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Elevated atmospheric CO_2 and soil N fertility effects on growth, mycorrhizal colonization, and xylem water potential of juvenile ponderosa pine in a field soil

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Abstract

Interactive effects of atmospheric CO_2 enrichment and soil N fertility on above- and below-ground development and water relations of juvenile ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) were examined. Open-top field chambers permitted creation of atmospheres with 700 μL L^{-1}, 525 μL L^{-1}, or ambient CO_2 concentrations. Seedlings were reared from seed in field soil with a total N concentration of approximately 900 μg g^{-1} or in soil amended with sufficient $(NH_4)_2SO_4$ to increase total N by 100 μg g^{-1} or 200 μg g^{-1}. The 525 μL L^{-1} CO_2 treatment within the intermediate N treatment was excluded from the study. Following each of three consecutive growing seasons, whole seedlings of each combination of CO_2 and N treatment were harvested to permit assessment of shoot and root growth and ectomycorrhizal colonization. In the second and third growing seasons, drought cycles were imposed by withholding irrigation during which predawn and midday xylem water potential and soil water potential were measured. The first harvest revealed that shoot weight and coarse and fine root weights were increased by growth in elevated CO_2. Shoot and root volume and weights were increased by CO_2 enrichment at the second harvest, but growth stimulation by the 525 μL L^{-1} CO_2 concentration exceeded that in 700 μL L^{-1} CO_2 during the first two growing seasons. At the third harvest, above- and below-ground growth increases were largely confined to the 700 μL L^{-1} CO_2 treatment, an effect accentuated by high soil N but evident in all N treatments. Ectomycorrhizal formation was reduced by elevated CO_2 after one growing season, but thereafter was not significantly affected by CO_2 and was unaffected by soil N throughout the study. Results of the xylem water potential measurements were variable, as water potentials in seedlings grown in elevated CO_2 were intermittently higher on some measurement days but lower on others than that of seedlings grown in the ambient atmosphere. These results suggest that elevated CO_2 exerts stimulatory effects on shoot and root growth of juvenile ponderosa pine under field conditions which are somewhat dependent on N availability, but that temporal variation may periodically result in a greater response to a moderate rise in atmospheric CO_2 than to a doubling of the current ambient concentration.

Introduction

Research to date concerned with the effects of atmospheric CO_2 enrichment on forest ecosystems has generally emphasized above-ground growth processes of tree seedlings, and the results have indicated that the stimulation of shoot growth by elevated CO_2 varies somewhat by species in both its magnitude and duration (Bazzaz et al., 1990; Brown and Higginbotham, 1986; Conroy et al., 1986; Kaushal et al., 1989; Norby and O'Neill, 1989; Norby et al., 1996; O'Neill et al., 1987a; Radoglou and Jarvis, 1990; Surano et al., 1986; Tissue et al., 1996). Due to the difficulty inherent in the examination of below-ground processes, less research has focused on effects of rising CO_2 on tree root systems. Although in some studies CO_2 enrichment promoted disproportionately greater root than shoot growth (Norby et al., 1986; O'Neill et

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al., 1987a; Walker et al., 1995a, b), others revealed that stimulation of below-ground tree growth was a short-term response only (Brown and Higginbotham, 1986; Radoglou and Jarvis, 1990). For effects of elevated CO_2 on mycorrhizal colonization in forest tree seedlings, a positive response was observed by Norby et al. (1987), and positive but ephemeral responses by Lewis et al. (1994) and O'Neill et al. (1987b). As root systems provide critical feedback to the whole-tree response by altering the capacity to acquire water and nutrients (Norby, 1994), greater elucidation of the impact of rising CO_2 on root development is deemed crucial.

Comparatively little research has focused on the impact of elevated CO_2 on water relations of forest trees. Documented studies in this realm have consisted of pot studies which demonstrated that CO_2 enrichment may ameliorate the effects of water stress on seedling growth. Hollinger (1987) and Conroy et al. (1988) reported increases in instantaneous transpiration efficiency, while the results of related experiments (Conroy et al., 1986, 1988; Norby et al., 1986; Norby and O'Neill, 1989; Tolley and Strain, 1984) indicate improvement of whole-seedling water use efficiency in CO_2-enriched atmospheres.

The study reported here concerns the response of juvenile ponderosa pine (*P. ponderosa* Dougl. ex Laws.) to the interaction of elevated atmospheric CO_2 and soil N fertility under field conditions, with emphasis on root system development and water relations. We hypothesized that 1) CO_2 enrichment would stimulate above- and below-ground growth of ponderosa pine, an effect that would be accentuated by increased soil N availability, and 2) partitioning of this added growth to favor below-ground organs would enhance the resource acquisition capability of the root systems sufficiently to ameliorate the impact of short-term drought cycles on moisture stress.

Materials and methods

This study was conducted at the USDA Forest Service Institute of Forest Genetics (Placerville, CA) on a level uniform site at an elevation of 843 m with a soil of Aiken clay loam, a Xeric Haplohumult derived from andesite. Prior to the installation of the study, the site was occupied by a sparse grass cover, and the surrounding vegetation consists primarily of mature ponderosa pine trees. Site preparation consisted solely of disking to a depth of 25 cm. In 1991, 24 hexagonal open-top chambers, each 3.6 m in diameter, were constructed of clear acrylic plastic sheeting to a height of 2.5 m with frustums of the same material extending above the chamber walls. These chambers were designed so that CO_2 could be pumped in from a central reservoir through a plenum at a set-point concentration. Selected concentrations were 700 μL L^{-1} CO_2, 525 μL L^{-1} CO_2, and ambient CO_2, with the latter predetermined as 352 μL L^{-1}. Automated monitoring of each chamber atmosphere revealed that actual CO_2 concentrations did not vary from the set points by more than 14 μL L^{-1}, and eight chambers were dedicated to each atmosphere. The distance between chambers was 5.5 m to prevent intermixing of the atmospheres. Irrigation water was delivered to each chamber via a standpipe to a looped 2.5-cm diameter manifold and distributed through low pressure spray heads on a schedule regulated by an electronic timer. The irrigation regime consisted of one 30-min period on each of three days per week from April through October, and approximately 0.7 cm of water was applied to each chamber during individual periods.

Intermediate and high soil N fertility treatments were imposed within individual chambers by annual application of sufficient $(NH_4)_2SO_4$ to increase total soil N by 100 μg g^{-1} and 200 μg g^{-1}, respectively. The low N treatment consisted of unamended soil which had a total N concentration of approximately 900 μg g^{-1}. Total soil N was monitored periodically using a Perkin-Elmer 2400 CHN Analyzer (Perkin-Elmer Corp., Norwalk, CT) with samples collected by coring to a depth of 30 cm, and its concentration was consistent across the study site prior to the initial $(NH_4)_2SO_4$ applications. Three chambers were dedicated to each combination of CO_2 and N treatment, but due to the limitation imposed by the number of available chambers, the intermediate N regime within the 525 μL L^{-1} CO_2 treatment was omitted from the study.

Half sib ponderosa pine seed (Eldorado County, CA seed source) were stratified at 3 °C for 30 days and then sterilized in 10% H_2O_2 for 10 min. In May 1991, 66 pretreated seeds were sown per chamber in groups of three on a 30-cm square spacing. These seeds were sown directly into the soil constituting the chamber floors so that root egress would not be constrained by artificial barriers. Irrigation was applied as needed to induce germination, which was followed by a thinning to 22 equally-spaced seedlings per chamber.

Following the 1991 and 1992 growing seasons, three whole seedlings from each chamber were harvested per year using water pressure to excavate complete root systems. One additional seedling from each chamber was harvested by this method following the 1993 growing season. Thus, the first, second, and third harvests were completed 6, 18, and 30 months, respectively, after the chambers were seeded. At each harvest, individual roots of the selected seedlings were excavated from the root collar to their terminus to provide assurance that complete root systems were obtained. This frequently necessitated sacrificing seedlings immediately adjacent to those selected for harvest, which increased the spacing between the remaining seedlings, but equal numbers of seedlings were maintained in each chamber throughout the study and the seedlings within individual chambers remained approximately equidistant. Immediately after each harvest, shoots were severed at the root collar, the root systems were washed, and shoot and root volumes were determined by water displacement using the apparatus of Yawney and Carl (1969).

Ectomycorrhizal colonization of harvested root systems was quantified by measuring the total length of each root system and counting the root segments (1.0 cm lengths) exhibiting characteristic monopodial, bifurcate, or coralloid short roots, or short roots with an obvious fungal mantle. The number of root segments with mycorrhizae was then expressed as a percentage of the total length of the root system (Grand and Harvey, 1982). Ectomycorrhizae resulting from infection by different mycobionts were grouped together in the quantification procedure, but the fungi forming these mycorrhizae were identified as to probable species when possible by the sporocarps appearing in the chambers and by mycorrhizal color and morphology. Following mycorrhizal quantification, the shoots and root systems of each seedling were dried at 75 °C for 36 hours and weighed. Root systems were divided into coarse ($\geq$2.0 mm diameter) and fine (<2.0 mm diameter) root fractions which were weighed and recorded separately but then combined for calculation of shoot/root ratios, whole-seedling volume/weight ratios, and specific root length.

In September 1992, predawn and midday measurements of xylem water potential (Ψ_p) were made on seedlings of all treatments by the method of Waring and Cleary (1967) using a PMS Model 600 portable pressure chamber (PMS Instrument Co., Corvallis, OR). These measurements were made on the first, third, fifth, and seventh day after discontinuing irrigation in order to examine seedling water relations as affected by treatment during short-term imposed drought episodes. Predawn measurements were completed between 0500 and 0600 hours and midday measurements were completed between 1300 and 1400 hours. During each measurement period, a needle from the upper crown of one seedling from every chamber was selected for measurement. Upon excision, the needles were immediately placed in the chamber, and the chamber pressure was then increased at a constant rate of 0.03 MPa s^{-1} until sap appeared uniformly over the cut surface. Between the predawn and midday Ψ_p measurements of each day, soil water potential (Ψ_s) was measured with a Soilmoisture Model 5910-A digital soil moisture meter and Model 5201 gypsum blocks (Soilmoisture Equipment Corp., Santa Barbara, CA). One gypsum block had been previously installed to a depth of 20 cm at the center of each chamber. The series of Ψ_p and Ψ_s measurements described above was repeated in May, July, and September of 1993 in order to examine seasonal differences in the effect of the CO_2 and N treatments on seedling water relations.

Two-way analysis of variance was performed on all data derived from this 3 × 3 (three atmospheric CO_2 × three soil N treatments) incomplete factorial experiment, with the intermediate soil N and 525 μL L^{-1} CO_2 treatment combination excluded. Type IV sum of squares was used to accommodate the missing treatment combination, and the CO_2 and N main treatment and $CO_2 \times N$ interaction effects were considered significant when $p \leq 0.05$ according to the F test. The arcsine transformation was performed on the mycorrhizal infection percentages prior to analysis. Data derived from each of the three harvests and each of the four series of water relations measurements were analyzed separately, with the data collected from seedlings in individual chambers constituting replications and thus three replications per treatment combination. All statistical analyses were accomplished using the Statistical Analysis System (SAS Institute, Inc., 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 (SE) of the mean for all variables presented in tables is included to provide an indication of the variation within treatment means, and the standard error of the difference (SED) among means for both main treatment and treatment interaction effects is included to facilitate comparisons among means for all growth variables.

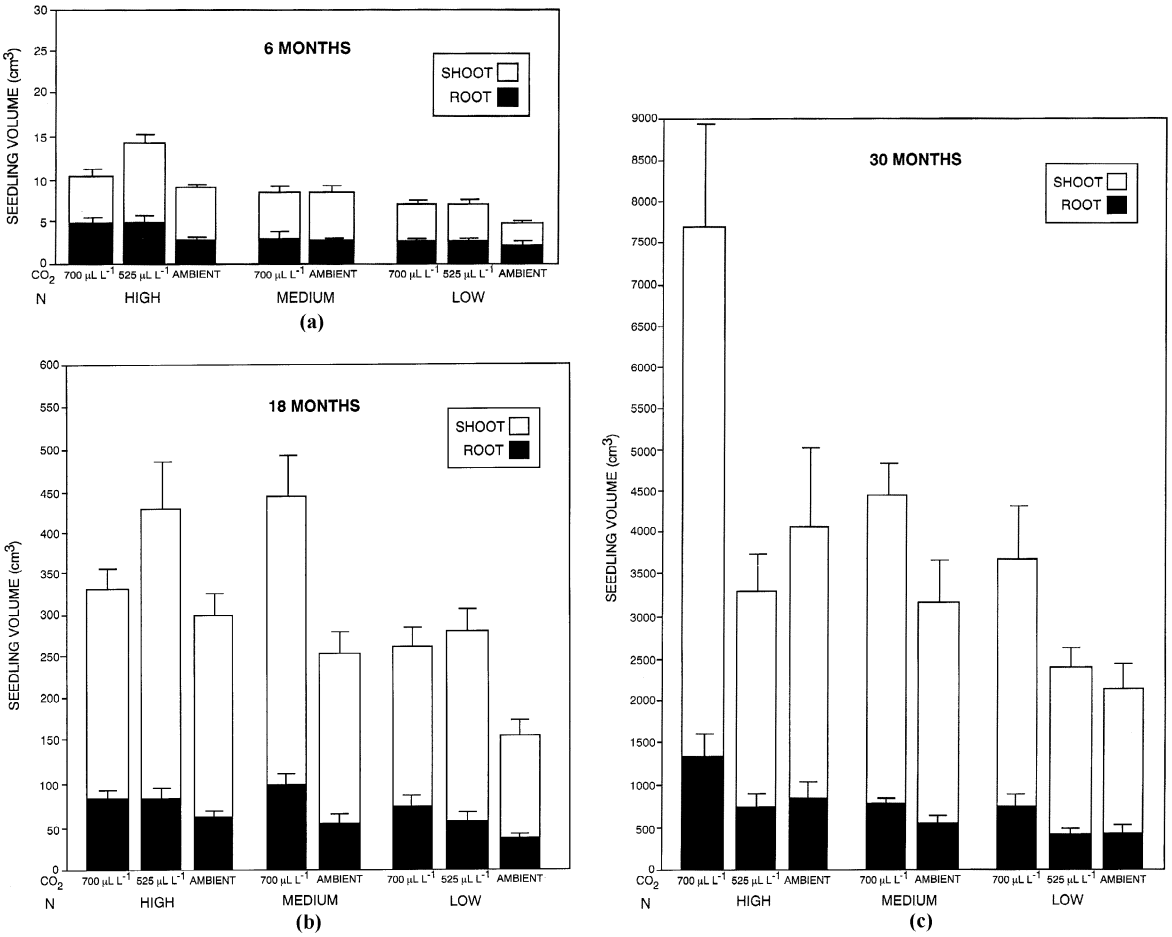

Figure 1. Shoot and root volumes after 6, 18, and 30 months of juvenile ponderosa pine as affected by atmospheric CO_2 and soil N concentration. Bars represent the SE of each mean. For shoot volume at 6 months, SED = 1.5 for CO_2, 1.5 for N, and 2.5 for $CO_2 \times$ N; for root volume at 6 months, SED = 0.8 for CO_2, 0.8 for N, and 1.3 for $CO_2 \times$ N; for shoot volume at 18 months, SED = 58 for CO_2, 58 for N, and 94 for $CO_2 \times$N; for root volume at 18 months, SED = 15 for CO_2, 15 for N, and 25 for $CO_2 \times$ N; for shoot volume at 30 months, SED = 876 for CO_2, 876 for N, and 1430 for $CO_2 \times$ N; and for root volume at 30 months, SED = 123 for CO_2, 123 for N, and 201 for $CO_2 \times$ N.

Results

Shoot growth

Analysis of variance revealed that shoot volume was significantly increased (p = 0.0245) by N fertilization but was unaffected by elevated CO_2 after one growing season (Figure 1). After 18 months, however, both the CO_2 (p = 0.0298) and N (p = 0.0129) treatments significantly influenced shoot growth, as a substantial volume increase was evident in the 525 μL L^{-1} CO_2 treatment in high soil N, in the 700 μL L^{-1} CO_2 treatment in medium soil N, and in both enriched atmospheres in low N. Furthermore, the smallest shoot volumes after two growing seasons were those of seedlings grown in low soil N and ambient CO_2. After 30 months, shoot volume was significantly increased (p = 0.0411) by the high CO_2 atmosphere in all three N treatments. The greatest response was evident in high soil N, however, as the volume of these seedlings exceeded that of any other treatment by $\geq$73%. Unlike the high CO_2 concentration, the 525 μL L^{-1} CO_2 atmosphere did not increase shoot volume after three growing seasons in high soil N and produced only a marginal increase in low N.

Although shoot volume was not significantly affected by the CO_2 concentration after 6 months, shoot dry weight was increased (p = 0.0498) by both the 700 μL L^{-1} and 525 μL L^{-1} CO_2 atmospheres, with the latter having a greater stimulatory effect than the former in high and low N (Table 1). Shoot weight was also affected by N fertility after one growing season ($p = 0.0027$), with the high N treatment producing the greatest biomass. The above pattern of treatment effects persisted through 18 months, as both CO_2 enrichment ($p = 0.0007$) and N fertilization ($p = 0.0187$) increased shoot weight, and seedlings grown in the intermediate CO_2 concentration were again larger than those grown in high CO_2 in high and low N. Unlike the first growing season, however, high CO_2 substantially increased shoot weight in the medium N treatment as well. At the third harvest, a shift in the effect of atmospheric CO_2 was revealed, as increases in shoot weight were largely a response ($p = 0.0417$) to the 700 μL L^{-1} concentration alone. Shoot weight was also increased by N fertility ($p = 0.0296$) after 30 months, and overall, the combination of high N and high CO_2 produced the greatest shoot biomass by a substantial margin.

Root growth

Root volume was not significantly affected by either atmospheric CO_2 or N fertility after 6 months (Figure 1). During the second growing season, however, elevated CO_2 ($p = 0.0202$) and N fertilization ($p = 0.0499$) stimulated root volume growth, as a stepwise response to increasing CO_2 was evident in medium and low N after 18 months while the 700 μL L^{-1} and 525 μL L^{-1} CO_2 atmospheres produced a comparable growth increase in high N. Low soil N produced the smallest root volumes overall after the second growing season, although the ambient atmosphere resulted in root growth in high and medium soil N similar to that in low N. The third harvest revealed a root growth response to CO_2 enrichment parallel to that of the shoots, as the high CO_2 atmosphere increased root volume ($p = 0.0077$) but this effect did not extend to the medium CO_2 concentration. Soil N also had a significant effect ($p = 0.0097$) on root growth, as root volumes generally increased with increasing N availability. Overall, the largest root volume was that of seedlings grown in high soil N and the 700 μL L^{-1} CO_2 atmosphere, which exceeded that of any other treatment by $\geq$ 56% after 30 months.

Elevated CO_2 and N fertilization increased root dry weight after one growing season in both the coarse ($p = 0.0464$ for CO_2 treatment and $p = 0.0015$ for N treatment) and fine ($p = 0.0414$ and $p = 0.0216$ for CO_2 and N treatments, respectively) root fractions (Table 1). The smallest root systems within N treatments were those of seedlings grown in ambient CO_2 and the smallest of any treatment were those of seedlings grown in ambient CO_2 and low N for both root fractions. The second harvest revealed a similar response in coarse root growth to the CO_2 ($p = 0.0026$) and N ($p = 0.0146$) concentrations. In the fine root fraction, however, CO_2 enrichment alone resulted in an increase ($p = 0.0270$) in dry weight, and the 525 μL L^{-1} CO_2 atmosphere did not stimulate fine root growth in low soil N. Root weight measurements after 30 months indicated a modification of treatment effects, although here also the CO_2 ($p = 0.0219$) and N ($p = 0.0128$) concentration effects were significant for coarse root growth. However, the increase in coarse root weight was largely confined to the high CO_2 treatment regardless of N availability, and neither CO_2 nor N significantly affected fine root growth. Nevertheless, the overall result of the root weight measurements after three growing seasons indicated a response in concert with that revealed previously for above- and below-ground growth, as the largest combined coarse and fine root weight of any treatment was that of seedlings grown in high CO_2 and high N.

Examination of specific root length revealed that the effect of CO_2 concentration on this root growth variable was nonsignificant throughout the study (Table 1). Specific root length was also unaffected by N treatment after 6 months, but was increased by low soil N after 18 months ($p = 0.0339$) and by medium N after 30 months ($p = 0.0133$).

Shoot/root and volume/weight ratios

Shoot/root ratio was not significantly affected by either CO_2 enrichment or N fertilization at the first and third harvests (Table 1). The second harvest revealed a conflicting, although significant ($p = 0.0454$), result with regard to elevated CO_2, however, as the lowest shoot/root ratios within the high and low N treatments were those of seedlings grown in 700 μL L^{-1} CO_2, but this atmosphere produced the highest ratio in medium N. Similarly, whole-seedling volume/weight ratio was unaffected by CO_2 and N after the first and third growing seasons, but volume/weight ratios were significantly higher ($p = 0.0002$) in the ambient CO_2 treatment after 18 months.

Mycorrhizal colonization

The initial harvest revealed that the percentage of total root system length colonized by ectomycorrhizal fungi was reduced by elevated CO_2 ($p = 0.0471$), as the

Table 1. Dry weight and ectomycorrhizal colonization after 6, 18, and 30 months of juvenile ponderosa pine as affected by atmospheric CO_2 and soil N concentration.The SE of each mean is in parentheses

Harvest	Soil N and CO_2 treatment	Shoot weight (g)	Root weight (g)		Shoot/root ratio	Volume/weight ratio ($cm^3\ g^{-1}$)	Specific root length ($m\ g^{-1}$)	Mycorrhizal infection (%)
			Coarse	Fine				
6 months	*High soil N*							
	700 $\mu L\ L^{-1}\ CO_2$	1.57 (0.26)	0.32 (0.05)	0.37(0.06)	2.25(0.21)	5.06(0.37)	3.09(0.34)	22(3)
	525$\mu L\ L^{-1}\ CO_2$	1.84 (0.33)	0.31 (0.07)	0.37(0.05)	2.68(0.25)	5.79(0.64)	3.83(0.48)	20(3)
	Ambient CO_2	1.28 (0.17)	0.20 (0.03)	0.26(0.04)	2.91(0.27)	7.53(2.52)	4.51(0.38)	32(9)
	Medium soil N							
	700 $\mu L\ L^{-1}\ CO_2$	0.95 (0.24)	0.17 (0.06)	0.28(0.05)	2.13(0.19)	5.33(0.63)	4.68(1.38)	24(3)
	Ambient CO_2	0.94 (0.20)	0.11 (0.02)	0.23(0.03)	2.74(0.29)	7.36(0.74)	4.78(0.20)	50(8)
	Low soil N							
	700 $\mu L\ L^{-1}\ CO_2$	0.81 (0.14)	0.14 (0.03)	0.22(0.04)	2.26(0.24)	6.83(0.63)	3.87(0.29)	27(4)
	525 $\mu L\ L^{-1}\ CO_2$	1.03 (0.17)	0.16 (0.03)	0.26(0.02)	2.44(0.24)	5.11(0.58)	4.09(0.47)	45(7)
	Ambient CO_2	0.54 (0.07)	0.08 (0.01)	0.17(0.01)	2.09(0.15)	6.77(0.77)	4.84(0.60)	52(9)
	SED: CO_2	0.32	0.06	0.06	0.35	1.61	0.94	10
	N	0.32	0.06	0.06	0.35	1.61	0.94	10
	$CO_2 \times N$	0.52	0.10	0.10	0.58	2.63	1.53	16
18 months	*High soil N*							
	700 $\mu L\ L^{-1}\ CO_2$	75.43 (10.29)	23.24 (3.04)	4.08(0.47)	2.82(0.26)	3.39(0.15)	0.59(0.08)	27(6)
	525 $\mu L\ L^{-1}\ CO_2$	107.82 (18.56)	27.93 (4.42)	4.09(0.85)	3.41(0.40)	3.20(0.14)	0.46(0.04)	35(3)
	Ambient CO_2	56.27 (7.34)	16.14 (1.95)	2.37(0.16)	3.01(0.16)	4.09(0.07)	0.56(0.07)	29(4)
	Medium soil N							
	700 $\mu L\ L^{-1}\ CO_2$	94.79 (15.95)	29.16 (5.50)	3.28(0.25)	3.00(0.21)	3.57(0.09)	0.39(0.04)	29(6)
	Ambient CO_2	47.75 (8.96)	13.67 (2.31)	3.23(0.39)	2.69(0.18)	4.12(0.16)	0.70(0.11)	30(4)
	Low soil N							
	700 $\mu L\ L^{-1}\ CO_2$	53.12 (11.83)	17.35 (3.62)	3.14(0.45)	2.57(0.27)	3.92(0.25)	0.65(0.09)	30(6)
	525 $\mu L\ L^{-1}\ CO_2$	68.28 (9.02)	17.58 (2.70)	2.45(0.33)	4.05(0.94)	3.25(0.24)	0.53(0.09)	30(6)
	Ambient CO_2	26.90 (3.28)	6.76 (1.09)	2.50(0.29)	3.18(0.56)	4.65(0.60)	0.91(0.18)	30(4)
	SED: CO_2	17.43	5.04	0.67	0.67	0.39	0.15	8
	N	17.43	5.04	0.67	0.67	0.39	0.15	8
	$CO_2 \times N$	28.46	8.22	1.10	1.09	0.64	0.24	12
30 months	*High soil N*							
	700 $\mu L\ L^{-1}\ CO_2$	2118.9 (696.1)	378.2 (82.1)	16.6(5.6)	5.1(0.8)	3.0 (0.1)	0.2 (0.1)	41 (8)
	525 $\mu L\ L^{-1}\ CO_2$	1036.5 (95.2)	204.0 (44.9)	18.5(3.1)	5.0(0.8)	2.6 (0.2)	0.2 (0.1)	27(3)
	Ambient CO_2	1235.8 (260.4)	234.5 (55.0)	11.2(5.0)	5.1(0.3)	2.7 (0.3)	0.2 (0.1)	21(3)
	Medium soil N							
	700 $\mu L\ L^{-1}\ CO_2$	1294.8 (165.8)	223.3 (14.5)	22.0(2.4)	5.3(0.7)	2.9 (0.1)	0.3 (0.1)	34(15)
	Ambient CO_2	841.9 (167.0)	160.7 (23.3)	14.1(3.3)	4.7(0.3)	3.1 (0.1)	0.3 (0.1)	32(10)
	Low soil N							
	700 $\mu L\ L^{-1}\ CO_2$	1068.3 (183.8)	208.0 (37.4)	12.0(2.2)	4.9(0.1)	2.9 (0.3)	0.2 (0.1)	38(8)
	525 $\mu L\ L^{-1}\ CO_2$	685.3 (93.3)	135.3 (32.2)	9.1(2.0)	5.0(0.6)	2.9 (0.1)	0.2 (0.1)	35(7)
	Ambient CO_2	619.0 (118.9)	123.5 (21.0)	10.9(3.3)	4.7(0.6)	2.9 (0.1)	0.2 (0.1)	49(11)
	SED: CO_2	251.3	38.0	3.1	0.5	0.2	0.1	8
	N	251.3	38.0	3.1	0.5	0.2	0.1	8
	$CO_2 \times N$	410.3	62.1	5.1	0.8	0.3	0.1	13

highest infection percentages within each N treatment were those of seedlings grown in ambient CO_2 (Table 1). After 18 months, however, infection percentages were generally consistent across all treatments. The third harvest provided conflicting results regarding the influence of CO_2 on mycorrhizal colonization, as the highest infection percentage in the high N treatment was exhibited by seedlings grown in 700 μL L^{-1} CO_2, while in low N, seedlings grown in the ambient atmosphere exhibited the highest infection level. Treatment effects were statistically nonsignificant after 30 months as well, however. The predominant mycobiont colonizing the roots of all seedlings throughout the study was *Suillus granulatus* (L. ex Fr.) Kuntze, readily identified due to the prevalence of its characteristic sporocarps (Lincoff, 1981) in the chambers and the color and morphology of its mycorrhizae (Riffle, 1973). Much less frequently encountered were ectomycorrhizae formed by *Cenococcum geophilum* Fr., which were identified by their unique coloration (Molina and Trappe, 1982).

Water relations

Substantial variability in predawn Ψ_p was evident both within and between the four series of water relations measurements (Figure 2). Predawn Ψ_p in the early fall of the second growing season was not affected on any of the four days of measurement by either the CO_2 or N treatment. Likewise, predawn measurements on the first and third days after discontinuing irrigation in the spring of the third growing season were unaffected by either treatment, but on the fifth day predawn Ψ_p of seedlings grown in 700 μL L^{-1} CO_2 and of seedlings grown in high soil N and 525 μL L^{-1} CO_2 were higher (p = 0.0286) than those of the other treatments. Also, predawn Ψ_p of unfertilized seedlings was lower, with the exception of those grown in 700 μL L^{-1} CO_2, than that of fertilized seedlings on this measurement day (p = 0.0329). The series of Ψ_p measurements completed in the summer of the third growing season also revealed that predawn Ψ_p of seedlings grown in elevated CO_2 exceeded that of seedlings grown in the ambient atmosphere on the fifth day after cessation of irrigation (p = 0.0129). However, measurements completed in the early fall of the third growing season were consistent with those made in the fall of the previous year in that predawn Ψ_p was unaffected by either CO_2 or N treatment on all measurement days.

The intermittent and inconsistent pattern of statistically significant treatment effects observed in the predawn measurements was also noted in midday Ψ_p (Figure 2). One day after suspending irrigation for the measurements made in the fall of the second growing season, midday Ψ_p of seedlings grown in 700 μL L^{-1} CO_2 and that of the 525 μL L^{-1} CO_2 treatment grown in high N were lower (p = 0.0498) than those of the other treatments, but this result did not persist through the remainder of this measurement series. Likewise, midday Ψ_p in the spring of the third growing season was not affected by CO_2 or N on any day of measurement. However, summer measurements of the third growing season revealed that midday Ψ_p of unfertilized seedlings grown in ambient CO_2 exceeded that of all other treatments on the seventh day after discontinuing irrigation (p = 0.0476 and p = 0.0435 for CO_2 and N treatments, respectively). Midday measurements made in the early fall of the third growing season revealed significant treatment effects on the third and seventh day after suspending irrigation. On the third day, midday Ψ_p of seedlings grown in elevated CO_2 was higher (p = 0.0444) than that of seedlings grown in the ambient atmosphere regardless of N treatment. Conversely, measurements on the seventh day revealed that midday Ψ_p of the elevated CO_2 treatments were lower (p = 0.0432) than that of the ambient treatment.

The Ψ_s measurements made in conjunction with the measurement of Ψ_p revealed that soil water availability did not differ significantly among the CO_2 or N treatments on any day within any series of the water relations measurements. Overall, Ψ_s declined on successive days of measurement after discontinuing irrigation within each of the four series (Table 2). Comparatively, higher Ψ_s values were encountered in the fall of the second growing season and lower values were prevalent in the spring of the third year, with an intermediate range of Ψ_s evident in the summer and fall of the third season.

Discussion

An array of results have been reported concerning the effect of CO_2 enrichment on shoot growth of forest tree seedlings. In previous experiments with ponderosa pine, which have primarily consisted of pot studies, Surano et al. (1986) observed an enhanced above-ground growth after 2.5 years in an atmosphere containing approximately twice the ambient CO_2 concentration. However, Walker et al. (1995a, b) found that 1.5×ambient CO_2 stimulated shoot growth of ponderosa pine seedlings more than a twice ambient atmosphere after one year if mineral nutrition was ade-

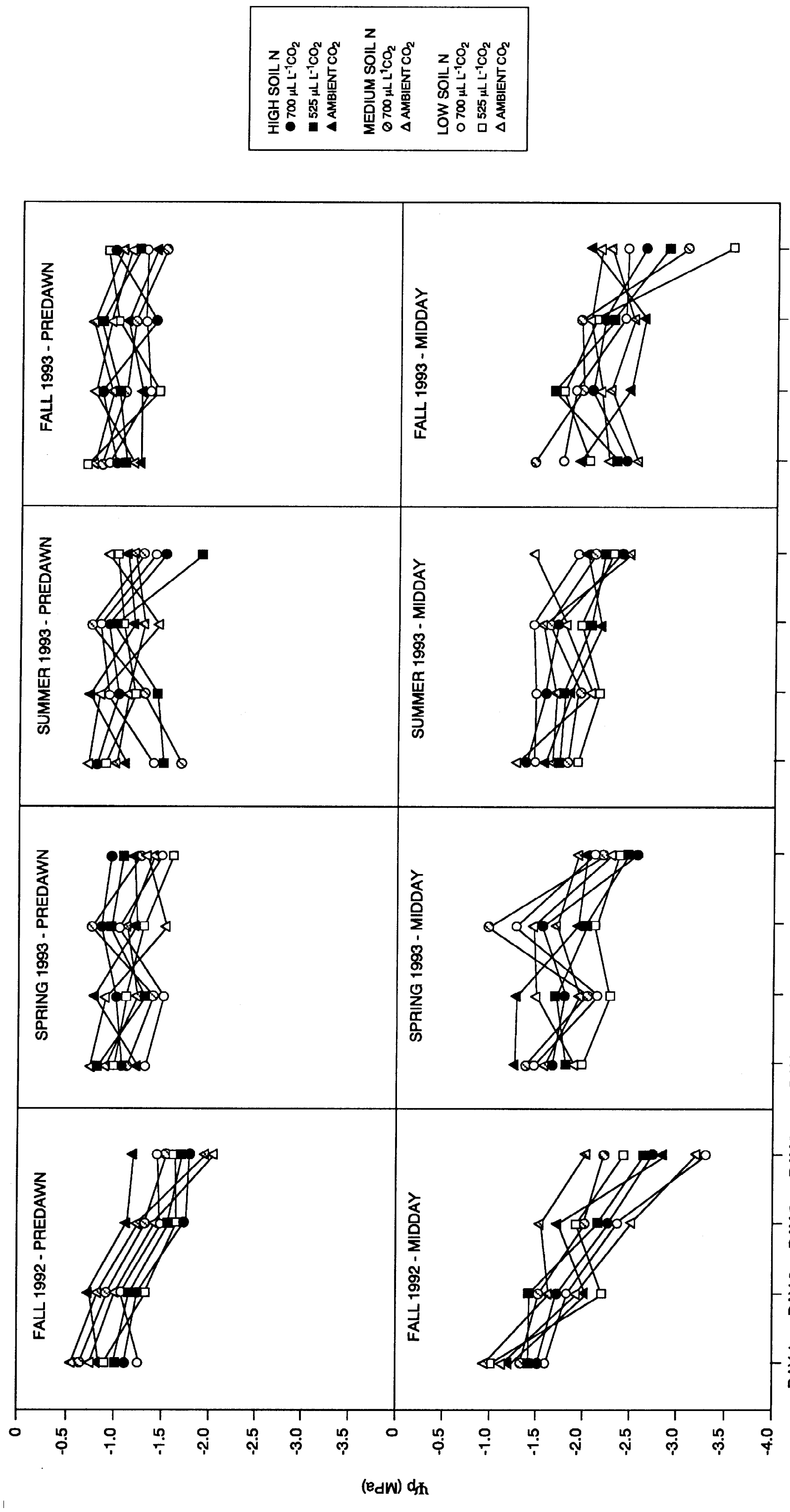

Figure 2. Predawn and midday xylem water potential (Ψ_p) of juvenile ponderosa pine as affected by atmospheric CO_2 and soil N concentration 1, 3, 5, and 7 days after cessation of irrigation in the fall of the second growing season and in the spring, summer, and fall of the third growing season.

Table 2. Soil water potential (Ψ_s) during measurements of xylem water potential in juvenile ponderosa pine as affected by atmospheric CO_2 and soil N concentration. The SE of each mean is in parentheses

Season and year of measurement	Soil N and CO_2 treatment	Ψ_s (MPa)			
		Day 1	Day 3	Day 5	Day 7
Fall 1992	*High soil N*				
	700 μL L^{-1} CO_2	-0.002(0.001)	-0.008(0.002)	-0.006(0.001)	-0.009(0.001)
	525 μL L^{-1} CO_2	-0.002(0.001)	-0.011(0.007)	-0.007(0.002)	-0.014(0.007)
	Ambient CO_2	-0.001 (0.001)	-0.006(0.001)	-0.004(0.001)	-0.007(0.002)
	Medium soil N				
	700 μL L^{-1} CO_2	-0.002(0.001)	-0.007(0.001)	-0.011(0.006)	-0.008(0.001)
	Ambient CO_2	-0.001(0.001)	-0.005(0.001)	-0.005(0.001)	-0.010(0.001)
	Low soil N				
	700 μL L^{-1} CO_2	-0.001(0.001)	-0.008(0.003)	-0.006(0.001)	-0.007(0.002)
	525 μL L^{-1} CO_2	-0.002(0.001)	-0.007(0.003)	-0.003(0.001)	-0.007(0.001)
	Ambient CO_2	-0.001(0.001)	-0.005(0.001)	-0.003(0.002)	-0.008(0.002)
Spring 1993	*High soil N*				
	700 μL L^{-1} CO_2	-0.082(0.047)	-0.053(0.019)	-0.137(0.035)	-1.167(0.167)
	525 μL L^{-1} CO_2	-0.067(0.032)	-0.052(0.012)	-0.452(0.216)	-1.052(0.448)
	Ambient CO_2	-0.042(0.007)	-0.045(0.005)	-0.467(0.033)	-1.133(0.367)
	Medium soil N				
	700 μL L^{-1} CO_2	-0.038(0.008)	-0.040(0.006)	-0.102(0.049)	-0.685(0.414)
	Ambient CO_2	-0.043(0.009)	-0.040(0.001)	-0.212(0.100)	-1.267(0.233)
	Low soil N				
	700 μL L^{-1} CO_2	-0.040(0.001)	-0.040(0.001)	-0.055(0.003)	-0.525(0.246)
	525 μL L^{-1} CO_2	-0.123(0.088)	-0.082(0.037)	-0.160(0.0721)	-0.933(0.067)
	Ambient CO_2	-0.033(0.003)	-0.033(0.003)	-0.163(0.118)	-0.510(0.257)
Summer 1993	*High soil N*				
	700 μL L^{-1} CO_2	-0.047(0.017)	-0.050(0.020)	-0.057(0.022)	-0.098(0.033)
	525 μL L^{-1} CO_2	-0.033(0.003)	-0.033(0.003)	-0.153(0.123)	-0.062(0.020)
	Ambient CO_2	-0.030(0.001)	-0.030(0.001)	-0.050(0.001)	-0.057(0.018)
	Medium soil N				
	700 μL L^{-1} CO_2	-0.027(0.003)	-0.027(0.003)	-0.030(0.006)	-0.090(0.055)
	Ambient CO_2	-0.030(0.001)	-0.030(0.001)	-0.033(0.003)	-0.050(0.020)
	Low soil N				
	700 μL L^{-1} CO_2	-0.033(0.003)	-0.033(0.003)	-0.040(0.001)	-0.093(0.031)
	525 μL L^{-1} CO_2	-0.027(0.003)	-0.027(0.003)	-0.040(0.006)	-0.147(0.053)
	Ambient CO_2	-0.030(0.001)	-0.030(0.001)	-0.030(0.001)	-0.043(0.007)
Fall 1993	*High soil N*				
	700 μL L^{-1} CO_2	-0.082(0.047)	-0.082(0.047)	-0.082(0.047)	-0.093(0.053)
	525 μL L^{-1} CO_2	-0.040(0.006)	-0.040(0.006)	-0.038(0.008)	-0.120(0.090)
	Ambient CO_2	-0.030(0.001)	-0.033(0.003)	-0.033(0.003)	-0.033(0.003)
	Medium soil N				
	700 μL L^{-1} CO_2	-0.037(0.007)	-0.033(0.003)	-0.033(0.003)	-0.037(0.003)
	Ambient CO_2	-0.030(0.001)	-0.033(0.003)	-0.033(0.003)	-0.033(0.003)
	Low soil N				
	700 μL L^{-1} CO_2	-0.040(0.001)	-0.040(0.001)	-0.037(0.003)	-0.040(0.001)
	525 μL L^{-1} CO_2	-0.030(0.006)	-0.033(0.003)	-0.037(0.007)	-0.085(0.045)
	Ambient CO_2	-0.037(0.007)	-0.037(0.007)	-0.037(0.007)	-0.040(0.006)

quate. Results reported here also provide evidence of a greater shoot response in juvenile ponderosa pine to the 1.5×ambient than the 2×ambient atmosphere for the first two growing seasons. Predictions of plant reactions to increasing CO_2 derived from the photosynthesis model of Farquhar et al. (1980) indicate that growth may be temporarily restricted in high CO_2 by dilution of foliar N to the extent that photosynthetic capacity declines, a limitation not imposed by a moderate rise in CO_2 above the ambient concentration (Y Luo, Biological Sciences Center, Desert Research Institute, Reno, NV, personal communication). This may partially explain the higher growth rate of the 525 μL L^{-1} CO_2 treatment relative to that in 700 μL L^{-1} CO_2 during the first two growing seasons observed here. Nevertheless, increases in shoot growth were largely confined to the high CO_2 treatment after 30 months, a response common to all three N fertility treatments but accentuated by high N. This suggests that low soil N does not preclude a positive response to elevated CO_2 in ponderosa pine, but that the effect is magnified when N is readily available.

Most studies concerned with effects of CO_2 enrichment on the root systems of forest tree seedlings have revealed that below-ground growth was stimulated by elevated CO_2 (Rogers et al., 1994). Root volume and dry weight measurements presented here support this conclusion for ponderosa pine, although significant CO_2 treatment effects on volume did not appear until the second growing season and CO_2 effects on fine root weight did not persist through the final harvest. It has been hypothesized that CO_2 enrichment may accelerate growth of below-ground tissues proportionally more than those above ground in forest trees (Norby, 1989), and subsequent research has shown that this is true for some species (Rogers et al., 1994). However, there was little evidence in the data presented here that elevated CO_2 favors root growth over that of the shoots in ponderosa pine, as shoot/root ratios were largely unaffected by CO_2 treatment throughout the study. Furthermore, the accentuation of root growth in the 700 μL L^{-1} CO_2 atmosphere by the high N treatment was less pronounced than that of the shoots after three growing seasons.

Ectomycorrhizal quantification of complete root systems harvested after 6 months of seedling growth revealed that the percentage of root length colonized decreased in elevated CO_2 relative to that of seedlings grown in the ambient atmosphere. Thereafter, atmospheric CO_2 was of little or no consequence in ectomycorrizal formation, as all treatment effects were nonsignificant after 18 and 30 months. Thus, results presented here are not in agreement with those of Norby et al. (1987) which indicated that ectomycorrhizal development of *P. echinata* Mill. was stimulated by a doubling of the ambient CO_2 concentration. Furthermore, they are not in concurrence with those of studies in which CO_2 enrichment resulted in a temporary increase in ectomycorrhizal colonization of *P. echinata* Mill., *Quercus alba* L. (O'Neill et al., 1987b), and *P. taeda* L. (Lewis et al., 1994) that did not persist through the end of the experiments. Previous research with ponderosa pine of somewhat longer duration than the above studies provided mixed results, including a finding of no CO_2 effect on ectomycorrhizal formation in one experiment (Walker et al., 1995a) but a significant increase in colonization after one year resulting from a doubling of ambient CO_2 in another (Walker et al., 1995b). It is apparent that the conclusions drawn from studies concerned with effects of CO_2 enrichment on the mycorrhizal development of forest trees is at least partially dependent upon the experimental conditions imposed as well as the species investigated. Given the episodic nature of ectomycorrhizal formation in most species, the duration of the experiment and the frequency and timing of sampling are particularly important in the interpretation of results. Nevertheless, the only reasonable conclusion that can be drawn from the data presented here is that CO_2 enrichment did not elevate ectomycorrhizal colonization of ponderosa pine during the first three years of growth.

The water relations measurements in the study reported here involved examination of the effects of growth in enriched CO_2 atmospheres on water stress of ponderosa pine in the field during drought episodes imposed in the early, middle, and late phases of the growing season. Overall, these results must be considered inconclusive, however, due to the intermittent pattern of significant treatment effects observed throughout the four series of predawn and midday Ψ_p measurements, and the propensity of seedlings grown in elevated CO_2 to exhibit a Ψ_p that was higher or lower than that of seedlings grown in the ambient atmosphere depending on when it was measured. This was exemplified in the last series of measurements, which were made at the end of the third growing season, when midday Ψ_p was higher in seedlings grown in elevated CO_2 on the third day after discontinuing irrigation but below that of the ambient treatment on the seventh day of this series. Furthermore, little can be deduced from these data about the linkage between proportionality of shoot and root biomass as affected by CO_2 treatment and the response of ponderosa pine to drought, as CO_2 enrichment did not definitively alter shoot/root ratio in this study. It is possible that greater clarity of results may have been achieved if the duration of the drought episodes had been extended in order to impart a higher degree of water stress, although seedling Ψ_p measured here was generally well below that at which water potential-mediated plant processes are impacted (Hsiao et al., 1976).

Conflicting results have been presented concerning the potential fertilization effect of rising atmospheric CO_2 in mature natural forests (Graumlich, 1991; Kienast and Luxmoore, 1988; LaMarche et al., 1984). Likewise, the growing body of information derived from the studies with seedlings previously cited indicates substantial interspecific, intraspecific, and temporal variation in the response to CO_2 enrichment. Continued research on the impact of elevated CO_2 during all phases of tree growth is thus necessary before definitive conclusions regarding this phenomenon can be reached. The results presented here suggest that atmospheric CO_2 enrichment administered under field conditions stimulates proportional above- and below-ground growth of juvenile ponderosa pine, and the magnitude of this response is dependent on soil N availability. However, they also suggest that temporal variation in this response may periodically result in greater growth stimulation by a moderate CO_2 increase than by a doubling of the ambient concentration.

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