Growth and carbon accumulation in root systems of *Pinus taeda* and *Pinus ponderosa* seedlings as affected by varying CO$_2$, temperature, and nitrogen

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Summary   It has been hypothesized that increasing atmospheric CO$_2$ concentration enhances accumulation of carbon in fine roots, thereby altering soil carbon dynamics and nutrient cycling. To evaluate possible changes to belowground pools of carbon and nitrogen in response to elevated CO$_2$, an early and a late successional species of pine (*Pinus taeda* L. and *Pinus ponderosa* Dougl. ex Laws, respectively) were grown from seed for 160 days in a 35 or 70 Pa CO$_2$ partial pressure at low or high temperature (30-year weekly mean and 30 year weekly mean + 5 °C) and a soil solution nitrogen concentration of 1 or 5 mM NH$_4$NO$_3$ at the Duke University Phytotron. Seedlings were harvested at monthly intervals and growth parameters of the primary root, secondary root and tap root fractions evaluated. Total root biomass of *P. ponderosa* showed a positive CO$_2$ response (105% increase) ($P = 0.0001$) as a result of significant increases in all root fractions in the elevated CO$_2$ treatment, but all other main effects and interactions were insignificant. In *P. taeda*, there were significant interactions between CO$_2$ and temperature ($P = 0.04$) and CO$_2$ and nitrogen ($P = 0.04$) for total root biomass. An allometric analysis indicated that modulation of the secondary root fraction was the main response of the trees to altered environmental conditions. In *P. ponderosa*, there was an increase in the secondary root fraction relative to the primary and tap root fractions under conditions of low temperature. In *P. taeda*, there was a shift in carbon accumulation to the secondary roots relative to the primary roots under low temperature and low nitrogen. Neither species exhibited shifts in carbon accumulation in response to elevated CO$_2$. We conclude that both species have the potential to increase belowground biomass substantially in response to rising atmospheric CO$_2$ concentration, and this response is sensitive to temperature and nitrogen in *P. taeda*. Both species displayed small shifts in belowground carbon accumulation in response to altered temperature and nitrogen that may have substantial ecosystem consequences over time.

Keywords: climate change, ecosystem response, elevated atmospheric carbon dioxide concentration, elevated temperature, nutrient availability, root allometry.

Introduction

Increasing atmospheric carbon dioxide (CO$_2$) concentration has the potential to raise global temperatures, alter nutrient cycling, and directly affect plant growth and carbon accumulation. Although the effects of elevated CO$_2$ on photosynthesis and aboveground plant growth have been studied extensively (Rogers and Runion 1994, Ceulemans and Mousseau 1994, Gunderson and Wullschleger 1994), our ability to make realistic predictions of ecosystem responses to global change is limited by a lack of knowledge of the belowground response of plants to elevated CO$_2$ (Strain and Cure 1985) and the ecosystem processes associated with that response. A detailed understanding of the interactive effects of elevated atmospheric CO$_2$ and temperature and altered nutrient availability would permit a better estimate of ecosystem responses to global climate change.

Increased photosynthetic rates in response to elevated CO$_2$ are not always accompanied by equivalent increases in aboveground biomass (Ceulemans and Mousseau 1994), leading some authors to hypothesize that part of the increase in carbon allocation is belowground (Oechel and Strain 1985, Bazzaz 1990). Increased allocation of carbon belowground could be manifested as greater root biomass, higher production or turnover of fine roots (Pregitzer et al. 1995), or increased root exudation of organic compounds (Zak et al. 1993). Each of these responses will cause increased delivery of reduced carbon to the soil, which will in turn affect soil microbial communities, decomposition rates, nutrient availability and carbon storage in soils (Curtis et al. 1994).

Proportionately greater accumulation of carbon in roots than in shoots will cause an increase in root/shoot ratio, and this has been observed in many studies of elevated CO$_2$ (Ceulemans and Mousseau 1994). However, root/shoot ratios in wild species often decline or are unresponsive to elevated CO$_2$ (Table 4 in Rogers and Runion 1994). A possible reason for this discrepancy is that woody species with functionally distinct root fractions (i.e., tap root, coarse roots, and fine roots) could shift carbon accumulation from one root fraction to another with no overall change in total root biomass. Increases in fine root

biomass in response to elevated CO_2 have been observed in artificial tropical ecosystems (Körner and Arnone 1992), but it remains unclear whether this is representative of other forest ecosystems.

Forest ecosystems are important in the assessment of global change because they cover one third of the Earth's land surface and account for 65 to 70 percent of the carbon exchange between the atmosphere and terrestrial biota (Waring and Schlesinger 1985). Loblolly pine (*Pinus taeda* L.) and ponderosa pine *(Pinus ponderosa* Dougl. ex Laws.) are important commercial species in the USA, covering extensive areas in their southeastern (11.7 million ha) and western (10.9 million ha) ranges, respectively (Baker and Langdon 1990, Oliver and Ryker 1990). Accordingly, ecosystems dominated by these species will be important components of the biosphere response to global change.

Although the two pine species are closely related (Shaw 1914), they have recently evolved in different environments resulting in contrasting life histories and growth strategies. Loblolly pine is an early successional exploitive species, whereas ponderosa pine is a late successional conservative species (Griffin et al. 1995). Loblolly pine is adapted to the colonization of recently disturbed sites (Wahlenberg 1960) and grows most rapidly prior to reproductive maturity (Baker and Langdon 1990). It is often found in areas of high light and water availabililily, such as are common throughout southeastern USA (Allen et al. 1990). Ponderosa pine is characteristic of later-stage ecosystems, having a slower growth rate and higher tolerance to shade (Griffin et al. 1995). It is common on xeric sites and is most often limited by available soil water throughout its range in the western USA (Bassman 1988, Oliver and Ryker 1990).

The exploitive versus conservative growth strategies of these two pine species have resulted in different root system architectures, which may influence root responses to changes in available resources and environmental conditions. Loblolly pine has a lower root/shoot ratio and shallower root system (shorter tap root) than ponderosa pine (Griffin et al. 1995).

Loblolly pine is also comparatively less efficient at absorbing water and nutrients at the lower range of availability than ponderosa pine (Griffin et al. 1995). Therefore, we might expect loblolly pine to show a greater response to an increase in resource availability or environmental stress. Further, the pattern of carbon accumulation among root fractions is likely to differ between the two species. The exploitive loblolly pine would be expected to maximize resource acquisition and therefore to have proportionately more biomass in the small diameter, high surface area root fraction. The conservative ponderosa pine would likely exhibit greater storage capacity and therefore allocate more biomass to the taproot. To investigate the relative responses of the root fractions to anticipated global climate change, *Pinus taeda* and *Pinus ponderosa* seedlings were grown under conditions of normal and elevated CO_2 and temperature and at two nitrogen availabilities.

Methods

Growth conditions

On March 31, 1994, seeds of loblolly and ponderosa pine were inoculated with *Pisolithus tinctorius* (Pers.; Coker and Couch (Mycorr Tech Inc., Pittsburgh, PA)), an ectomycorrhizal associate of pines (Marx 1977), and planted in sterilized sand in 3.5-liter pots located in the Duke University Phytotron (Kramer et al. 1970). The plants were grown for 160 days in natural light in two greenhouses maintained at 35 Pa partial pressure CO_2 and two greenhouses maintained at 70 Pa partial pressure CO_2 (Hellmers and Giles 1979). Temperature in one greenhouse for each CO_2 regime was maintained at the 30-year weekly mean for the Raleigh–Durham area (low temperature treatment, L). The other greenhouse in each CO_2 regime was maintained at the 30-year weekly mean plus 5 °C (high temperature treatment, H; Figure 1). Treatments were randomized among greenhouses weekly to avoid bias in the data caused by slight differences among greenhouses. Plants were fertilized every morning with a modified Hoagland's solution containing
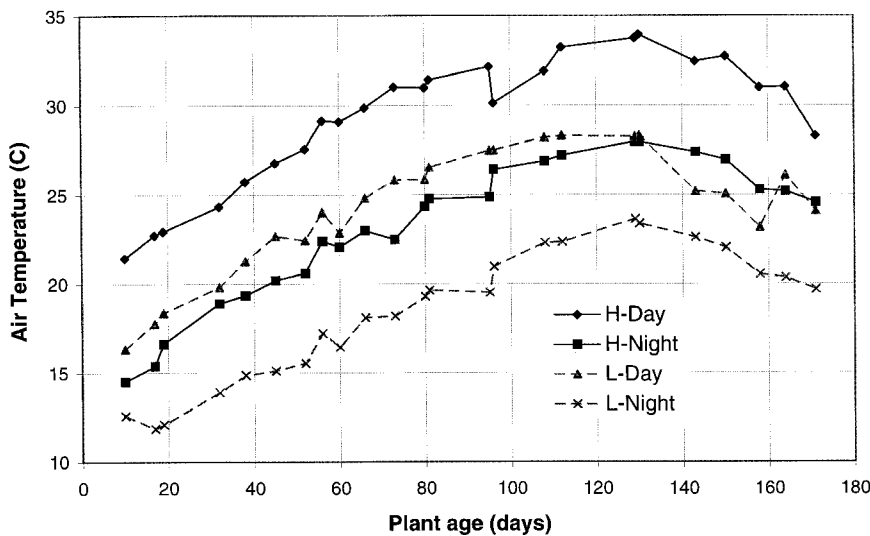

Figure 1. Mean weekly day/night temperatures in the high and low temperature greenhouses during the growing season. The low temperature treatment tracked the 30-year weekly mean for the Raleigh–Durham area. The high temperature treatment tracked the 30-year weekly mean + 5 °C.

1 mM KH$_2$PO$_4$, 2 mM K$_2$SO$_4$, 1.5 mM MgSO$_4$7H$_2$O, 3 mM CaCl$_2$, 0.167 NaOH, 0.14 mM FeSequestrene, micronutrients, and 1 and 5 mM NH$_4$NO$_3$ for the low (LN) and high (HN) nitrogen treatments, respectively. Plants were watered to saturation with de-ionized water at noon and every evening to prevent desiccation and salt accumulation.

Harvests

Five plants of each species were selected at random for each factorial combination of CO$_2$, temperature and nitrogen treatments and harvested at Days 37, 69, 98, 128, and 160 after germination. Root systems of the plants were carefully removed from the pots with the soil intact and washed under running water over a basin. The soil in the basin was wet-sieved to recover root fragments. Once removed from the soil, root systems were spread out and separated into tap root, secondary roots and primary roots. Secondary roots were operationally defined as roots showing an increase in diameter and continued development resulting in the formation of woody roots. Primary roots showed no increase in diameter (often < 1 mm in diameter) and remained unlignified or non-woody.

During the experiment, most roots growing from the tap root were less than 2 mm in diameter, a commonly used size class to discriminate between fine roots and coarse roots (Sutton and Tinus 1983, Norby 1994). Roots in the primary root fraction were much smaller in diameter than roots in the secondary root fraction. Even though all roots contribute to the uptake of water and nutrients (MacFall et al. 1992, Kramer and Boyer 1995), the greater surface area of the primary root fraction accounts for a greater proportion of the total uptake. Therefore, because of potential differences in chemical composition and function, separation into different fractions was based on tissue quality (i.e., woody versus non-woody) rather than size class only. After separation, each root fraction was frozen in liquid nitrogen and immediately placed in a forced-air oven and dried to constant weight at 70 °C.

Growth, carbon accumulation, and statistical methods

Total root biomass data from all five harvests were used to determine relative growth rate (RGR) by the method of Hunt (1990) and dry matter production, both with 95% confidence intervals. Simple linear regressions were used to summarize growth data over the course of the experiment and to make basic comparisons between species. Data from the final harvest were used to determine total biomass and carbon accumulation in each root fraction for each treatment. Data were tested for normality and natural log transformed where necessary to normalize variances among treatments. One was added to all values before transformation because many weights were less than 1 g (Steel and Torrie, 1980). Treatment main effects and interactions were tested by standard analysis of variance techniques (Steel and Torrie 1980). Because of physical constraints, there was no greenhouse replication and interactions of main effects were tested against the residual term in the model. Treatment effects were considered significant if $P \leq$ 0.05 or if there was no overlap of the 95% confidence intervals.

Changes in carbon accumulation among the different root fractions were detected by allometric analysis. The allometric analysis fitted linear functions to root fraction dry weight by treatment for trees ranging in age from 98 to 160 days. Because the allometric analysis used a wide range of plant root sizes (0.35–15.28 g for *P. ponderosa* and 0.22–15.16 g for *P. taeda*), it should provide a powerful test to check for shifts in carbon accumulation between the root fractions. The procedure involved plotting the log dry weight of one root fraction against the log dry weight of another fraction and noting significantly different slopes between treatments (Niklas 1994). An analysis of covariance (ANCOVA) was used to determine if the non-homogeneity between slopes was significant and was indicated by significant interactions with the covariate.

In cases where significant interactions were detected, the models were constrained to have parallel slopes between treatment levels by eliminating the interaction terms with the covariate (Burdick, personal communication). This was done to examine the magnitude of the effect, in terms of a percent change, of the interaction on the response variables. Marginal means for the response variables by treatment were compared for the constrained (parallel slopes) and unconstrained (interactions) models as follows:

$$\% \text{ difference} = \{(\text{MMR}_{unc} - \text{MMR}_{con})/\text{MMR}_{unc}\}100,$$

where MMR = marginal mean of the response variable, unc = unconstrained model, and con = constrained model.

Results

Growth and production

Averaged over all treatments, the relative dry weight growth rate of loblolly pine in the first interval (0.14 day^{-1}) was higher than that of ponderosa pine (0.12 day^{-1}). During the growing season, the relative growth rate of loblolly pine decreased more rapidly than that of ponderosa pine so that by the end of the experiment, which coincided with the end of the growing season, both species grew at approximately the same rate (0.02 day^{-1}) (Figure 2). Differences between treatments were highly variable and usually insignificant (confidence limits not shown). Dry matter production showed a similar response to that of RGR (Figure 3). Rates of dry matter production were slightly higher for loblolly pine than for ponderosa pine at Day 128, but were comparable at other times during the experiment. In addition, elevated CO$_2$ caused a consistent but not significant increase in dry matter production for both species (Figure 3). Effects of temperature and nitrogen within a CO$_2$ treatment were highly variable and usually not significant (confidence limits not shown).

Total root biomass

The cumulative effects of the treatments on total root biomass at the end of the experiment are shown in Figure 4. Total root biomass production was similar for the two species in all treatments except the 70 Pa CO$_2$ + high temperature treatment, in which loblolly pine produced considerably more root
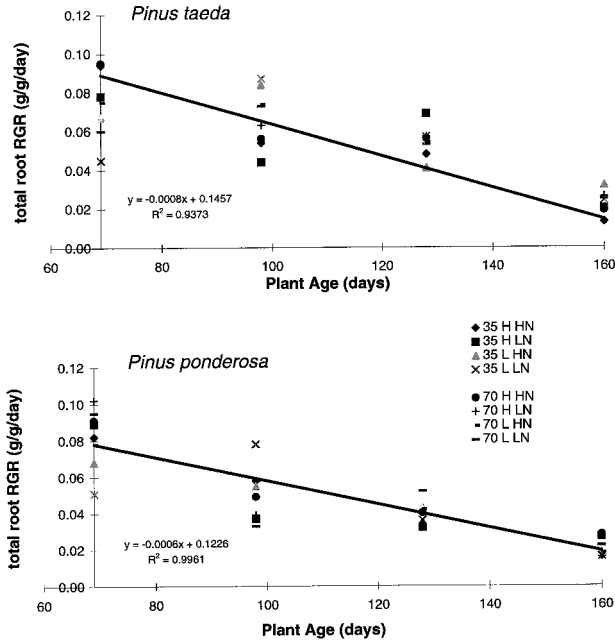
Figure 2. Simple regressions of relative growth rate of the root systems of *P. taeda* and *P. ponderosa* grown at 35 and 70 Pa CO_2, high (H) and low (L) temperature, and high (HN) and low (LN) nitrogen for 160 days. Regressions summarize growth data for basic comparison between species, but should not be used to make statistical inferences about the relative growth rate.
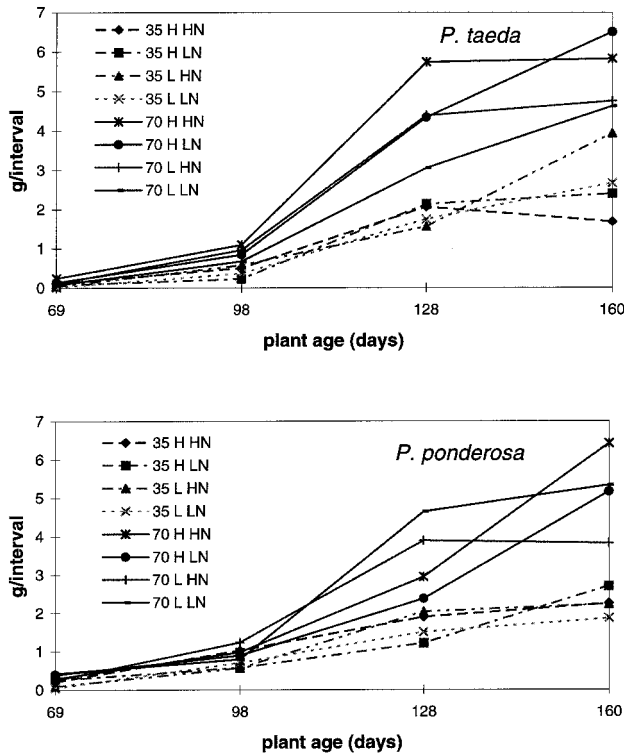

Figure 3. Root system dry matter production between harvests (g per interval) of *P. taeda* and *P. ponderosa* grown at 35 and 70 Pa CO_2, high (H) and low (L) temperature, and high (HN) and low (LN) nitrogen for 160 days.

biomass than ponderosa pine. Loblolly pine total root biomass showed significant $CO_2 \times$ temperature ($P = 0.04$) and $CO_2 \times$ nitrogen ($P = 0.04$) interactions (Table 1). Within a given nitrogen treatment, the increase in root biomass in response to elevated CO_2 was much greater at high temperature (Figure 4). Likewise, CO_2 stimulation of root growth was much greater in the low nitrogen regime within a given temperature treatment. The total root biomass of ponderosa pine increased on average 105% ($P = 0.0001$) in response to elevated CO_2, but did not change in response to elevated temperature and high nitrogen availability (Table 1). However, the elevated-CO_2-induced response occurred in both temperature and nitrogen regimes (Figure 4).

Root fractions

Responses of individual root fractions to the treatments differed slightly between the two species. In loblolly pine, the tap root biomass fraction showed an average 94% increase in response to elevated CO_2 and an average 29% increase in response to high nitrogen treatment (Table 2), whereas the secondary root biomass fraction showed a significant $CO_2 \times$ temperature interaction ($P = 0.001$; Table 1) and the primary root biomass fraction increased 160% in response to elevated CO_2 treatment ($P = 0.0003$). In ponderosa pine, the elevated CO_2 treatment increased tap root biomass by 85% (Table 2) and there was some evidence of a $CO_2 \times$ temperature interaction ($P = 0.09$; Table 1). Secondary root biomass fraction increased on average 115% and the primary root biomass fraction increased 120% in response to elevated CO_2 ($P = 0.0001$), but there were no other main effects or interactions (Tables 1 and 2).

Carbon accumulation

To determine if the percentage increases in root fraction biomass represented shifts in carbon accumulation, an analysis of covariance was used to detect significant interactions between the experimental factors and covariates in linear models for loblolly and ponderosa pine (Table 3). In loblolly pine, a significant interaction ($P \leq 0.04$) occurred in the model with primary root dry weight as the response variable and secondary root dry weight as the covariate (Figure 5). The interaction involved secondary root dry weight with temperature and nitrogen (Table 3). In ponderosa pine, a significant interaction ($P = 0.05$) occurred in the model with primary root dry weight as the response and secondary root dry weight as the covariate interacting with temperature (Table 3, Figure 6). In addition, this interaction was significant ($P = 0.007$) in the model with secondary root dry weight as the response and tap root dry weight as the covariate (Table 3, Figure 7).

The comparison of constrained and unconstrained models provided estimates of the actual percentage change in biomass caused by the interactions (Table 4). Differences ranged from 0.2 to 6.3%, indicating that the effects of the interaction terms were small, i.e., only minimal shifts in carbon accumulation resulted in response to the treatments.
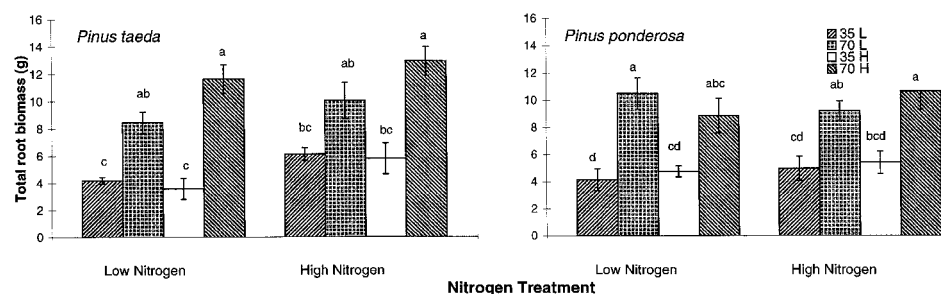
Figure 4. Total root system biomass of *P. taeda* and *P. ponderosa* grown at 35 and 70 Pa CO_2, high (H) and low (L) temperature, and high and low nitrogen for 160 days.

Table 1. The *P*-values of treatment main effects and interactions on total root biomass and root fractions for *Pinus taeda* and *Pinus ponderosa* grown for 160 days at ambient (35 Pa) and elevated (70 Pa) CO_2, high and low temperature, and high and low nitrogen.

	CO_2	Temperature	Nitrogen	$CO_2 \times$ Temp	$CO_2 \times$ N	Temp $\times$ N	3-Way
P. taeda							
Total root	0.0001	0.11	0.0008	0.04	0.04	0.52	0.35
Tap	0.0001	0.79	0.008	0.19	0.14	0.35	0.65
Secondary	0.0001	0.005	0.0001	0.001	0.76	0.93	0.82
Primary	0.0003	0.21	0.41	0.40	0.13	0.30	0.49
P. ponderosa							
Total root	0.0001	0.60	0.37	0.40	0.61	0.54	0.30
Tap	0.0001	0.53	0.36	0.09	0.43	0.43	0.88
Secondary	0.0001	0.13	0.38	0.91	0.90	0.77	0.10
Primary	0.0001	0.88	0.64	0.77	0.53	0.43	0.46

Table 2. Total and fraction dry weights (g) for *P. taeda* and *P. ponderosa* grown for 160 days at ambient (35 Pa) and elevated (70 Pa) CO_2, high (H) and low (L) temperature, and high (HN) and (LN) low nitrogen. Standard error and % of total root in parentheses.

	Total root	Tap root	Secondary root	Primary root
P. taeda				
35 H HN	5.77 (1.14)	2.04 (0.34; 35)	1.43 (0.32; 25)	2.29 (0.82; 40)
35 H LN	3.58 (0.77)	1.47 (0.21; 41)	0.90 (0.12; 25)	1.20 (0.47; 34)
35 L HN	6.13 (0.48)	2.56 (0.21; 42)	1.77 (0.17; 29)	1.79 (0.23; 29)
35 L LN	4.19 (0.25)	1.66 (0.23; 39)	0.99 (0.11; 23)	1.54 (0.16; 38)
70 H HN	12.92 (1.04)	3.72 (0.49; 29)	4.44 (0.27; 34)	4.74 (0.88; 37)
70 H LN	11.64 (1.16)	3.64 (0.16; 31)	3.12 (0.34; 27)	4.88 (0.99; 42)
70 L HN	10.05 (1.47)	3.71 (0.06; 37)	2.86 (0.4 ; 28)	3.48 (1.19; 35)
70 L LN	8.45 (0.8)	3.03 (0.32; 36)	1.78 (0.2 ; 21)	3.63 (0.68; 43)
P. ponderosa				
35 H HN	5.41 (0.83)	2.05 (0.20; 38)	1.79 (0.44; 33)	1.57 (0.25; 29)
35 H LN	4.76 (0.41)	1.57 (0.14; 33)	1.86 (0.28; 39)	1.32 (0.17; 28)
35 L HN	4.98 (0.9)	1.74 (0.32; 35)	1.65 (0.25; 33)	1.58 (0.38; 32)
35 L LN	4.15 (0.8)	1.56 (0.30; 37)	1.24 (0.23; 30)	1.35 (0.3 ; 33)
70 H HN	10.66 (1.31)	2.94 (0.28; 27)	4.21 (0.27; 39)	3.50 (0.79; 34)
70 H LN	8.88 (1.27)	2.76 (0.53; 31)	3.26 (0.62; 37)	2.85 (0.36; 32)
70 L HN	9.22 (0.72)	3.35 (0.27; 36)	2.96 (0.29; 32)	2.91 (0.29; 32)
70 L LN	10.51 (1.28)	3.63 (0.45; 34)	3.38 (0.65; 32)	3.50 (0.35; 34)

Discussion

Root systems of loblolly pine and ponderosa pine had similar rates of growth and dry matter production during the experiment (cf. Griffin et al. 1995), suggesting that growth was controlled to a greater extent by environmental conditions than by heritable factors. Initially, relative growth rate of loblolly pine roots was higher than that of ponderosa pine, but declined more rapidly during the growing season so that both species

Table 3. The *P*-values for interactions between experimental factors and covariate in analysis of covariance. Significant values ($P \leq 0.05$) indicate non-parallel slopes.

Treatment[1]	Pinus taeda			Pinus ponderosa		
	Primary/ Secondary	Primary/Tap	Secondary/Tap	Primary/ Secondary	Primary/Tap	Secondary/Tap
$Cov \times CO_2$	0.63	0.24	0.26	0.62	0.38	0.19
$Cov \times temp$	0.47	0.95	0.61	0.05	0.43	0.007
$Cov \times CO_2 \times Temp$	0.23	0.86	0.16	0.08	0.27	0.88
$Cov \times N$	0.02	0.39	0.23	0.74	0.6	0.95
$Cov \times CO_2 \times N$	0.16	0.86	0.77	0.76	0.84	0.37
$Cov \times Temp \times Nit$	0.04	0.5	0.43	0.32	0.29	0.84
$Cov \times CO_2 \times Temp \times Nit$	0.96	0.22	0.99	0.37	0.76	0.18

[1] Treatment column indicates interactions between the covariate and experimental factor(s). Species column headings indicate response variable:covariate.
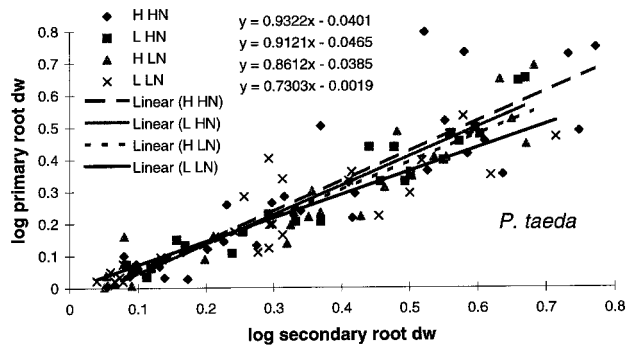

Figure 5. Log of primary root dry weight on log secondary root dry weight for *P. taeda* grown for 98 to 160 days at 35 and 70 Pa CO_2, high (H) and low (L) temperature, and high (HN) and low (LN) nitrogen. A significant interaction occurred between temperature, nitrogen and the covariate secondary root dry weight.
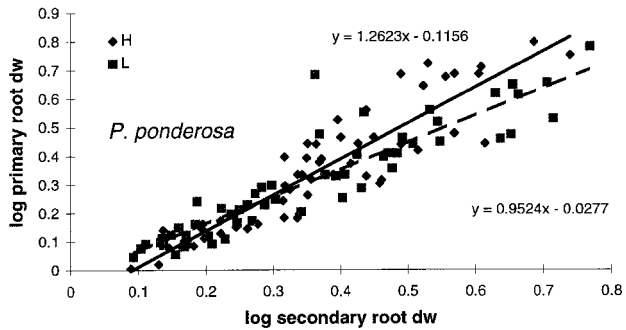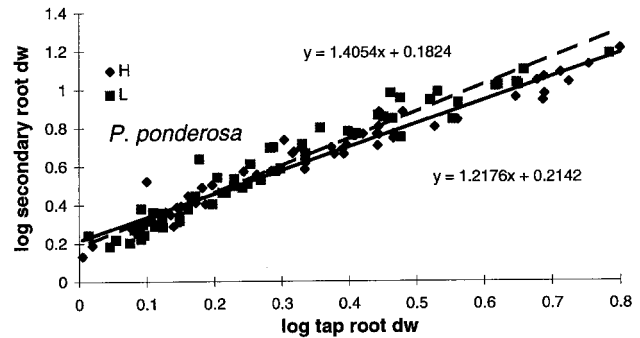

Figure 7. Log of secondary root dry weight on log tap root dry weight for *P. ponderosa* grown for 98 to 160 days at 35 and 70 Pa CO_2, high (H) and low (L) temperature, and high and low nitrogen. A significant interaction occurred between temperature and the covariate tap root dry weight.

Figure 6. Log of primary root dry weight on log secondary root dry weight for *P. ponderosa* grown for 98 to 160 days at 35 and 70 Pa CO_2, high (H) and low (L) temperature, and high and low nitrogen. A significant interaction occurred between temperature and the covariate secondary root dry weight.

Table 4. Comparison of the constrained and unconstrained models in the analysis of covariance by treatment. Data are percent difference in response variable.

Treatment[1]	Pinus taeda	Pinus ponderosa	
	Primary/ Secondary	Primary/ Secondary	Secondary/ Tap
35 H HN	1.8	−0.2	−0.3
35 H LN	2.2	−5.1	3.8
35 L HN	−1.6	2.3	−3.4
35 L LN	2.1	6.3	−5.8
70 H HN	3.6	0.9	−3.3
70 H LN	−1.3	−0.2	−0.9
70 L HN	4.2	0.2	2.9
70 L LN	0.3	−0.6	0.7

[1] Treatments are as follows: 35 and 70 = 35 and 70 Pa CO_2, respectively; H, L = High and low temperature, respectively; HN, LN = High and low nitrogen, respectively.

accumulated about the same amount of dry matter belowground by the end of the experiment.

Although total root system responses to CO_2 were similar for the two species, differences occurred in sensitivity to the temperature and nitrogen regimes. Root systems of both spe-

cies had a strong positive growth response to elevated CO_2, which mirrored the whole plant responses (Thomas et al. unpublished observations). The CO_2 growth response of lob-

lolly pine, however, was significantly greater in the high temperature treatment, suggesting that this species has a greater capacity to overcome carbon losses from increased respiration and photorespiration at high temperature (Taiz and Zeiger 1991) when supplied with additional carbon than ponderosa pine. In addition, the CO_2 root growth response of loblolly pine root systems was also greater in the low nitrogen regime, which might be expected given its exploitive growth strategy.

Ponderosa pine root systems were not responsive to the high nitrogen or elevated temperature regime. This presumably reflects the conservative growth strategy of this species. However, other studies have reported positive growth responses in ponderosa pine to added nitrogen (Johnson et al. 1994), and it may be that the nitrogen concentrations used in this experiment were above the limiting conditions experienced by this species in the field. The lack of a belowground response to elevated temperature in ponderosa pine is consistent with other studies (Callaway et al. 1994) and suggests that projected increases in global temperatures will not directly affect root growth of this species. This could place ponderosa pine at a competitive disadvantage with vegetation that exhibits a strong belowground response to elevated temperatures.

Differences were found in the responsiveness of individual root fractions to elevated CO_2, temperature and nitrogen. All root fractions of both species showed a significant growth stimulation in response to elevated CO_2, which cumulatively accounted for the large CO_2 response in total root biomass. In loblolly pine, tap root growth also responded to nitrogen treatment and the secondary root biomass fraction showed a significant $CO_2 \times$ temperature interaction. In ponderosa pine, there was a strong, but statistically insignificant ($P = 0.09$) $CO_2 \times$ temperature interaction on tap root growth. Of the root fractions, the primary root fraction in both species was the least responsive to the temperature and nitrogen treatments (Table 1), indicating that the plant response to variation in these factors is to modify the amount of long-lived tissue (i.e., lignified) retained, rather than the amount of short-lived tissue (i.e. non-lignified) produced. This was confirmed by a lack of change in the primary root/total plant ratio in response to the temperature and nitrogen treatments (data not shown).

We used the root fraction data and allometric analysis to characterize belowground carbon accumulation patterns in the two species of pine, and to determine the sensitivity of these accumulation patterns to changes in environmental conditions. The root fraction data indicated that loblolly pine allocated more carbon to the primary roots (41% on average) than ponderosa pine (31% on average), which is not surprising given the contrasting (exploitive versus conservative) growth strategies of the two species. The allometric analysis illustrated that the pattern of carbon accumulation among root fractions is subject to change under altered environmental conditions.

Shifts in carbon accumulation were detected for both species in response to temperature and nitrogen, and always involved the secondary root fraction. In loblolly pine, accumulation in secondary roots relative to primary roots increased under conditions of low temperature and low nitrogen. Similarly, ponderosa pine showed greater accumulation of carbon in secondary roots relative to both primary and tap roots

in the low temperature treatment. These results support the idea that modulation of the secondary root fraction is a long-term response of the plant to altered resource availabilities or stress. Under high temperature stress there is potentially less carbon available for growth because of increased respiration and photorespiration (Taiz and Zeiger 1991) and therefore accumulation to secondary roots might decrease. Likewise, increased accumulation of carbon in fine roots under conditions of low nutrient availability has been reported (Cannell 1989), so increased accumulation to the secondary root fraction under low nitrogen would be expected. Furthermore, in other studies, no shift in carbon accumulation among root fractions was detected in response to elevated CO_2 (Larigauderie et al. 1994).

Although the observed shifts in carbon accumulation were not large (cf. constrained and unconstrained ANCOVA models), the allometric analysis showed that the potential for belowground shifts exists. The linear models used in the analysis of covariance fitted the data well (large R^2 values, small coefficients of variation) and the direction of the shifts agree with established concepts in the literature (Cannell 1989, Taiz and Zeiger 1991). Therefore, over several growing seasons the effects of altered carbon accumulation in response to elevated temperature and nitrogen availability could become important. The lack of a shift in belowground carbon accumulation in response to elevated CO_2 suggests that, in loblolly and ponderosa pine ecosystems at least, the standing pool of carbon in fine roots will not be altered by rising atmospheric CO_2 concentrations. However, a CO_2 response may be present in the form of altered flux of carbon to the soil from increased fine root production and turnover (Pregitzer et al. 1995).

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