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Interactive effects of soil nitrogen and atmospheric carbon dioxide on root/rhizosphere carbon dioxide efflux from loblolly and ponderosa pine seedlings

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Abstract

We measured CO_2 efflux from intact root/rhizosphere systems of 155 day old loblolly (*Pinus taeda* L.) and ponderosa (*Pinus ponderosa* Dougl. ex Laws.) pine seedlings in order to study the effects of elevated atmospheric CO_2 on the below-ground carbon balance of coniferous tree seedlings. Seedlings were grown in sterilized sand culture, watered daily with either 1, 3.5 or 7 m*M* NH_4^+, and maintained in an atmosphere of either 35 or 70 Pa CO_2. Carbon dioxide efflux (μmol CO_2 plant^{-1} s^{-1}) from the root/rhizosphere system of both species significantly increased when seedlings were grown in elevated CO_2, primarily due to large increases in root mass. Specific CO_2 efflux (μmol CO_2 g root^{-1} s^{-1}) responded to CO_2 only under conditions of adequate soil nitrogen availability (3.5 m*M*). Under these conditions, CO_2 efflux rates from loblolly pine increased 70% from 0.0089 to 0.0151 μmol g^{-1} s^{-1} with elevated CO_2 while ponderosa pine responded with a 59% decrease, from 0.0187 to 0.0077 μmol g^{-1} s^{-1}. Although below ground CO_2 efflux from seedlings grown in either sub-optimal (1 m*M*) or supra-optimal (7 m*M*) nitrogen availability did not respond to CO_2, there was a significant nitrogen treatment effect. Seedlings grown in supra-optimal soil nitrogen had significantly increased specific CO_2 efflux rates, and significantly lower total biomass compared to either of the other two nitrogen treatments. These results indicate that carbon losses from the root/rhizosphere systems are responsive to environmental resource availability, that the magnitude and direction of these responses are species dependent, and may lead to significantly different effects on whole plant carbon balance of these two forest tree species.

Introduction

It is estimated that between ten to forty percent of the total carbon fixed by crop plants is released into the soil system, either as respired CO_2 or as organic compounds from roots (Van Veen et al., 1991). Of the total carbon translocated to roots, Lambers (1987) estimates that twelve to twenty nine percent is respired, an amount nearly equal to that used in root growth (11-35%). Elevated CO_2 partial pressures frequently increase the rate of carbon fixation in C_3 plants and can alter the allocation of carbon to roots (Lewis et al., 1994; Norby, 1994; Rogers et al., 1994; Strain and Cure, 1985). There is mounting evidence that atmospheric CO_2 can strongly affect belowground processes and allocation and that these changes can initiate a complex set of system level responses (Diaz et al., 1993; Körner and Arnone, 1992; Johnson et al., 1994; Zak et al., 1993). Although the role root mass and root turnover play in the overall plant response to elevated CO_2 is beginning to be recognized (Norby et al., 1992; Pregitzer et al., 1995), the role of root function, physiology and biochemistry has not been well studied.

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Root respiration can dominate the carbon budget of forest tree seedlings (Reid and Strain, 1994) and thus understanding the effects of global environmental change on seedling growth requires a mechanistic understanding of root physiology. The ecological, economic and geographic dominance of forest tree species requires that any attempt to predict biospheric response to global change must focus, at least in part, on forest tree species. Here we compare the results of root system respiration measurements of seedlings of two important forest tree species, loblolly pine (*Pinus taeda* L.), a dominant forest tree in southern US, and ponderosa pine (*Pinus ponderosa* Dougl. Ex Laws.) a dominant forest tree in the western US. We grew seedlings of both species in a "common garden" type experiment to examine the effects of elevated CO_2 and soil N availability on root system form and function.

In a previous study of the patterns of biomass accumulation annd allocation we report that root biomass and root:shoot ratio are responsive to the availability of atmospheric carbon and soil nitrogen in loblolly and ponderosa pine (Griffin et al., 1995). This study expands on these findings, incorporating the results of plants grown in toxic nitrogen concentrations, and examine the role of root respiration in regulating the response of these two closely related species to changing environmental resource availability. Since concentration of CO_2 in soils is often quite high ($> 10^3$ μmol mol^{-1}, Russell, 1961) it is generally believed that the effects of elevated CO_2 on root physiology are indirect in nature. Direct effects of a doubling of the atmospheric CO_2 partial pressure on root/rhizosphere respiration are unlikely (but see Qi et al., 1994) and was not considered in this study. To interpret the observed responses we examined the relationships between respiratory carbon substrates (sugars and starch), root nutrient concentrations (C and N) and whole plant growth.

Materials and methods

Plant material, propagation and harvest

Seeds from a single, wild-type of loblolly pine from the North Carolina piedmont (North Carolina Forestry Commission, Lot # LB-NC-P-84-27) and a half-sib family of ponderosa pine collected at 915 m elevation in El Dorado County California (California Department of Forestry Lot # CDF 526) were germinated and grown in the Duke University Phytotron. The growth period lasted 166 days, from May through October 1993, the approximate length of one growing season for these species (Oliver and Ryker, 1990; Wahlenberg, 1960).

Three seeds of either species were planted in each 3L PVC pot filled with sterilized, acid washed river sand and covered with 1 cm of similarly prepared gravel to reduce evaporative water loss. Two weeks after seedling emergence, all pots were thinned to a single seedling. Seven to 10 seedlings per treatment were harvested 166 days after planting, immediately following root system gas exchange measurements. All seedlings were placed in a 10 °C darkened chamber to minimize growth during the 3 day harvest. Plants were separated into roots, stems, primary leaves and fascicle leaves, and dried to a constant mass at 60 °C.

Carbon and N concentrations were measured with an elemental analyzer (Carlo Erba NA 1500 nitrogen/carbon analyzer, Carlo Erba strumentazione, Milano, Italy). The analyzer was calibrated from a Atropine Std. of 70.56% Carbon and 4.84% Nitrogen. Soluble sugar, starch and total non-structural carbohydrate (TNC) concentrations were colorimetrically determined using a chloroform, methanol, water extraction from dried root tissue (Tissue et al., 1993). Pinitol, a compound that can act as a storage carbohydrate in leaves, was not assayed since it has not been reported to contribute significantly to root TNC.

Environmental treatments

Atmospheric CO_2 partial pressures of 35 or 70 Pa were automatically maintained in two greenhouses in the Duke University Phytotron (Hellmers and Giles, 1979). These partial pressures were chosen to simulate current ambient CO_2 and a predicted doubling by the end of the next century (Watson et al., 1990). Phytotron greenhouses also controlled air temperature to 27/22 °C (day/night), with the thermoperiod adjusted to follow the photoperiod. Relative humidity inside the chambers was approximately 70% during the day, and greater than 95% at night. Greenhouse transmission of solar radiation was greater than 90%, with a natural photoperiod throughout the duration of the experiment (May to November).

Nutrient treatments were initiated 3 weeks after planting by applying nutrient solutions each morning, followed by deionized water each evening to prevent water stress and salt buildup. Prior to this time all pots were watered to saturation twice daily with deionized water. Nutrient treatments differed only in ammonium concentration, with the low N treatments receiving 1.0

m*M* N, the medium N treatments receiving 3.5 m*M* N and the high N treatments receiving 7.0 m*M* N from NH_4Cl (Griffin et al., 1995). These N concentrations were chosen to result in a range of nitrogen concentrations and whole plant growth from sub-optimal to supra-optimal. Ammonium was used as the sole N source to facilitate calculations of tissue construction cost (Griffin et al., 1996b). Although ammonium-based nutrient solutions have been shown to undergo conversion from ammonium to nitrate by nitrifying organisms during storage (Padgett and Leonard, 1993), we found no such conversion. No nitrate was detected in analysis of either the irrigation solution or solutions caught as through-flow from the pots. Nitrifying organisms were most likely inhibited due to the low pH of the nutrient solution (4.5) and the use of the nutrient solution within 3 days of preparation. Alternatively some undetected nitrification may have taken place if the nitrate produced was complete and immediately taken up by the plants.

Gas exchange measurements

Root/rhizosphere respiration was measured as net CO_2 efflux from the entire intact soil volume using a custom built gas exchange system. This system consisted of mass flow controllers and meters (MKS Instruments, Andover, Mass., USA), to regulate and measure the flow through the system, a distribution manifold to allow continuous gas flow through 5 pots simultaneously, and a LiCor. 6262 infra-red gas analyzer (LiCor Inc., Lincoln, Neb., USA) for differential measurements of CO_2. Data acquisition and system control were maintained via personal Computer running Lab View software (National Instruments Corporation, Austin, TX).

During the gas exchange measurements, all plants were kept in a growth chamber maintained at 27 °C, 70% relative humidity, 1000 μmol m^{-2} s^{-1} PAR, and the growth CO_2 concentration. All plants to be measured from a given CO_2 treatment were placed in the growth chamber at least 12 hr. prior to the actual measurement. During each run, the PVC pots of five replicate treatments were sealed with pipe caps and turnicate straps. The top cap was slotted to accommodate the stem that was sealed with putty. Gas flow was from the bottom of the pot to the soil surface. All plants acclimated to the flow-through system with a measurement gas with 45 Pa CO_2 for a period of time sufficient to reach steady state (a minimum of one hour) prior to the initiation of the measurements.

Statistical analysis

The normality of all data was tested with a Shipiro-Wilk W test (JMP statistical software version 2.0.5, SAS Institute Inc., Cary, North Carolina) and a log transformation was performed prior to analysis when necessary. Treatment means from the log transformed data were back-transformed to geometric means and presented with asymmetric 95% confidence limits as an indication of variance (Sokal and Rohlf, 1981). The effect of species, CO_2 partial pressure, and nitrogen fertilizer were tested by ANOVA (Data Desk 4.1 statistical software, Data Description Inc., Ithaca, New York). Means separation based on planned comparisons were accomplished with a protected LSD test. Treatment effects and means separation were considered significant only when $p \leq 0.05$. Species effects were determined on a full factorial, three way ANOVA, but all other results are reported from two way ANOVA within either species.

Results

The response of biomass production to resource availability was similar in loblolly and ponderosa pine after 166d (Table 1). Elevated atmospheric CO_2 tends to increase total plant biomass yet the difference between the high and low CO_2 grown plants was significant only when plants were grown with optimal N (3.5 m*M*). Increasing N availability ultimately led to nitrogen toxicity, manifested as reduced growth, in all plants. In loblolly pine, nitrogen toxicity occurred only at the highest level of N (7.0 m*M*), while in ponderosa pine N toxicity occurred at 3.5 m*M* N when ambient CO_2 levels were low (35 Pa). Root mass was not significantly altered in loblolly pine in response to changing CO_2 availability within a N treatment. Ponderosa pine root mass was significantly increased by CO_2 at both 3.5 and 7.0 m*M* N levels. Toxic nitrogen concentrations reduced total root biomass in loblolly pine at both CO_2 partial pressures and at 35 Pa CO_2 in ponderosa pine, suggesting that elevated CO_2 can at least partially alleviate the toxic effects of 3.5 and 7.0 m*M* N in this species. As a result of commensurate changes in total dry mass and root mass, the root:shoot ratios of loblolly pine were unchanged by CO_2 when N was supplied at 3.5 m*M*. Loblolly pine root:shoot ratio was also unresponsive to N at low CO_2. Ponderosa pine root:shoot ratio was not significantly affected by CO_2 at any level of N availability or by N at either CO_2 partial pressure.

Table 1. Total plant mass, root mass and root:shoot ratio of loblolly and penderosa pine seedlings grown in either 35 or 70 Pa CO_2 partial pressure and 1.0, 3.5 or 7.0 m*M* N for 166 days. Data are presented as geometric means and the asymmetrical 95% confidence limit. Root:shoot ratio was naturally normally distributed and thus the arithmetic mean and symmetrical 95% confidence limits are presented (n = 7 - 10)

	1.0 m*M* N		3.5 m*M* N		7.0 m*M* N	
	35 Pa $\bar{X}$ (95% C.I.)	70 Pa $\bar{X}$ (95% C.I.)	35 Pa $\bar{X}$ (95% C.I.)	70 Pa $\bar{X}$ (95% C.I.)	35 Pa $\bar{X}$ (95% C.I.)	70Pa $\bar{X}$ (95% C.I.)
Loblolly						
Total mass (g)	5.33(3.84 - 7.41)	7.28 (3.67 - 14.42)	7.96 (5.20 - 12.19)	14.45 (8.45 - 24.72)	2.08 (0.92 - 4.72)	2.27 (1.02 - 5.05)
Root mass (g)	2.19 (1.54 - 3.13)	3.86 (1.64 - 9.08)	2.72 (1.61 - 4.61)	4.68 (2.31 - 9.46)	0.88 (0.39 - 1.99)	1.17 (0.61 - 2.27)
Root:shoot	0.73 (0.33 - 1.13)	1.31 (0.02 - 2.64)	0.53 (0.32 - 0.75)	0.51 (0.22 - 0.79)	0.76 (0.58 - 0.94)	1.24 (0.70 - 1.78)
Ponderosa						
Total mass (g)	6.46 (5.24 - 7.96)	9.53 (7.16 - 12.68)	2.31 (0.89 - 6.00)	11.75 (5.60 - 24.66)	2.13 (0.81 - 5.62)	5.92 (2.14 - 16.34)
Root mass (g)	3.37 (2.64 - 4.32)	5.04 (3.69 - 6.87)	1.19 (0.47 - 3.01)	5.25 (2.20 - 12.53)	1.15 (0.48 - 2.76)	2.92 (1.09 - 7.85)
Root:shoot	1.13 (0.65 - 1.61)	1.18 (0.56 - 1.80)	1.14 (0.52 - 1.76)	0.96 (0.01 - 1.90)	1.36 (0.61 - 2.11)	0.99 (0.85 - 1.14)

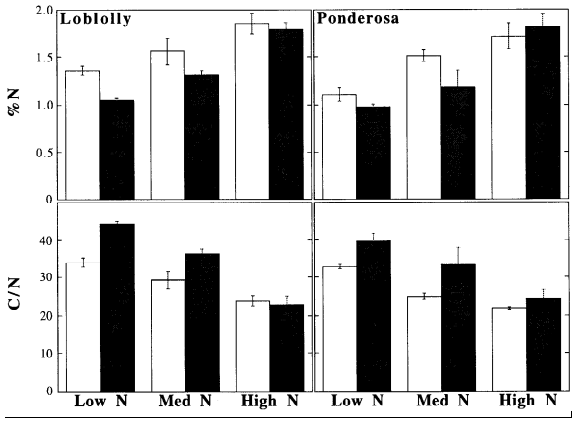

Figure 1. Nitrogen concentration and C/N ratio of loblolly (left) and ponderosa (right) pine roots from seedlings grown for 166 days in 3.5 (open bars) or 70 Pa. (closed bars) ambient CO_2 partial pressure. Nitrogen treatments were daily applications of 1.0, 3.5 or 7.0 m*M* NH_4^+ (low, med and high N respectively). Each bar represents the arithmetic mean of 5 replicates and is subtended by one standard error of the mean.

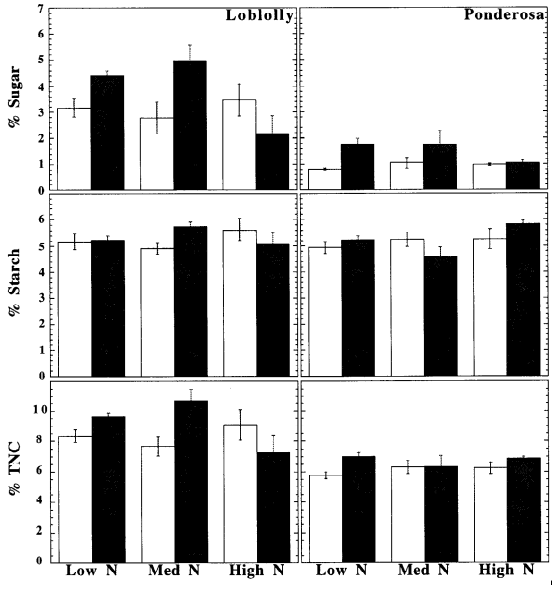

Figure 2. Sugar, starch and total non-structural carbohydrate (TNC) levels of loblolly (left) and ponderosa (right) pine roots from seedlings grown for 166 days in 35 (open bars) or 70 (closed bars) Pa. ambient CO_2 partial pressure. Nitrogen treatments were daily applications of 1.0, 3.5 or 7.0 m*M* NH_4^+ (low, med and high N respectively). Each bar represents the arithmetic mean of 5 replicates and is subtended by one standard error of the mean.

Loblolly and ponderosa pine roots had increased nitrogen concentrations when grown in increased N availability at both CO_2 partial pressures, and decreased nitrogen concentrations when grown in elevated CO_2, at medium and low N availibility (Figure 1). Both species had similar root nitrogen concentrations (1.81%) when grown with 7.0 m*M* N availability and these concentrations were not affected by CO_2. Similarly, C/N ratio of roots from both species were similar and unresponsive to CO_2 at High N (Figure 1). When grown in low or medium N availability both species showed a significantly increased C/N ratio when grown in elevated CO_2.

Starch concentrations between the two species and six treatment combinations were similar (Figure 2). The only significant treatment effect on root starch concentrations was an increase in high CO_2, medium N grown loblolly pine. Sugar concentrations were

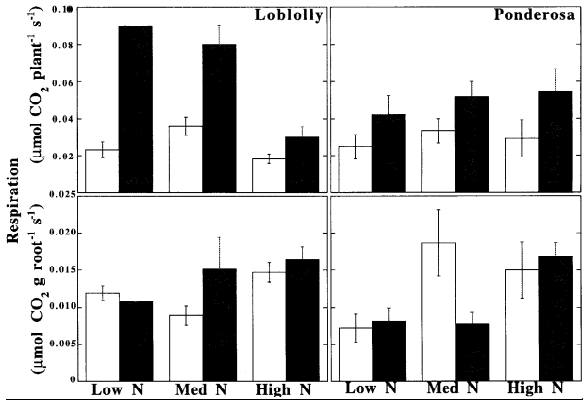

Figure 3. Root/rhizosphere respiration (μmol CO_2 plant^{-1} sec^{-1}, top) and Specific root/rhizosphere respiration (μmol CO_2 g^{-1} root dry mass s^{-1}, bottom) of loblolly (left) and ponderosa (right) pine seedlings grown for 166 days in 35 (open bars) or 70 (closed bars) Pa. ambient CO_2 partial pressure. Nitrogen treatments were daily applications of 1.0, 3.5 or 7.0 m*M* NH_4^+ (low, med and high N respectively). Each bar represents the arithmetic mean of 3 to 5 replicates and is subtended by one standard error of the mean.

significantly higher in loblolly pine roots than in ponderosa pine roots. Elevated CO_2, resulted in increased root sugar concentration in low and medium N plants, but did not significantly affect high N plants. Total non-structural carbohydrate levels were also higher in loblolly as compared to ponderosa pine. TNC was significantly affected by CO_2 at low and medium N in loblolly pine, and at low N in ponderosa pine.

Below ground carbon flux (μmol CO_2 plant^{-1} s^{-1}) was significantly increased when plants were grown in high CO_2 as compared to low CO_2 in all N treatments and both species (Figure 3a). Loblolly pine below ground fluxes were significantly larger than ponderosa pine fluxes at high CO_2, and similar at low CO_2. These differences in whole plant below ground carbon fluxes were mainly the result of increases in root biomass (Table 1). When below ground CO_2 efflux is normalized to root biomass, CO_2 significantly affected only plants grown in medium N (Figure 3b). Under these conditions loblolly pine root/rhizosphere respiration increased in response to elevated CO_2 while ponderosa pine root/rhizosphere decreased. However, it is important to point out that if the naturally elevated partial pressure of CO_2 found in forest soils directly inhibits root respiration, then our estimates made at a single low CO_2 partial pressure may overestimate root respiration, and only relative treatment differences are meaningful.

Increasing N availability generally lead to increased respiration. Root N concentration (%) was significantly correlated to root respiration, such that seedlings with higher root N concentrations had higher rates of specifc root respiration (μmol g^{-1} root mass s^{-1}, Figure 4).

Discussion

Ponderosa pine seedlings grown in ambient CO_2 have previously been shown to exhibit symptoms of nitrogen toxicity when grown in 3.5 m*M* N (Griffin et al., 1995). The results of this study indicate that 7.0 m*M* N is toxic to seedlings of both species. Interestingly, elevated CO_2 provided some alleviation of this stress in ponderosa pine even at 7.0 m*M* N. Although root biomass was consistently reduced under toxic nitrogen conditions, ponderosa pine root:shoot ratio was not affected. Loblolly pine root:shoot ratio increased, indicating that the toxic effects of 7.0 m*M* N on this species severely limited shoot growth. Root morphology of both species was affected by the toxic N treatment, limiting pine root production and leading to fewer but thicker roots (data not shown). The response of these two species to N availability/toxicity appears to be driving the physiological root system responses.

The instantaneous rates of CO_2 flux from the root/rhizosphere system measured in this study are similar to the few other system measurements that exist (Edwards, 1991; Reid and Strain, 1994; Van de Geijn et al., 1993) and to isolated root measurements from loblolly pine (Boyer et al., 1971; Drew and Ledig, 1981). Reid and Strain (1994) compared root maintenance respiration in two co-dominant, shade tolerant tree species and found diverging CO_2 effects. Although the daily rates of carbon loss frorn the root systems were not affected by growth CO_2 conditions, seasonal average maintenance respiration of *Fagus grandifolia* was stimulated by growth in elevated CO_2 while in *Acer saccharum*, the faster growing species, respiration rates were decreased. Based on comparative studies of plant species with inherently different growth rates Lambers et al. (1990) makes the more general conclusion that fast growing species lose a smaller fraction of their fixed carbon via root respiration than inherently slower growing species. Here results are inconsistent with both these findings because loblolly is considered to be the faster growing of the two species, yet generally had higher respiration rates and these rates were increased by CO_2 when soil nitro-

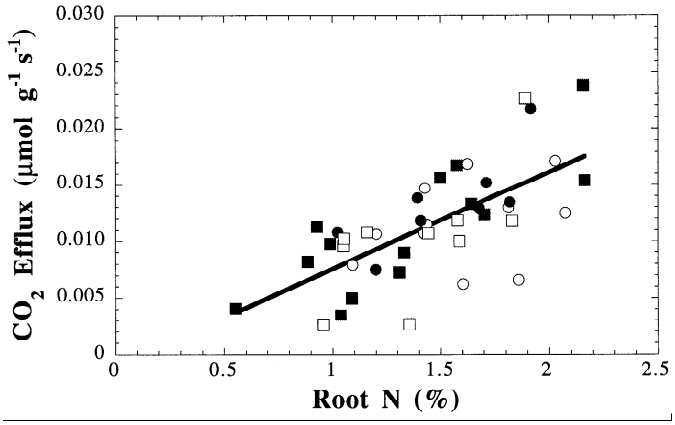

Figure 4. Specific root/rhizosphere respiration (μmol CO_2 g^{-1} root dry mass s^{-1}) as a function of root system N (%). Measured as CO_2 efflux from the intact below ground root/soil system of loblolly (m 1) and ponderosa pine (o n) seedlings grown in 35 or 70 Pa CO_2 (open and filled symbols respectively) for 166 days. CO_2 efflux = 0.00105 - 0.0086 $\times$ Root N, r^2 = 0.45, p<0.01. n=3-5.

gen was adequate but non toxic. Spring wheat grown in the Wageningen Rizolab under elevated CO_2 had 20% greater root system respiration (per m^2 ground area) than ambient CO_2 grown wheat (Van de Geijn et al., 1993). Similar to our results this stimulation was driven by increases in root mass. Complex seasonal patterns of belowground CO_2 flux found in the rizolab study should add a note of caution to all studies such as ours that make single point in time measurements. Clearly root phenology and whole plant nutrient and water demands driven by whole plant phenology have tremendous impacts on below ground CO_2 flux.

In this study there were no correlations between the supply of C as a respiratory substrate (sugar, starch or TNC) and root respiration. Similar results have been reported for crop species such as wheat and alfalfa (Barta, 1988; Huang and Johnson, 1995) and are consistent with the current thinking that mitochondrial respiration is generally regulated by the use of respiratory products, and not by the supply of substrates (Ap Rees, 1990; Copeland and Turner, 1987; Dry et al., 1987; Lambers,1990; Møller and Palmer, 1984; Palmer, 1984). However, during periods of rapid. growth, substrate utilization in the formation of new tissues can lead to substrate limitations of respiration (Amthor, 1996). Here respiration measurements were made at the end of the growing season and thus the observed rates are more likely related to maintenance or ion uptake processes than to growth processes.

Nitrogen concentrations are often used as a proxy for protein concentrations, and therefore the relationship between root nitrogen concentrations and root/rhizosphere respiration may be indicative of increased maintenance respiration (Amthor, 1989; Ryan 1991). Alternatively Pregitzer et al. (1995) suggests that increased soil N availability speeds the rate of fine root turnover, indicating that increased respiration rates may be associated with growth functions. In a long term study of CO_2 and nitrogen availability in field grown ponderosa pine seedlings opposite results were obtained (Dr J T Ball, pers. comm.). Elevated levels of resource availability, led to lower root turnover rates and lower fine root biomass. Yet another possibility is that the increased N availability affected the release of organic compounds into the rhizosphere stimulating microbial respiration. Such a correlation between increased N availability and increased release of root derived carbon compounds has been made for crop species (Liljeroth et al., 1990; Merckx et al., 1987).

Our data suggest that under the experimental conditions root respiration was driven primarily by nitrogen uptake and detoxification rather than by growth or substrate availability. However, if root system respiration is related to NH_4^+ detoxification alone, we would expect, but did not find, a correlation between root respiration (μmol g $root^{-1}$ s^{-1}) and total plant N uptake (g N g $root^{-1}$). This may be the result of nitrogen toxicity causing a disproportional increase in respiration compared to N uptake. When only the non-toxic N treatments are considered there is the suggestion of an increasing function between respiration and whole plant N uptake (r^2 = 0.20, p = 0.01). Furthermore, the two measurements have inherently different time scales, with whole plant N uptake being an integrat-

ed function of the plant life span, and root respiration being an instantaneous measure of respiratory activity. A direct test of this relationship would require the measurement of instantaneous nitrogen uptakes rates or integrated respiratory activity.

Apparent species differences may be related to the evolutionary history of, and environmental conditions native to, each species (Griffin et al., 1995). In a parallel study we found that when presented with equal concentrations of both NH_4^+ and NO_3^- more than 80% of the total N taken up by loblolly pine was NH_4^+ and between 15 and 45% (low and high CO_2 respectively) of the total N taken up by ponderosa pine was NH_4^+ (BassiriRad and Griffin, 1996). Not only did ponderosa pine prefer NO_3^-, it also had much lower total uptake capacity than loblolly pine. These differences resulted in a lower NH_4^+ threshold in ponderosa pine, the species not being adapted to detoxify large amounts of this N substrate.

Although of the mechanisms responsible for the observed results have not been identified, it is obvious that the availability of both carbon and nitrogen have both direct and indirect effects on root system respiration. Furthermore these effects are interactive both with each other and with species characteristics. In our study root N concentration, N species preference and ammonium detoxification can be used to explain the majority of the treatment effects on root system respiration and total biomass accumulation. Human activities are known to be altering the global cycling of both carbon and nitrogen (Aber et al., 1989; Keeling et al., 1989; Schlesinger, 1991), two important regulators of tree seedling growth (Bazzaz, 1990; Eamus and Jarvis, 1989). Understanding the physiological mechanisms involved in seedling growth regulation is the first step in developing predictive models of forest regeneration and response to future environmental conditions of altered atmospheric CO_2 and soil N availability. Clearly root system physiology must be considered as primary factor in this response.

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