**Growth and ectomycorrhizal colonization of ponderosa pine seedlings
supplied different levels of atmospheric CO₂ and soil N and P**

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Abstract

Individual and interactive effects of atmospheric CO₂ enrichment and soil N and P fertility on above- and below-ground growth and mycorrhizal colonization of juvenile ponderosa pine (Pinus ponderosa Dougl. ex Laws.) were examined. Seedlings were grown from seed in atmospheres with 700 $\mu\text{l l}^{-1}$, 525 $\mu\text{l l}^{-1}$, or ambient CO₂ concentrations. High and low soil N treatments were created by adding sufficient (NH₄)₂SO₄ to an infertile soil mixture to establish total N concentrations of 500 $\mu\text{g g}^{-1}$ and 400 $\mu\text{g g}^{-1}$, respectively, while high and low P treatments consisted of 68 $\mu\text{g g}^{-1}$ and 43 $\mu\text{g g}^{-1}$ concentrations, respectively, of extractable P created by additions of CaHPO₄. All seedlings were inoculated with the mycobiont Pisolithus tinctorius (Pers.) Coker and Couch shortly after emergence. Three whole-seedling harvests at 4-month intervals permitted assessment of treatment effects on shoot and root growth and ectomycorrhizal development. Initially, 525 $\mu\text{l l}^{-1}$ CO₂ and high N and P were all influential factors in above-ground growth, with each of these treatments increasing shoot weight while the latter increased height, diameter, and volume. Stimulation of root growth was evident in dry weight and length measurements at the first harvest, with N and P main treatment effects again evident, but the response to elevated CO₂ was most pronounced in the 700 $\mu\text{l l}^{-1}$ atmosphere. After 8 months, soil P was of little consequence above- or below-ground, but high N increased shoot dimensions, volume, and weight and root weight and length. Furthermore, the 525 $\mu\text{l l}^{-1}$ CO₂ treatment emerged as the dominant stimulatory atmosphere both above- and below-ground, as seedlings grown in intermediate CO₂ exhibited the largest shoot diameters, greatest shoot

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and root weights, and the longest root systems at the second harvest. At the final harvest, interactive effects of $525 \mu\text{l l}^{-1} \text{CO}_2$ and high N were prominent, as this treatment combination produced the largest shoot dimensions, volume, and weight and the greatest root volume and coarse and fine root weights. Intermediate CO_2 also produced the longest root systems after 12 months. Shoot/root ratios were lowered by growth in $700 \mu\text{l l}^{-1} \text{CO}_2$ after 4 months and by both enriched atmospheres after 8 months, but this effect was no longer evident at the final harvest. Greater numbers of mycorrhizae were formed by seedlings grown in $700 \mu\text{l l}^{-1} \text{CO}_2$ after 4 months and by those grown in $525 \mu\text{l l}^{-1} \text{CO}_2$ after 8 months. Both enriched atmospheres increased mycorrhizal counts after 12 months, and seedlings grown in high CO_2 and low N exhibited the highest percentage of total root system length colonized at the final harvest as well. Overall, these results indicate that CO_2 enrichment stimulates shoot and root growth of juvenile ponderosa pine, a response dependent on soil N rather than P availability, and that the magnitude of the growth increase is greater in $1.5 \times$ ambient than in $2 \times$ ambient CO_2 .

Keywords: Elevated CO_2 ; Soil fertility; Mycorrhizae; Pinus ponderosa

Introduction

Early investigations concerned with the effects of atmospheric CO₂ enrichment on forest ecosystems emphasized above-ground growth processes of tree seedlings, and the results indicated that the stimulation of shoot growth by elevated CO₂ varies somewhat by species in both its magnitude and duration (Brown and Higginbotham, 1986; Conroy et al., 1986; Surano et al., 1986; O'Neill et al., 1987a; Kaushal et al., 1989; Norby and O'Neill, 1989; Bazzaz et al., 1990; Radoglou and Jarvis, 1990). Because of the difficulty inherent in investigations of below-ground processes, comparatively less research has examined the effects of rising CO₂ on tree root systems. Nevertheless, some studies have revealed that CO₂ enrichment promoted disproportionately greater root than shoot growth (Norby et al., 1986; O'Neill et al., 1987a), but others have provided evidence that stimulation of below-ground tree growth may be a short-term response (Brown and Higginbotham, 1986; Radoglou and Jarvis, 1990). Equally perplexing have been the results of studies concerned with effects of elevated CO₂ on mycorrhizal colonization, as reports to date have indicated both a positive response (Norby et al., 1987; O'Neill et al., 1987b) and positive but ephemeral responses (O'Neill et al., 1987b; Lewis et al., 1994) in forest tree seedlings. Greater elucidation of the impact of rising CO₂ on root development is deemed crucial because root systems provide critical feedback to the whole-tree response by altering the capacity to acquire water and nutrients (Norby, 1994). It has been hypothesized that an increased efficiency of nutrient and water uptake afforded forest trees by enhanced root system development may permit a positive growth response to CO₂ enrichment in infertile and droughty soils.

Reported here are results derived from an investigation of the response of juvenile ponderosa pine to the interaction of elevated atmospheric CO₂ and soil N and P fertility. Emphasis was placed

on root system development and ectomycorrhizal colonization. This study is one of a series currently underway which examine the impact of rising atmospheric CO₂ on the development and physiology of ponderosa pine growing in nutrient-limited soils.

Methods

Ponderosa pine seed (half-sib seed lot, Eldorado County, CA source) were stratified for 30 days at 3°C followed by sterilization in 10% H₂O₂ for 10 min. The seeds were then dusted with Captan (FMC Corp., Fresno, CA) to inhibit pathogenic fungi. Three seeds were sown in each of 108 Roottrainer (1100 cm³ capacity) containers (Spencer-Lemaire Industries, Edmonton, Alberta, Canada) filled with a soil mixture consisting of 60% sand, 20% soil, 10% peat moss, and 10% perlite by volume. The soil component of this mix was collected from the upper 15 cm of a hydrothermally altered Zephan very gravelly sandy loam (USDA Soil Conservation Service, 1983), and the mix was formulated specifically to provide low levels of plant-essential nutrients, especially N and P. Deionized water mists were applied frequently until emergence was complete, and the seedlings were then thinned to one per container. Thereafter, the irrigation regime was varied as required to maintain moisture availability conducive to seedling growth.

High and low soil N fertility treatments were created by adding sufficient (NH₄)₂SO₄ to the soil mixture to increase total N by 200 µg g⁻¹ and 100 µg g⁻¹, respectively. The unamended soil mix had a total N concentration of 300 µg g⁻¹ as determined with a Perkin-Elmer 2400 CHN Analyzer (Perkin-Elmer Corp., Norwalk, CT), but this N was largely unavailable for plant growth. The (NH₄)₂SO₄ additions were made prior to filling the containers, and a second fertilization, identical to the first except that it was applied topically to avoid disturbing the root systems, was necessary

to maintain the integrity of the N treatments and followed 6 months later. Within each of the two soil N treatments, high and low P treatments were created by adding sufficient CaHPO_4 to increase the extractable P concentration by $50 \mu\text{g g}^{-1}$ and $25 \mu\text{g g}^{-1}$, respectively. Unamended soil mix had an extractable P concentration of $18 \mu\text{g g}^{-1}$ as determined by the method of Olsen and Sommers (1982), and the CaHPO_4 additions were also made prior to filling the containers. Periodic monitoring of soil nutrients indicated that a second P fertilization was not needed to maintain the desired extractable P concentrations. The Ca additions resulting from fertilization with CaHPO_4 to create high and low P treatments increased the exchangeable Ca concentrations by only 2.1% and 1.1%, respectively, and were thus considered inconsequential. As determined by the method of Johnson et al. (1994), concentrations of K, Ca, and Mg in the soil mix were 309, 1292, and $320 \mu\text{g g}^{-1}$, respectively, prior to the $(\text{NH}_4)_2\text{SO}_4$ and CaHPO_4 additions.

All seedlings were inoculated 1 week after emergence with the mycobiont Pisolithus tinctorius (Pers.) Coker and Couch using a pelletized basidiospore inoculum consisting of spores (Alpine County, CA source) attached to vermiculite particles with a water-soluble adhesive (International Forest Seed Co., Odenville, AL). These spores had been extracted from sporocarps found growing in association with ponderosa pine seedlings, and a preliminary trial verified their suitability as an ectomycorrhizal inoculum for this host species. Each pellet had approximately 3×10^6 spores, which were dislodged upon watering to permeate the soil mixture, and each container received 20 pellets to ensure the mycorrhization of the seedling root systems.

Within each of the four combinations of soil N and P treatments, high, medium, and low atmospheric CO_2 treatments were imposed consisting of $700 \mu\text{l l}^{-1}$, $525 \mu\text{l l}^{-1}$, and ambient CO_2 , respectively, with the latter predetermined as $350 \mu\text{l l}^{-1}$. These atmospheres were maintained by

regulated injection of gaseous CO₂ into the airstreams entering six controlled-environment growth chambers, with two chambers dedicated to each CO₂ concentration. Constructed to permit automated additions as needed based on the monitored concentrations in individual chambers, actual CO₂ concentrations did not deviate from the set points by more than 20 µl l⁻¹. A KMnO₄ scrubber installed upstream from the chambers eliminated C₂H₂ contamination in the airstreams. A photoperiod of 16 hr, created with multi-vapor HID lamps providing 600 µmol PAR m⁻² s⁻¹, and an air temperature averaging 25°C (range 23-27°C) were maintained in all six chambers. The containers were placed in the chambers at seeding, so all seedlings were grown in the appropriate atmosphere from germination. To minimize chamber effects on growth, seedlings within CO₂ treatments were alternated monthly between the two chambers dedicated to each atmosphere, and the N and P treatment combinations were randomized and rearranged monthly within the appropriate chambers as well.

There were nine replications of each of 12 factorial combinations of three CO₂ treatments, two N treatments, and two P treatments, with each replication consisting of a single seedling. At 4-month intervals, three seedlings of each treatment combination were randomly selected for harvest for a total of three harvests over the 1-year duration of the study. Following measurement of height and stem diameter, shoots of seedlings to be harvested were severed at the root collar and their volumes were measured by water displacement (Burdett, 1979). Complete root systems of these seedlings were then extracted from the soil mix and washed. At the third harvest, root system volumes were also measured by water displacement, but after 4 and 8 months the root systems were too small for accurate volume measurement.

Ectomycorrhizal colonization of harvested root systems was quantified by two methods.

First, a count of the total number of mycorrhizae, identified by the characteristic monopodial, bifurcate, or coralloid short roots or short roots with an obvious fungal mantle, was determined for each root system. Second, the total length of each root system was measured, the root segments (1.0 cm length) bearing mycorrhizae as identified above were counted, and the number of segments with mycorrhizae was then expressed as a percentage of total root system length (Grand and Harvey, 1982). Ectomycorrhizae resulting from infection by different mycobionts were identified as to probable fungal species but were then grouped together in both quantification procedures. Following mycorrhizal quantification, the shoots and root systems of harvested seedlings were dried at 75°C for 36 h and weighed, permitting the calculation of shoot/root ratios. At the third harvest, root systems were divided into coarse (≥ 2.0 mm diameter) and fine (< 2.0 mm diameter) root fractions which were weighed and recorded separately, but their weights were then combined for shoot/root ratio calculations. Root diameters of < 2.0 mm predominated after 4 and 8 months, thus no attempt was made to divide the root systems into size fractions at the first two harvests.

Three-way analysis of variance was performed on all data derived from this $3 \times 2 \times 2$ (three atmospheric $\text{CO}_2 \times$ two soil N treatments $\times$ two soil P treatments) factorial experiment. Data collected at each of the three harvests were analyzed separately with three replications per treatment combination. Mycorrhizal infection percentages were subjected to arcsine transformation prior to analysis. The CO_2 , N, and P main treatment and $\text{CO}_2 \times \text{N}$, $\text{CO}_2 \times \text{P}$, $\text{N} \times \text{P}$, and $\text{CO}_2 \times \text{N} \times \text{P}$ interaction effects were considered significant only when $P \leq 0.05$ according to the F test. All statistical analyses were accomplished using the Statistical Analysis System (SAS Institute, Cary, NC). In the presentation of results that follows, P values are included in the text when either a main treatment or treatment interaction effect proved significant as determined through analysis of

variance. Also, the standard error of the mean for all variables presented in tables and figures is included to provide an indication of the variation within treatment means.

Results

Among the main treatments, analysis of variance revealed that soil P fertility alone significantly affected seedling height ($P = 0.0256$) and stem diameter ($P = 0.0048$) after 4 months. This was most apparent in the latter measurements, as seedlings of the high P treatment had the largest diameters within each of the three CO₂ treatments at the first harvest (Table 1). The interaction of CO₂, N, and P also had a significant height effect ($P = 0.0188$), however, as low N and P fertility produced the shortest seedlings within the 700 $\mu\text{l l}^{-1}$ and ambient CO₂ treatments. After 8 months, CO₂ enrichment had opposing effects on these two dimensions, resulting in a decrease in height ($P = 0.0291$) but an increase in diameter ($P = 0.0305$), with the latter most apparent in 525 $\mu\text{l l}^{-1}$ CO₂. A soil N fertility effect was also evident at the second harvest, with high N generally increasing both height ($P = 0.0279$) and diameter ($P = 0.0001$). At the third harvest, CO₂ enrichment also increased both height ($P = 0.0179$) and diameter ($P = 0.0001$), most notably in 525 $\mu\text{l l}^{-1}$ CO₂ (Table 2). Furthermore, this effect was accentuated by high soil N, as the interaction of the intermediate CO₂ atmosphere and high N resulted in the largest seedlings overall ($P = 0.0419$ and $P = 0.0404$ for the CO₂ $\times$ N effects on height and diameter, respectively). Nevertheless, high N stimulated height ($P = 0.0071$) and diameter ($P = 0.0001$) growth in all CO₂ atmospheres after 12 months, and high soil P increased diameter growth ($P = 0.0059$) within all CO₂ and N treatments.

Significant main treatment effects on shoot volume were confined to soil P ($P = 0.0039$) after 4 months and to soil N ($P = 0.0001$) after 8 months, and in both cases the high fertilization rate

proved stimulatory (Fig. 1). However, the $\text{CO}_2 \times \text{N} \times \text{P}$ interaction effect was also significant ($P = 0.0167$) after 8 months, a result most apparent in the exceedingly small volumes of seedlings grown in ambient CO_2 and low N and P. At the third harvest, individual and interactive effects of CO_2 and N on shoot volume proved significant ($P = 0.0001$ for CO_2 and N, $P = 0.0335$ for $\text{CO}_2 \times \text{N}$). Overall, the enriched atmospheres increased volume growth 51% in comparison with ambient CO_2 , and high N increased shoot volume 79% relative to the low N treatment. Nevertheless, the most notable result after 12 months was again produced by the $\text{CO}_2 \times \text{N}$ interaction, as the volume of seedlings grown in $525 \mu\text{l l}^{-1}$ CO_2 and high soil N exceeded those of all other treatments by $\geq 55\%$. Root volume measurements at the final harvest revealed a result somewhat parallel to that of the shoots, as here also, effects of CO_2 and N proved significant ($P = 0.0001$ for CO_2 , $P = 0.0081$ for N). Comparisons among treatments after 12 months revealed that elevated CO_2 increased root volume 67% relative to the ambient CO_2 treatment, and root volumes in the high N treatment exceeded those in low N by 30%. Again, however, the prominence of the stimulatory growth effects of $525 \mu\text{l l}^{-1}$ CO_2 and high N were noteworthy, as the root volumes of seedlings that received these treatments were $\geq 23\%$ greater than those of the other treatments.

In contrast to shoot dimensions and volume, shoot dry weight was significantly affected by atmospheric CO_2 concentration ($P = 0.0218$) after 4 months (Table 1). With the exception of seedlings grown in high CO_2 and low N and P, CO_2 enrichment increased shoot weight, particularly the intermediate atmosphere. Stimulation of root growth was also evident in the dry weight measurements at the first harvest ($P = 0.0001$), but unlike that of the shoots, was most pronounced in the $700 \mu\text{l l}^{-1}$ CO_2 concentration rather than intermediate CO_2 . Root weight was again increased by elevated CO_2 after 8 months ($P = 0.0001$), but in contrast to the first harvest, was evident

primarily in the intermediate concentration. The $525 \mu\text{l l}^{-1}$ CO_2 atmosphere also increased shoot weight after 8 months ($P = 0.0050$), but the $700 \mu\text{l l}^{-1}$ concentration generally resulted in lighter shoots than the ambient atmosphere. In addition to CO_2 effects on dry weight during the first 8 months, individual fertilization responses to N and P were noted at the first harvest in both shoots ($P = 0.0037$ for N, $P = 0.0009$ for P) and roots ($P = 0.0080$ for N, $P = 0.0011$ for P), and the positive response to N was evident at the second harvest as well ($P = 0.0001$ for both shoot and root weights).

At the final harvest, CO_2 and N effects on shoot weight were again significant, both individually and interactively ($P = 0.0001$ for CO_2 and N, $P = 0.0307$ for $\text{CO}_2 \times \text{N}$ interaction). Comparison of treatments producing the greatest and least shoot dry weights revealed that shoot weight of seedlings grown in $525 \mu\text{l l}^{-1}$ CO_2 and high N exceeded that of seedlings grown in ambient CO_2 and low N by 182% (Table 2). An even greater disparity of 224% between these treatments was revealed by measurements of coarse root weight, with individual and interactive effects of CO_2 and N proving significant for this root fraction as well ($P = 0.0001$ for CO_2 and N, $P = 0.0005$ for $\text{CO}_2 \times \text{N}$ interaction). A somewhat smaller, but nevertheless substantial, difference of 108% was noted for the fine root fraction ($P = 0.0001$ for CO_2 , $P = 0.0073$ for N). For both the shoots and coarse and fine roots, seedlings grown in $700 \mu\text{l l}^{-1}$ CO_2 also exhibited substantial increases in dry weight after 12 months, particularly those of the high N treatment.

Attributable largely to the enhanced root growth in $700 \mu\text{l l}^{-1}$ CO_2 during the first 4 months, seedlings of this atmospheric treatment had significantly lower shoot/root ratios ($P = 0.0001$) overall than those of seedlings grown in the other atmospheres at the first harvest (Table 1). Elevated CO_2 also significantly affected shoot/root ratio after 8 months, when it was revealed that ratios of seedlings grown in both the 700 and $525 \mu\text{l l}^{-1}$ atmospheres were lower ($P = 0.0001$) than that of

seedlings grown in ambient CO₂. At the final harvest, however, shoot/root ratio was affected by soil N ($P = 0.0057$) rather than CO₂ (Table 2), as high N produced higher ratios within the 525 $\mu\text{l l}^{-1}$ and ambient CO₂ treatments.

Total root system length was significantly affected by CO₂ ($P = 0.0001$), N ($P = 0.0003$), P ($P = 0.0105$), and the interaction of N and P ($P = 0.0258$) after 4 months. Overall, 700 $\mu\text{l l}^{-1}$ CO₂ and N and P fertilization stimulated root elongation, and the high N and P interaction resulted in the longest root systems within each CO₂ treatment (Table 1). Elevated CO₂ ($P = 0.0012$) and soil N ($P = 0.0005$) effects persisted through 8 months, but root system length was enhanced more by 525 than by 700 $\mu\text{l l}^{-1}$ CO₂ at the second harvest. By the third harvest, the CO₂ effect alone was significant ($P = 0.0001$), and again the 525 rather than the 700 $\mu\text{l l}^{-1}$ CO₂ atmosphere produced the longest root systems (Table 2). Overall, intermediate and high CO₂ increased total root length by 132% and 40%, respectively, compared to the ambient concentration after 12 months.

Quantification of ectomycorrhizal formation yielded conflicting results regarding CO₂ enrichment effects on mycorrhization, as at each of the three harvests, the CO₂ main treatment effect on the total number of mycorrhizae per seedling was significant but its effect on the percentage of total root system length colonized was nonsignificant. Nevertheless, seedlings grown in 700 $\mu\text{l l}^{-1}$ CO₂ had greater numbers of mycorrhizae ($P = 0.0007$) after 4 months, while those grown in the 525 $\mu\text{l l}^{-1}$ concentration had greater numbers ($P = 0.0055$) after 8 months (Table 1). The final harvest also revealed that seedlings of the intermediate CO₂ treatment formed the most mycorrhizae ($P = 0.0027$), but those grown in the high CO₂ atmosphere exhibited greater numbers than seedlings grown in ambient CO₂ as well (Table 2). As for N and P, significant N $\times$ P interaction effects on both the total number of mycorrhizae ($P = 0.0045$) and the percentage of root length colonized (P

= 0.0259) were noted at the first harvest (Table 1), with high N and P resulting in the highest mycorrhizal counts while high N and low P produced the lowest percentages of root length infected within each CO₂ treatment. Furthermore, a CO₂ × N interaction effect on the latter was evident after 12 months (Table 2), as seedlings grown in 700 µl l⁻¹ CO₂ and low N exhibited the highest percentage of root length infected ($P = 0.0498$) among all treatments at the final harvest.

The predominant mycobiont colonizing the root systems of all seedlings was P. tinctorius, which was easily identified due to its unique gold-yellow pigmentation (Marx and Bryan, 1975; Walker, 1989). Infrequently, ectomycorrhizae of Suillus granulatus (L.ex Fr.) Kuntze, a common mycobiont of ponderosa pine (Trappe, 1962) which was readily identified by both morphotype (Riffle, 1973) and the occasional appearance of its characteristic sporocarps (Lincoff, 1981) in some seedling containers, were noted. These likely originated from wind-dispersed spores or mycelia in the soil component of the potting mix and never constituted more than an exceedingly small proportion of the mycorrhizae on any seedling.

Discussion

A preponderance of the research to date concerned with CO₂ effects on above-ground growth of forest trees has indicated a positive response to CO₂ enrichment (Conroy et al., 1986; Surano et al., 1986; Kaushal et al., 1989; Norby and O'Neill, 1989; Bazzaz et al., 1990; Mousseau et al., 1996; Norby et al., 1996; Tissue et al., 1996), although several positive but ephemeral (Brown and Higginbotham, 1986; Radoglou and Jarvis, 1990; Walker et al., 1995a), negligible (O'Neill et al., 1987a; Bazzaz et al., 1990; Curtis et al., 1994; Norby et al., 1996), and negative responses (Mousseau and Enoch, 1989) have also been reported. The inconsistencies among these results are

likely attributable in part to interspecific variability, given the wide array of species investigated. However, differences in experimental duration and approach undoubtedly contributed as well, especially in regard to the extent to which CO₂ concentrations were elevated above ambient levels. Of particular importance in the evaluation of the above-ground response to CO₂ enrichment of ponderosa pine in the study reported here is the disparity in shoot growth between the 1.5 × ambient and 2 × ambient treatments. Specifically, shoot dimensions, volume, and dry weight of seedlings grown in 525 µl l⁻¹ CO₂ generally exceeded those of seedlings grown in 700 µl l⁻¹ CO₂, particularly at the final harvest, although both enriched atmospheres produced a positive above-ground response overall. A definitive explanation for this anomaly was beyond the scope of the experiment, but predictions of plant reactions to increasing CO₂ derived from the photosynthesis model of Farquhar et al. (1980) indicate that growth may be constrained in high CO₂ concentrations by foliar N dilution of sufficient magnitude to decrease photosynthetic capacity, a limitation on growth not imposed by more moderate increases in CO₂ above the ambient concentration (Luo et al., 1997). This explanation for the potency of the intermediate CO₂ treatment ultimately may or may not prove satisfactory, but the linkage between N and the CO₂ response was clearly evident in these results, as the largest shoots were consistently those of seedlings grown in high N in combination with intermediate CO₂. Limitations imposed by N supply are a commonality shared by this and enough previous studies (Brown and Higginbotham, 1986; Brown, 1991; Silvola and Ahlholm, 1993; Walker et al., 1995; Mousseau et al., 1996) to assume that the magnitude of the response of natural forest stands to continuing increase in atmospheric CO₂ may be restricted by soil N availability, although results of some studies (Norby et al., 1986; O'Neill et al., 1987a) suggest that growth in enriched atmospheres may enhance N-use efficiency and thus offset this limitation.

In comparison with soil N, P supply was of minimal importance in the above-ground response of the seedlings in this study, producing an overall growth increase during the first 4 months only. Thereafter, it proved to be of little consequence, with main treatment effects largely confined to shoot diameter. A limited number of previous studies (Conroy et al., 1986, 1988; Walker et al., 1995b) have revealed a proclivity of P fertilization to accentuate the stimulation of shoot growth resulting from CO₂ enrichment. Results presented here, however, largely indicate the absence of this interactive effect or perhaps that it was masked by the more prominent interaction of CO₂ and N.

The extensive literature review of plant root responses to CO₂ enrichment by Rogers et al. (1994) revealed that virtually all studies with forest trees that examined below-ground growth have reported an increase in root mass, and of the few that included measurements of length, an increase in root elongation. The dry weight and length measurements of ponderosa pine root systems presented here are thus in concurrence with previous findings. The root response diverged somewhat from that of the shoots in that the initial growth stimulation resulting from exposure to 700 $\mu\text{l l}^{-1}$ CO₂ exceeded that from exposure to the intermediate atmosphere, but after the first harvest the 525 $\mu\text{l l}^{-1}$ concentration again surpassed high CO₂ in growth production. Dry weight measurements at the final harvest revealed that this additional below-ground biomass was divided between the coarse and fine root fractions to somewhat favor the former, suggesting that ponderosa pine may invest more of the additional photosynthate produced in enriched atmospheres in the storage function predominating in coarse roots than in the resource capture function of fine roots. Prominent also in the measurements of root development, as discussed previously with regard to above-ground tissues, were the interactive effects of CO₂ and N, with high N again accentuating the growth stimulation of

CO₂ enrichment. This was exemplified by the greater root weight and length after 8 months and greater weight, length, and volume after 12 months of seedlings grown in elevated CO₂ and high N, and particularly in the 1.5 × ambient atmosphere and high N. The dominance of N over P in the mineral nutrition of the seedlings in this study was also evident in the root measurements, as increases in root weight and length resulting from P fertilization did not persist beyond the first harvest.

Evidence of a partitioning of the added growth attributable to CO₂ enrichment to favor below-ground over above-ground tissues was provided by the shoot/root ratios at the first two harvests. Initially, this effect was confined to the 700 µl l⁻¹ CO₂ treatment, but the second harvest revealed that it prevailed in 1.5 × ambient CO₂ also. Thus, these results were in concurrence, at least initially, with those of a majority of the studies reviewed by Ceulemans and Mousseau (1994), who reported that elevated CO₂ preferentially induced extra root rather than shoot growth. Baker and Enoch (1983), by way of an explanation for this asymmetric growth response, postulated that higher CO₂ levels should preferentially increase the growth of plant organs in closest proximity to supplies of limiting resources, which are often mineral nutrients. Nevertheless, this result did not persist to the final harvest, as N replaced CO₂ as the significant factor influencing shoot/root ratios at the end of the study and with an effect opposite that produced by CO₂ at the earlier harvests. Thus, after 12 months, these results were in agreement with a majority of the studies reviewed by Rogers et al. (1994) who noted that few investigations of the impacts of rising CO₂ on tree root systems had provided evidence of a disproportionate allocation of the added growth to below-ground tissues.

Reports to date of the effects of CO₂ enrichment on the mycorrhizal development of forest tree seedlings have been sufficiently disparate to create uncertainty about the impact rising CO₂ will

have on the mycorrhization of natural forests. Norby et al. (1987) documented an increase in the ectomycorrhizal colonization of *P. echinata* Mill. attributable to a doubling of the ambient CO₂ concentration. However, O'Neill et al. (1987b), in another study with this species and *Quercus alba* L., and Lewis et al. (1994), working with *P. taeda* L., found increases in mycorrhizal formation to be only a temporary response to atmospheric enrichment. Previous research with ponderosa pine has done little to alleviate this uncertainty, as one study indicated elevated CO₂ to have a negligible effect on mycorrhizal development (Walker et al., 1995a), but another demonstrated that a 2 × ambient atmosphere decreased colonization near mid study but increased colonization at the conclusion of the experiment (Walker et al., 1995b). Results reported here reveal that 700 µl l⁻¹ CO₂ induced an increase in the number of mycorrhizae formed by ponderosa pine after 4 months, 525 µl l⁻¹ CO₂ increased the number formed after 8 months, and both enriched atmospheres elevated mycorrhizal counts after one year, with the number formed in the 525 µl l⁻¹ atmosphere exceeding that in high CO₂. Thus, based on mycorrhizal counts alone, it is reasonable to deduce from these results that ponderosa pine invests some of the additional photosynthate it generates in elevated CO₂ atmospheres in ectomycorrhizal formation, and also that its proclivity to do so is more pronounced with a moderate elevation of CO₂ than with a doubling of the current concentration. This conclusion must be tempered somewhat, however, by the discrepancies between the mycorrhizal counts and the percentages of total root system length that were colonized, as the latter did not vary significantly among CO₂ treatments during the first 8 months of the study, and increased only in 700 µl l⁻¹ CO₂ after 12 months when in combination with low soil N. Thus, it is apparent that even though the number of mycorrhizae formed by the seedlings in this study was increased by atmospheric enrichment, a parallel increase in root system length resulted in a significant difference among CO₂

treatments in the proportion of root system colonized at only one of the three harvests, albeit the last one. Furthermore, the higher percentage of root length infected in high CO₂ and low N noted at the end of the experiment likely resulted as much from the moderately short root systems this treatment combination produced as from the fairly high number of mycorrhizae formed. Therefore, any conclusion that elevated CO₂ precipitated overall enhancement of mycorrhizal development in this study must be viewed with caution.

Given that research thus far has revealed substantial interspecific, intraspecific, and temporal variation in the response of forest trees to elevated CO₂, further examination of the potential impacts of this atmospheric change on tree growth is warranted before definitive conclusions are finalized. Data presented here indicate that atmospheric CO₂ enrichment administered under greenhouse conditions stimulates above- and below-ground growth of juvenile ponderosa pine, with initial allocation of the added biomass favoring root tissues modulating over time such that proportional whole-seedling growth prevails. Furthermore, they reveal a moderate CO₂ increase to be more stimulatory overall for this species during early growth stages than a doubling of the current ambient concentration, and that the magnitude of the CO₂ response is dependent upon soil N to a far greater extent than soil P availability.

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Table 1

Ponderosa pine seedling dimensions, dry weights, root elongation, and ectomycorrhizal colonization after 4 and 8 months as affected by atmospheric CO₂ and soil N and P concentration*

Harvest	Atmospheric CO ₂ and soil N and P treatment	Height (cm)	Stem diameter (mm)	Shoot weight (g)	Root weight (g)	Shoot/root ratio	Total root length (cm)	Total ectomycorrhizae	Ectomycorrhizal infection (%)
1	700 μ l l ⁻¹ CO ₂								
	High N, high P	8.2 (0.4)	3.3 (0.2)	1.33 (0.16)	1.09 (0.10)	1.22 (0.12)	1304 (131)	784 (118)	60 (4)
	High N, low P	8.3 (0.5)	2.9 (0.1)	1.07 (0.17)	0.78 (0.10)	1.36 (0.04)	894 (98)	474 (143)	51 (10)
	Low N, high P	9.6 (0.6)	3.1 (0.2)	1.22 (0.14)	1.01 (0.11)	1.20 (0.02)	1031 (97)	622 (64)	60 (1)
	Low N, low P	6.9 (0.9)	2.4 (0.2)	0.58 (0.13)	0.68 (0.11)	0.82 (0.06)	921 (123)	565 (60)	62 (4)
	525 μ l l ⁻¹ CO ₂								
	High N, high P	9.6 (0.3)	3.2 (0.1)	1.84 (0.20)	1.05 (0.08)	1.78 (0.28)	938 (146)	610 (69)	66 (4)
	High N, low P	8.4 (0.3)	2.8 (0.2)	1.07 (0.10)	0.53 (0.09)	2.09 (0.19)	704 (68)	296 (40)	42 (2)
	Low N, high P	8.8 (0.6)	3.2 (0.1)	1.30 (0.14)	0.59 (0.04)	2.20 (0.11)	603 (55)	360 (61)	60 (10)
	Low N, low P	9.1 (0.3)	2.7 (0.6)	1.09 (0.43)	0.52 (0.18)	2.04 (0.07)	561 (100)	343 (79)	62 (10)
	Ambient CO ₂								
	High N, high P	8.7 (0.4)	3.0 (0.3)	1.24 (0.14)	0.57 (0.07)	2.19 (0.17)	1010 (98)	572 (54)	57 (5)
	High N, low P	9.5 (0.6)	2.7 (0.3)	1.13 (0.19)	0.54 (0.07)	2.21 (0.46)	803 (78)	416 (46)	52 (2)
	Low N, high P	9.7 (0.6)	2.8 (0.1)	0.93 (0.08)	0.37 (0.02)	2.51 (0.31)	535 (50)	303 (25)	57 (2)
	Low N, low P	7.9 (0.7)	2.4 (0.2)	0.49 (0.07)	0.43 (0.03)	1.12 (0.10)	621 (24)	390 (8)	63 (3)
2	700 μ l l ⁻¹ CO ₂								
	High N, high P	11.8 (0.6)	5.0 (0.2)	3.68 (0.50)	2.72 (0.13)	1.36 (0.19)	1685 (19)	420 (19)	25 (1)
	High N, low P	12.3 (1.1)	5.9 (0.4)	5.94 (0.22)	3.37 (0.38)	1.80 (0.17)	1867 (436)	669 (125)	37 (4)
	Low N, high P	11.0 (0.8)	4.6 (0.2)	3.10 (0.19)	1.92 (0.04)	1.61 (0.06)	1281 (196)	537 (83)	46 (14)
	Low N, low P	10.9 (0.7)	5.0 (0.3)	4.19 (0.54)	1.87 (0.37)	2.32 (0.31)	1480 (227)	697 (222)	45 (10)
	525 μ l l ⁻¹ CO ₂								
	High N, high P	12.4 (0.6)	5.8 (0.6)	6.17 (0.38)	3.70 (0.29)	1.68 (0.11)	2439 (224)	1109 (81)	46 (1)
	High N, low P	12.3 (1.1)	6.1 (0.1)	6.30 (0.50)	3.62 (0.54)	1.82 (0.29)	2174 (95)	878 (120)	40 (5)
	Low N, high P	11.5 (1.4)	4.7 (0.3)	4.14 (0.25)	2.14 (0.23)	1.99 (0.26)	1481 (308)	664 (144)	45 (3)
	Low N, low P	9.9 (0.6)	5.2 (0.3)	4.68 (1.01)	3.36 (0.21)	1.37 (0.22)	2281 (79)	886 (121)	39 (5)
	Ambient CO ₂								
	High N, high P	14.8 (1.4)	5.5 (0.7)	6.46 (0.12)	2.81 (0.25)	2.34 (0.23)	2147 (257)	880 (245)	40 (6)
	High N, low P	13.4 (0.8)	5.5 (0.1)	7.06 (0.21)	2.74 (0.30)	2.64 (0.28)	1523 (219)	556 (66)	37 (1)
	Low N, high P	15.1 (1.6)	4.5 (0.3)	4.72 (0.55)	1.32 (0.10)	3.66 (0.62)	1136 (75)	425 (78)	38 (7)
	Low N, low P	10.2 (1.4)	3.4 (0.2)	2.54 (0.36)	1.08 (0.21)	2.44 (0.32)	1094 (139)	569 (93)	52 (4)

*The standard error of each mean is in parentheses.

Table 2

Ponderosa pine seedling dimensions, dry weights, root elongation, and ectomycorrhizal colonization after 12 months as affected by atmospheric CO₂ and soil N and P concentration^a

Harvest	Atmospheric CO ₂ and soil N and P treatment	Height (cm)	Stem diameter (mm)	Shoot weight (g)	Root weight (g)		Shoot/root ratio	Total root length (cm)	Total ectomycorrhizae	Ectomycorrhizal infection (%)
					Coarse	Fine				
3	700 µl l ⁻¹ CO ₂									
	High N, high P	14.5 (0.3)	9.0 (0.3)	11.11 (0.79)	2.53 (0.25)	5.47 (0.46)	1.42 (0.12)	2365 (376)	413 (76)	18 (3)
	High N, low P	14.2 (0.9)	8.1 (0.6)	8.76 (0.26)	2.03 (0.57)	5.75 (0.21)	1.14 (0.07)	3398 (84)	422 (64)	12 (2)
	Low N, high P	12.5 (0.5)	7.1 (0.5)	6.46 (0.22)	1.19 (0.09)	4.35 (0.52)	1.19 (0.14)	2103 (417)	545 (111)	26 (1)
	Low N, low P	14.4 (2.0)	6.3 (0.5)	6.39 (0.53)	1.17 (0.33)	3.41 (0.21)	1.40 (0.05)	2177 (559)	496 (137)	27 (10)
	525 µl l ⁻¹ CO ₂									
	High N, high P	17.0 (1.3)	10.0 (0.3)	14.67 (1.92)	3.69 (0.41)	6.55 (0.82)	1.47 (0.17)	4356 (520)	576 (139)	14 (3)
	High N, low P	17.7 (1.1)	9.8 (0.3)	15.89 (0.82)	3.16 (0.14)	6.47 (0.80)	1.66 (0.07)	4394 (957)	867 (394)	18 (5)
	Low N, high P	12.8 (1.1)	8.3 (0.5)	8.26 (1.27)	1.21 (0.18)	5.32 (0.40)	1.26 (0.11)	3499 (288)	389 (72)	11 (1)
	Low N, low P	13.3 (0.3)	7.4 (0.6)	8.52 (0.56)	1.54 (0.08)	5.42 (0.46)	1.22 (0.02)	4434 (613)	914 (311)	20 (5)
	Ambient CO ₂									
	High N, high P	14.0 (0.6)	7.6 (0.3)	7.92 (0.39)	1.46 (0.17)	3.23 (0.33)	1.77 (0.18)	1891 (211)	364 (103)	21 (7)
	High N, low P	12.4 (1.1)	6.5 (0.7)	7.14 (1.39)	1.03 (0.24)	3.33 (0.57)	1.64 (0.06)	1632 (254)	198 (34)	12 (1)
	Low N, high P	13.3 (1.6)	6.6 (0.1)	5.34 (0.43)	1.12 (0.18)	3.15 (0.36)	1.25 (0.10)	1965 (127)	200 (6)	10 (1)
	Low N, low P	12.8 (0.9)	6.3 (0.1)	5.48 (0.57)	1.00 (0.16)	3.10 (0.37)	1.35 (0.04)	1710 (51)	254 (53)	15 (3)

^aThe standard error of each mean is in parentheses.

Fig. 1. Ponderosa pine seedling shoot volumes after 4 and 8 months and shoot and root volumes after 12 months as affected by atmospheric CO₂ and soil N and P concentration. Bars represent the standard error of each mean. For N and P, H and L indicate high and low fertility treatments, respectively.