorrhizal cover (cover %) is the degree of infection for mycorrhizal roots measured as the number of hyphal line intersects. 'Root density' is the number of mycorrhizal branch roots per unit length (cm) of lateral root, and 'Root area' is the area covered by branch roots per unit length of lateral root. For treatments and symbols see Fig. 2. There were significant CO₂, temperature and CO₂ × temperature effects ($P < 0.05$) in (A), (B) and (C) and CO₂ only in (D). Bars with different superscripts are significantly different. Error bars represent s.e. and $n = 12$.

Rhizosphere phosphatase activity was measured as in Adams (1992), except that CaCl₂ was omitted as it flocculated with our soil and prevented accurate spectrophotometer readings.

Oxalate was extracted from 0.5 g of ground rhizosphere soil with 1 ml of 0.024 N HCl. After shaking for 1 h the filtered extract (0.45- μ m millipore filter) was run on an ion chromatograph (Dionex 2010).

Statistical analyses

Prior to statistical analyses the data were tested for normality and heteroscedasticity. Data transformations were applied where appropriate but the means and error terms presented in the figures are nontransformed values. As mentioned previously, the non-mycorrhizal treatment was

dropped from our initial experimental design and we used a two-way ANOVA ($P < 0.05$) to test for significant CO₂ and temperature effects on the remaining plants. If significant treatment effects were detected, means were compared with a Ryan-Einot-Gabriel Welsch Multiple F -Test. A two-tailed t test was used to compare the means for plants grown on organic P vs. inorganic P (IO vs. CT), and for comparing soil phosphatase activity and oxalate concentration for mycorrhizal and 'non-mycorrhizal' seedlings. Statistical analyses were done with SAS for PC (v 6.04).

RESULTS

Elevated CO₂ concentration significantly increased final biomass and relative growth rate of ponderosa pine seedlings grown on organic P, but had no main effect on root/shoot

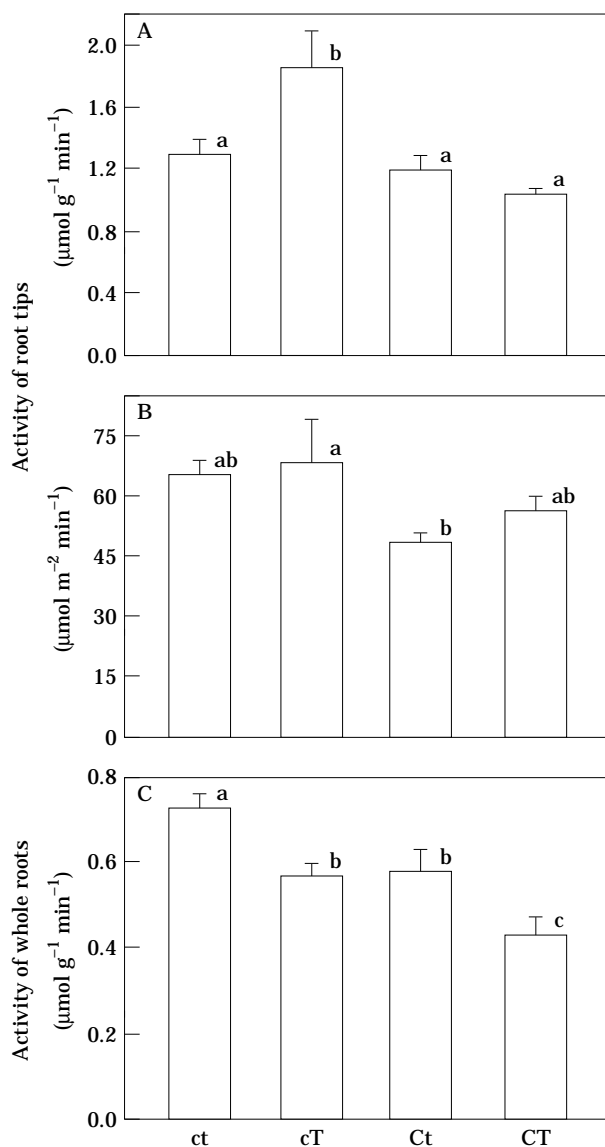


FIG. 5. The effect of atmospheric CO_2 concentration and growth temperature on root phosphatase activity for fine roots (area and dry mass basis) and the whole root system. For treatments and symbols see Fig. 2. There were significant ($P < 0.05$) CO_2 and $\text{CO}_2 \times$ temperature effects in (A), CO_2 only in (B) and CO_2 and temperature in (C). Bars with different superscripts are significantly different. Error bars represent s.e. and $n = 12$.

ratio (Fig. 2). Elevated temperature had no main effect on growth, but at low CO_2 it significantly decreased the root/shoot ratio. The proportion of fine roots (< 1 mm diameter) increased from 24 to 37% with an increase in CO_2 ($T = 2.883$, $P = 0.018$).

The effect of inorganic P on growth is evident in comparisons of the 'CT' and 'IO' treatments. These two groups of pots were maintained at high CO_2 and elevated temperature (CT), but the former received P in organic form whereas the latter received P_i . Growth with inorganic P decreased R/S by the final harvest but had no effect on final biomass or relative growth rate. The higher growth rates under elevated atmospheric CO_2 were sustained by increased

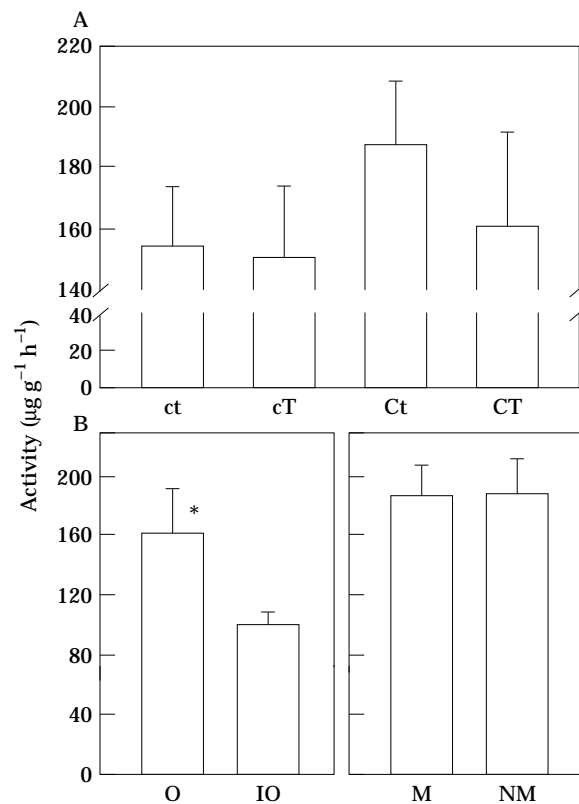


FIG. 6. The effect of atmospheric CO_2 concentration and growth temperature on rhizosphere phosphatase activity, where activity is expressed per unit mass of rhizosphere soil. The treatments and symbols in the upper panel are the same as Fig. 2. Error bars represent s.e. and $n = 24$. Plants were grown with organic (O) or inorganic (IO) P and were mycorrhizal (M) or non-mycorrhizal (NM).

rates of P uptake and greater specific utilization rate. Elevated CO_2 and elevated temperature increased the specific absorption rate for P (SAR_p , Fig. 3). This is a measure of the integrated rate of root P uptake over the harvest interval. In contrast to P, there were no significant treatment effects on the rate of N uptake (SAR_N). The specific utilization rate for N or P (SUR_N , SUR_P) is the biomass increment per unit nutrient in the tissue, which provides a measure of nutrient-use efficiency. SUR_P was increased by elevated CO_2 and higher temperatures, but only CO_2 affected SUR_N (Fig. 3). The form of P, organic or inorganic, had no effect on N uptake but plants grown on P_i had higher SAR_p and SUR_p .

Mycorrhizal development was quantified by two methods. Percent infection (infection %, Fig. 4), defined as the number of infected branch roots / total number of branch roots, increased at $700 \mu\text{l l}^{-1} \text{CO}_2$ but was reduced, under the low CO_2 treatment, by increased temperature. However, over 93% of all branch roots were infected across the treatments. Similarly, elevated temperature decreased hyphal cover (% cover) at low CO_2 concentrations (significant $C \times T$ interaction), but elevated CO_2 increased hyphal cover. As for percent infection and percent cover, elevated CO_2 increased the density and area-based cover of branch roots (Fig. 4). In contrast to the first two variables,

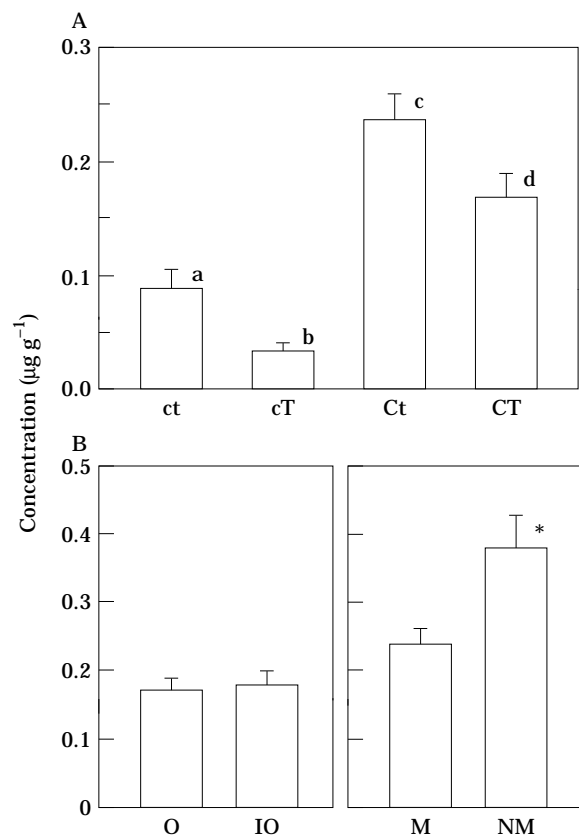


FIG. 7. The effect of atmospheric CO_2 concentration and growth temperature on rhizosphere oxalate concentration. The treatments and symbols in the upper panel are the same as in Fig. 2. There were significant ($P < 0.05$) CO_2 , temperature, and $\text{CO}_2 \times$ temperature effects in (A). Bars with different superscripts are significantly different. Error bars represent s.e. and $n = 12$. Plants were grown with organic (O) or inorganic (IO) P and were mycorrhizal (M) or non-mycorrhizal (NM).

elevated temperature increased the density of mycorrhizal-supporting branch roots (root density and root cover).

Phosphatase activity of white root tips per root area and mass, and of the entire root system, was suppressed in plants grown under elevated atmospheric CO_2 (Fig. 5). For root tips, temperature was not a significant main effect; however, when expressed on a dry mass basis, elevated temperature increased phosphatase activity in the low CO_2 treatments. The phosphatase activity of whole roots decreased at higher temperatures. There was no effect of the CO_2 or temperature treatments on the activity of rhizosphere phosphatases (Fig. 6). Growth on inorganic P suppressed phosphatase activity but there was no difference between fully mycorrhizal (M) and the non-mycorrhizal (NM) treatments.

The concentration of oxalate in the rhizosphere was higher at elevated CO_2 but decreased at increased temperature (Fig. 7). This significant $C \times T$ interaction term indicates that the inhibitory effect of temperature was stronger at low CO_2 . The form of P, organic or inorganic, had no effect on oxalate concentration, but the non-mycorrhizal plants produced higher concentrations than the mycorrhizal plants.

DISCUSSION

Whether growth enhancements observed for tree seedlings under simulated future atmospheric CO_2 will be sustained for large trees under nutrient-limited field conditions, remains a central question in 'climate change' research (Bazzaz, 1990). Plants grown under elevated atmospheric CO_2 can compensate, to some degree, for nutrient deficiencies (Strain, 1987). Higher intercellular CO_2 concentrations for plants in an enriched atmosphere increase the carboxylation rate of RUBP carboxylase (Rubisco), even though the content of this enzyme may be reduced for plants grown under elevated CO_2 (Sage, Sharkey and Seemann, 1989; Tissue *et al.*, 1993). Because so much leaf N is invested in photosynthetic enzymes, increased rates of CO_2 assimilation per unit Rubisco and therefore per unit leaf N, result in higher photosynthetic-nitrogen use efficiency and increased growth per unit plant N (Thomas, Lewis and Strain, 1994). For ponderosa pine the biomass increment per unit plant N and P increased significantly for seedlings grown under elevated CO_2 (Fig. 3). Assuming similar mechanisms of enhanced nutrient-use efficiency operated in trees under field conditions, we would expect that with no additional inputs or enhanced ability to acquire these nutrients, growth of ponderosa pine will increase under elevated atmospheric CO_2 . Feedbacks at the ecosystem level may, however, mitigate this response for native trees.

Increased nutrient-use efficiency results in lower nutrient concentration and an increase in the C/N ratio of leaves and other plant parts. The biogeochemical cycles of N (or P) and C are linked by the C/N ratio of plant litter, with high C/N ratios resulting in lower rates of litter decomposition (Swift, Heal and Anderson, 1979; Schlesinger, 1991) and the potential for microbial sequestration of soil nutrients (Diaz *et al.*, 1993). As a consequence of this feedback at the ecosystem level, increased nutrient-use efficiency under future elevated atmospheric CO_2 , measured in this study as the specific utilization rate for N and P, may not sustain increased growth of ponderosa pine under nutrient-limited conditions. In this scenario the enhanced ability of potted plants to acquire organic P under elevated CO_2 is most significant.

Because of the low solubility of P_i and accumulation of P in organic forms in most forest soils (Wood, Bormann and Voigt, 1984; Attiwill and Adams, 1993; Cross and Schlesinger, 1995), P acquisition by trees is problematic, and several mechanisms of P acquisition have evolved. Paramount among these mechanisms is the symbiotic relationship between tree roots and fungi to form mycorrhizae. Mycorrhizal hyphae greatly increase the effective absorptive area and extend soil exploration. Inorganic P taken up by the fungi is transferred to the host plant where it is rapidly assimilated into sugar phosphates, nucleotides, and other P-rich compounds (Harley and Smith, 1983). The fungus depends on its host for reduced carbon compounds, and the degree of mycorrhizal function (amount and activity) is linked to plant carbon balance (Marx, Hatch and Mendicino, 1977). Using the pulse-chase technique, Rygielwicz and Anderson (1994) have shown that *Hebeloma crustuliniforme* in mycorrhizal association with ponderosa

pine seedlings receives approx. 7% of total plant C and can alter substantially root morphology and biomass allocation.

Our results do not support a strict carbon economy interpretation of the mycorrhizal association with ponderosa pine seedlings under different climate regimes. Considering the effect of temperature first, at low atmospheric CO₂ elevated temperature caused a decrease in the percent infection and the percent hyphal cover of branch roots. Considering the decrease in root/shoot ratio in this treatment, mycorrhizal infection per plant may have decreased. This temperature effect was eliminated when seedlings were grown under high CO₂ (Fig. 4). The temperature rise may have increased root respiration and therefore decreased the amount of carbon available to support the fungus at low but not high growth CO₂. However, the effect of temperature on the percent infection and percent cover was small and, in contrast to these variables, elevated temperature caused a significant increase in the number of branch roots that support mycorrhizae (Fig. 4; root density). Although a quantitative estimate of the amount of hyphae supported by each plant was not possible in this study, the increase in the number of mycorrhizal-supporting branch roots suggests that the cumulative effect of elevated temperature was to increase the amount of mycorrhizal infection in ponderosa pine seedlings.

Ineichen, Wiemkin and Wiemkin (1995) recently reported an increase in the number of ectomycorrhizal clusters per root and an increase in the biomass of extraradical mycelium for *Pinus sylvestris* seedlings grown under elevated CO₂. The most meaningful expression of the degree of mycorrhizal development would be the total hyphal area per unit plant root and some measure of the actual rates of exchange of C and P between the plant and fungus. This was, however, not practical, and we relied instead on two indirect or partial measures of fungal association. In a similar experiment Lewis, Thomas and Strain (1994) did not find an increase in the percent infected root tips of loblolly pine seedlings grown in elevated CO₂. This is consistent with our observation for ponderosa pine when comparing plants grown under the low temperature/low CO₂ with the low temperature/high CO₂ treatments ('ct' versus 'Ct' in Fig. 4). As noted by Lewis *et al.* (1994), this one measure of infection may be inadequate for describing mycorrhizal association as it does not address the degree of infection of infected roots or morphological changes in root architecture caused by the mycorrhizae.

Lewis *et al.* (1994) confirmed the linkage between root carbohydrate status and the degree of infection; mycorrhizal seedlings had significantly lower root starch, soluble sugars, and total non-structural carbohydrate concentrations than non-mycorrhizal seedlings. They also found that elevated CO₂ increased root carbohydrates. Although we did not measure root carbohydrate status in this study, it is reasonable to expect that enhanced carbohydrate supply for ponderosa pine seedlings grown under elevated atmospheric CO₂ increased the degree of mycorrhizal development. Similar results have been reported for other ectomycorrhizal tree species (O'Neill, 1995).

Phosphatases, produced by plant roots, mycorrhizae and

soil microbes initiate P mineralization by hydrolyzing the ester linkage between C and P in soil organic material (Tarafdar and Claassen, 1988; Dinkelaker and Marschner, 1992). This important class of enzymes may be associated with cell walls directly or exist freely in the rhizosphere. For ponderosa pine seedlings the phosphatase activity of fine roots was not significantly affected by temperature but decreased with elevated CO₂ (Fig. 5). The pattern of activity across treatments, expressed on a dry mass basis, was the inverse of the pattern of mycorrhizal infection illustrated in Fig. 4. This suggests a linkage between support of mycorrhizae and the production of root phosphatases, whereby roots with a high level of mycorrhizae utilize fungal phosphatases rather than maintaining high levels of root enzyme activity. In addition to the inducible nature of phosphatases in response to exogenous organic P (McLachlan and De Marco, 1982; Fig. 6), root phosphatase activity also may be modulated by root carbohydrate status.

Besides phosphatases, oxalate also is released to the rhizosphere by roots, mycorrhizae and microbes. By chelating P_i bound to Ca⁺⁺ or other soils cations, this low molecular weight organic acid renders P available for plant uptake (Fig. 1). The concentration of oxalate in the rhizosphere increased for ponderosa pine seedlings grown under elevated CO₂ and decreased for plants grown under high temperature (Fig. 7), suggesting that the production of this compound may also be linked to root carbohydrate status and ultimately the carbon economy of the seedlings. The source of oxalate cannot be ascertained from our data. Oxalate concentrations were highest for plants supporting the greatest level of mycorrhizal association but, within the CT treatment, the concentration of oxalates was also greater for the 'non-mycorrhizal' seedlings. We speculate that fine root tips may have been the primary source of rhizosphere oxalate in this study.

As a result of complementary mechanisms of P acquisition, elevated atmospheric CO₂ significantly increased RGR and total biomass for ponderosa pine seedlings grown on an organic P source. Increased mycorrhizal infection and the production and release of rhizosphere oxalate increased P availability and uptake, as indicated by significant increases in the specific uptake rate (Fig. 3), for seedlings grown under CO₂ enrichment. The importance of various mechanisms of P acquisition varied across treatments, and it appeared that when the development of mycorrhizae was pronounced, as in the CT treatment, the production of root phosphatases was lower. Support of the mycorrhizal symbiosis and the activity of root phosphatases and production of oxalate seem to be regulated by the carbohydrate status of the root, but in a complex manner. The fact that there was no difference in growth for plants fertilized with organic or inorganic P suggests that the difference in metabolic costs of maintaining these mechanisms of P uptake may be small relative to the direct maintenance costs of the root system.

With the exception of oxalate (Fig. 7), our hypothesis that elevated CO₂ and temperature act antagonistically on P uptake was not supported. The elevated temperature treatment was 5 °C greater than the mean growing season temperature for May and June on the east slope of the

Sierra Nevada. This temperature was not, however, limiting and may actually have been beneficial for the growth of ponderosa pine seedlings (Callaway *et al.*, 1994). At the organismal level, elevated levels of atmospheric CO₂ increased the capacity for P uptake by ponderosa pine seedlings grown under controlled conditions. Whether this enhancement will occur when potential feedbacks at the ecosystem level are considered remains unclear.

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LITERATURE CITED

- Adams MA. 1992. Phosphatase activity and phosphorus fractions in Karri (*Eucalyptus diversicolor* F. Muell.) forest soils. *Biological Fertility of Soils* **14**: 200–204.
- Allen MF. 1991. *The ecology of mycorrhizae*. Cambridge: Cambridge University Press. 184 pp.
- Attiwill PM, Adams MA. 1993. Nutrient cycling in forests. *New Phytologist* **124**: 561–582.
- Bazzaz FA. 1990. The response of natural ecosystems to the rising global CO₂ levels. *Annual Review of Ecology and Systematics* **20**: 167–196.
- Callaway RM, DeLucia EH, Thomas EM, Schlesinger WH. 1994. Compensatory responses of CO₂-exchange and biomass allocation and their effects on the relative growth rate of ponderosa pine in different CO₂ and temperature regimes. *Oecologia* **98**: 159–166.
- Ceulemans R, Mousseau M. 1994. Effects of elevated atmospheric CO₂ on woody plants. *New Phytologist* **127**: 425–426.
- Chapin FS. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**: 233–260.
- Cross AF, Schlesinger WH. 1995. A literature review and evaluation of the Hedley fractionation: Application to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* **64**: 197–214.
- DeLucia EH, Callaway RM, Schlesinger WH. 1993. Offsetting changes in biomass allocation and photosynthesis in ponderosa pine (*Pinus ponderosa*) under future climatic conditions. *Tree Physiology* **14**: 669–677.
- Diaz S, Grime JP, Harris J, McPherson E. 1993. Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature* **364**: 616–617.
- Dinkelaker B, Marschner H. 1992. *In vivo* demonstration of acid phosphatase activity in the rhizosphere of soil-grown plants. *Plant and Soil* **144**: 199–205.
- Downs RJ, Hellmers H. 1975. *Environment and the experimental control of plant growth*. New York: Academic Press.
- Duff SMG, Sarath G, Plaxton WC. 1994. The role of acid phosphatases in plant phosphorus metabolism. *Physiologia Plantarum* **90**: 791–800.
- Fox TR, Comerford NB. 1992. Influence of oxalate loading on phosphorus and aluminum solubility in spodosols. *Soil Science Society of America Journal* **56**: 290–294.
- Graustein WC, Cromack K, Sollins. 1977. Calcium oxalate: Occurrence in soil and effect on nutrient and geochemical cycles. *Science* **198**: 1252–1254.
- Green K, Wright R. 1977. Field response of photosynthesis to CO₂ enhancement in ponderosa pine. *Ecology* **58**: 687–692.
- Griffin KL, Thomas RB, Strain BR. 1993. Effects of nitrogen supply and elevated carbon dioxide on construction costs in leaves of *Pinus taeda* (L.) seedlings. *Oecologia* **95**: 575–580.
- Grulke NE, Horn JL, Roberts SW. 1993. Physiological adjustment of two full-sib families of ponderosa pine to elevated CO₂. *Tree Physiology* **12**: 391–401.
- Harley JL, Smith SE. 1983. *Mycorrhizal symbiosis*. New York: Academic Press. 483 pp.
- Houghton JT, Jenkins GJ, Ephraums JJ, eds. 1990. *Climate change: The IPCC scientific assessment*. Cambridge: Cambridge University Press. 365 pp.
- Hunt R. 1990. *Basic growth analysis*. London: Unwin Hyman Ltd.
- Ineichen K, Wiemkin V, Wiemkin A. 1995. Shoots, roots and ectomycorrhizae formation of pine seedlings at elevated atmospheric carbon dioxide. *Plant, Cell and Environment* **18**: 703–707.
- Johnson DW, Ball T, Walker RF. 1995. Effects of elevated CO₂ and nitrogen on nutrient uptake in ponderosa pine seedlings. *Plant and Soil* **168**: 535–545.
- Jurinak JJ, Dudley LM, Allen MF, Knight WG. 1986. The role of calcium oxalate in the availability of phosphorus in soils of semiarid regions: a thermodynamic study. *Soil Science* **142**: 255–261.
- Kroehler CJ, Linkins AE. 1988. The root surface phosphatases of *Eriophorum vaginatum*: effects of temperature, pH, substrate concentration and inorganic phosphorus. *Plant and Soil* **105**: 3–10.
- Lewis JD, Thomas RB, Strain BR. 1994. Effect of elevated CO₂ on mycorrhizal colonization of loblolly pine (*Pinus taeda* L.) seedlings. *Plant and Soil* **165**: 81–88.
- Lowther JR. 1980. Use of a single sulfuric acid-hydrogen peroxide digest for the analysis of *Pinus radiata* needles. *Communications in Soil Science and Plant Analysis* **11**: 175–188.
- McLachlan KD, De Marco DG. 1982. Acid phosphatase activity of intact roots and phosphorus nutrition in plants. 3. Its relation to phosphorus garnering by wheat and a comparison with leaf activity as a measure of phosphorus status. *Australian Journal of Agricultural Research* **33**: 1–11.
- Mitchell JFB, Manabe S, Meleshko V, Tokioka T. 1991. Equilibrium climate change—and its implications for the future. In: Houghton JT, Jenkins GJ, Ephraums JJ, eds. *Climate Change. The IPCC scientific assessment*. New York: Cambridge University Press 134–169.
- Marx DH, Hatch AB, Mendicino JF. 1977. High soil fertility decreases sucrose content and susceptibility of loblolly pine roots to ectomycorrhizal infection by *Pisolithus tinctorius*. *Canadian Journal of Botany* **55**: 1569–1574.
- Norby RJ, Gunderson CA, Wullschlegel SD, O'Neill EG, McCracken MK. 1992. Productivity and compensatory response of yellow poplar trees in elevated CO₂. *Nature* **357**: 322–324.
- Norton JM, Firestone MK. 1991. Metabolic status of bacteria and fungi in the rhizosphere of ponderosa pine seedlings. *Applied and Environmental Microbiology* **57**: 1161–1167.
- Norton JM, Smith JL, Firestone MK. 1989. Carbon flow in the rhizosphere of ponderosa pine seedlings. *Soil Biology and Biochemistry* **22**: 449–455.
- O'Neill EG. 1995. Responses of soil biota to elevated atmospheric carbon dioxide. *Plant and Soil* **165**: 55–65.
- O'Neill EG, Luxmoore RJ, Norby RJ. 1987. Elevated atmospheric CO₂ effects on seedling growth, nutrient uptake, and rhizosphere bacterial populations of *Liriodendron tulipifera* L. *Plant and Soil* **104**: 3–11.
- Rovira AD. 1969. Plant root exudates. *Botanical Review* **35**: 35–57.
- Rygielcz PT, Andersen CP. 1994. Mycorrhizae alter quality and quantity of carbon allocated below ground. *Nature* **369**: 58–60.
- Sage RF, Sharkey TD, Seemann JR. 1989. Acclimation of photosynthesis to elevated CO₂ in five C₃ species. *Plant Physiology* **89**: 590–596.
- Schlesinger WH. 1991. *Biogeochemistry: an analysis of global change*. San Diego: Academic Press. 443 pp.
- Strain BR. 1987. Direct effects of increasing atmospheric CO₂ on plants and ecosystems. *Trends in Ecology and Evolution* **2**: 18–21.
- Strain BR, Thomas RB. 1992. Field measurements of CO₂ enhancement and climate change in natural vegetation. *Water, Air and Soil Pollution* **64**: 45–60.

- Swift MJ, Heal OW, Anderson JM. 1979.** *Decomposition in terrestrial ecosystems*. Berkeley: University of California Press.
- Tarafdar JC, Claassen N. 1988.** Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biology and Fertility of Soils* **5**: 308–312.
- Thomas RB, Lewis JD, Strain BR. 1994.** Effects of leaf nutrient status on photosynthetic capacity in loblolly pine (*Pinus taeda* L.) seedlings grown in elevated atmospheric CO₂. *Tree Physiology* **14**: 947–960.
- Tissue DT, Thomas RB, Strain BR. 1993.** Long-term effects of elevated CO₂ and nutrients on photosynthesis and RUBISCO in loblolly pine seedlings. *Plant, Cell and Environment* **16**: 859–865.
- Wood T, Bormann FH, Voigt GK. 1984.** Phosphorus cycling in a northern hardwood forest: Biological and chemical control. *Science* **223**: 391–393.