

Direct and indirect effects of elevated CO₂ on whole-shoot respiration in ponderosa pine seedlings

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Summary We determined the short-term direct and long-term indirect effects of CO₂ on apparent dark respiration (CO₂ efflux in the dark) in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) seedlings grown in 35 or 70 Pa CO₂ partial pressure for 163 days in naturally lit, controlled-environment chambers. Two soil N treatments (7 and 107 ppm total N, low-N and high-N treatments, respectively) were imposed by watering half the plants every 2 weeks with 15/15/18 fertilizer (N,P,K) and the other half with demineralized water. Direct effects of ambient CO₂ partial pressure on apparent dark respiration were measured during short-term manipulations (from minutes to hours) of the CO₂ environment surrounding the aboveground portion of individual seedlings. Short-term increases in the ambient CO₂ partial pressure consistently resulted in significant decreases in CO₂ efflux of seedling in all treatments. Efflux of CO₂ decreased by 3 to 13% when measurement CO₂ partial pressure was increased from 35 to 70 Pa, and by 8 to 46% over the entire measurement range from 0 to 100 Pa. No significant interactions between the indirect effects of growth CO₂ partial pressure and the direct effects of the measurement CO₂ partial pressure were found. Seedlings grown in the high-N treatment were significantly less sensitive to short-term changes in CO₂ partial pressures than seedlings grown in the low-N treatment. Apparent respiration tended to decrease in seedlings grown in elevated CO₂, but the decrease was not significant. Nitrogen had a large effect on CO₂ efflux, increasing apparent respiration more than twofold on both a leaf area and a leaf or shoot mass basis. Both the direct and indirect effects of elevated CO₂ were correlated with changes in the C/N ratio. A model of cumulative CO₂ efflux for a 160-day period demonstrated that, despite a 49% increase in total plant biomass, seedlings grown in the high-N + high-CO₂ treatment lost only 2% more carbon than seedlings grown in the high-N + low-CO₂ treatment, suggesting increased carbon use efficiency in plants grown in elevated CO₂. We conclude that small changes in instantaneous CO₂ efflux, such as those observed in ponderosa pine seedlings, could scale to large changes in carbon sequestration.

Keywords: carbon flux, C/N ratio, dark respiration, nitrogen, *Pinus ponderosa*, seasonal carbon flux.

Introduction

Atmospheric CO₂ partial pressure, directly and indirectly, affects many plant physiological processes (reviewed in Kramer 1981, Lemon 1985, Strain 1985, Strain 1987, Eamus and Jarvis 1989, Bazzaz 1990, Mooney et al. 1991, Grodzinski 1992, Schulze and Mooney 1993, Gunderson and Wullschleger 1994). The effects of elevated CO₂ on growth, photosynthesis, and stomatal conductance are well documented (reviewed in Amthor 1991, Ryan 1991, Poorter et al. 1992, Wullschleger et al. 1994); however, much less is known about the effects of CO₂ on plant respiration, even though plant respiration is intricately linked to all metabolic processes, providing the energy and biochemicals required to maintain plant function. At the global scale, plant respiration returns approximately half of the photosynthetically fixed carbon to the atmosphere, accounting for the release of as much as 60 GT of carbon annually (Amthor 1995). Given the importance of plant respiration from the cellular to the global scale, it is essential to improve our understanding of the rates and control of respiration and the effects of changing atmospheric CO₂ partial pressures on respiratory CO₂ release.

Amthor (1991, 1995) divides the effects of CO₂ partial pressure on plant respiration into two categories: (1) direct effects observed during short-term changes in the CO₂ environment; and (2) indirect effects observed during long-term growth in a particular CO₂ partial pressure. The direct effects of CO₂ on apparent respiration have not been intensively studied. (Plant respiration is properly defined as the combined activities of glycolysis, the oxidative pentose phosphate pathway, the tricarboxylic acid cycle and mitochondrial electron transport chain. However, it is normally measured as either CO₂ release or O₂ consumption in the dark. Because other plant processes can contribute to the exchange of these gases, and the activity of the respiratory processes are not directly measured, it is more accurate to define the measured CO₂ efflux as apparent respiration (cf. Amthor et al. 1992)). However, several studies indicate that there is an immediate and fully reversible decrease in apparent respiration rates when atmospheric CO₂ partial pressures are increased (e.g., Bunce 1990, Amthor et al. 1992, Thomas and Griffin 1994, but see Hellmuth 1971, Byrd 1992 (as cited in Amthor 1995), Ryle et al. 1992a, 1992b,

Ziska and Bunce 1994). The mechanism(s) of this reduction has not been elucidated, but may be related to dark CO₂ fixation by phosphoenolpyruvate carboxylase (PEPc), altered cytosolic pH, or direct effects on membranes or enzymes (Amthor et al. 1992).

Indirect effects of CO₂ on apparent respiration, which have been the focus of many studies, are measured from steady-state apparent respiration rates of plants grown in two or more CO₂ partial pressures (reviewed in Poorter et al. 1992, Wullschlegel et al. 1994, Amthor 1995). Although Amthor (1995) defines indirect effects as secondary responses to altered growth and photosynthetic rates as well as changes in plant tissue composition resulting from long-term growth in different CO₂ environments, Azcón-Bieto et al. (1994) have demonstrated that indirect effects can include reductions in mitochondrial enzyme activities and concentrations. Based on a review of results published through 1992, Poorter et al. (1992) conclude that, in response to elevated CO₂, apparent respiration rates increase an average of 16% when expressed on a leaf area basis, but decrease an average of 14% when expressed on a leaf mass basis. Few studies have simultaneously examined both the direct and indirect effects of elevated CO₂ on apparent respiration (Bunce 1990, Thomas and Griffin 1994).

We examined the direct and indirect effects of a doubling of atmospheric CO₂ partial pressures on the apparent respiration rates of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) seedlings. Ponderosa pine is the most widely distributed conifer in North America and is an important species in four western North American forest types (Fowells 1965). Changes in the respiration rate of this long-lived coniferous species have the potential to significantly influence biospheric carbon flux and global carbon sequestration. Direct and indirect effects of CO₂ were studied simultaneously by measuring apparent respiration versus intercellular CO₂ curves (R/C_i) on whole shoots of seedlings grown for 80 or 163 days in either 35 or 70 Pa CO₂. We manipulated plant response to CO₂ by varying carbon and nitrogen supplies, and used a simple model to consider the influence of these observed changes in CO₂ efflux on seasonal carbon flux.

Materials and methods

Plant material and propagation

Ponderosa pine seeds from a half-sib family were collected at 915 m elevation in El Dorado County, California (California Department of Forestry Lot CDF 526). In March 1992, three seeds were planted in each of a number of 2.7-liter pots filled with a 1/1 (v/v) mix of sand and Aiken clay loam field soil (Xeric Haplohumult derived from andesite, D. Johnson, personal communication), and germinated and grown in controlled-environment growth chambers at the Desert Research Institute, Reno, Nevada. One month after seedling emergence, the plants were thinned to one per pot and subjected to the experimental treatments. The experimental growth period lasted 163 days, the approximate length of one growing season (Oliver and Ryker 1990). Seven plants per treatment were randomly selected for each of the two harvests (Days 80 and

163). Seedlings were separated into leaves, stems and roots, and dried to a constant mass at 60 °C. Tissue nitrogen concentrations of homogenized samples were determined with an elemental analyzer (2400 CHN, Perkin Elmer Corp., Norwalk, CT, USA).

Environmental treatments

Atmospheric CO₂ partial pressures of either ambient (approximately 35 Pa) or ambient + 35 Pa (approximately 70 Pa) were maintained in two naturally lit, controlled-environment growth chambers. These partial pressures were chosen to simulate the current ambient CO₂ concentration and a predicted doubling by the end of the next century (Watson et al. 1990). Ambient air temperature was maintained at 25 °C. Relative humidity inside the chambers was approximately 30% during the day, and 40% at night. Solar radiation was greater than 85% of ambient (typically between 1700 and 2400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, at solar noon), with a natural photoperiod throughout the experiment.

Nutrient treatments were initiated approximately 90 days after planting and were applied once every 2 weeks as Plantex 15/15/18 (N,P,K, Plantco Inc., Ontario, Canada). Based on the known soil nitrogen content, it was assumed that, before fertilization, the availability of soil N was adequate to supply the low N requirement of small seedlings. The nutrient solution contained 15% total N (3% NH₄⁺, 8.3% NO₃⁻ and 3.7% urea), 15% P₂O₄, 18% K₂O, 0.02% B, 0.05% Cu, 0.1% Fe, 0.05% Mn, 0.015% Mo and 0.05% Zn, and was added volumetrically to yield a final soil N concentration of 107 ppm (high-N treatment). The remainder of the pots received no fertilization, resulting in a final N concentration of 7 ppm (low-N treatment, D. Johnson, personal communication). All pots were watered to saturation daily with deionized water.

Gas exchange

Before each harvest, apparent dark respiration was measured as CO₂ efflux in the dark with an infrared gas analyzer in an open-flow gas exchange system. Nitrogen, oxygen and carbon dioxide from high pressure cylinders were passed through mass-flow controllers (MKS Instruments, Inc., Andover, MA, USA) to produce an air stream of the desired CO₂ concentration and a 21% O₂ concentration. Water vapor was then added to a known vapor pressure with a dew point generator (LI-610, Li-Cor, Inc., Lincoln, NE, USA). The conditioned air stream flowed continuously through three 0.185-liter cuvettes. By means of a series of nine solenoid valves (Clippard Inst. Laboratory, Inc., Cincinnati, OH, USA), the air stream to the cuvette of interest was first diverted through a mass flow meter (Li-Cor, Inc., Model LI-200 portable photosynthesis system) and, similarly, the return flow from this cuvette was sent to a humidity sensor (Humicap, Vaisala, Inc., Woburn, MA, USA) and the sample side of the infrared gas analyzer (Li-Cor, Inc., Model LI-6250).

Cuvettes were constructed from 7.5-cm diameter Plexiglas tubes lined with Teflon to minimize water sorption. Each cuvette contained a fan to ensure adequate mixing and to decrease the boundary layer (Micronel US, Vista, CA, USA).

The entire aboveground portion of the seedling was enclosed within the cuvette and the space between the stem and the bottom cap of the cuvette, was filled with Apiezon sealing compound (James Biddle Co., Plymouth, PA, USA)

All gas exchange parameters were calculated as described by Field et al. (1991), with the exception of the intercellular CO₂ partial pressure, C_i , which was calculated as in Amthor et al. (1992). All gas exchange parameters expressed on a leaf area basis were calculated from total leaf surface area rather than projected surface area.

Gas exchange measurements were initiated no sooner than 2 h after sundown and were completed during the natural dark period. Plants were allowed to acclimate to the cuvette environment for a minimum of 45 min before initiation of respiration measurements. Gas exchange parameters were recorded only after CO₂ efflux and the vapor pressure deficit had reached a steady state, usually requiring 10 to 20 min. Respiration versus C_i curves were made by varying the partial pressure of CO₂ entering the cuvette (C_e) from 0 to 105 Pa in seven 17.5-Pa steps. Indirect effects were analyzed from these curves by comparing the respiration rates measured at the growth CO₂ partial pressure of the seedlings from the different treatment combinations.

Carbon flux model

Seasonal shoot respiration (SR, $\mu\text{mol CO}_2$) and mass specific seasonal shoot respiration (SR_m, $\mu\text{mol g}^{-1}$) can be described as:

$$SR = \sum_{d=1}^{160} W_d S_d R_d \tau_d \quad (1)$$

or

$$SR_m = \sum_{d=1}^{160} R_d \tau_d \quad (2)$$

where W is the total plant dry mass (g), S is the shoot fraction (shoot mass/ W), R is the apparent respiration rate ($\mu\text{mol CO}_2 \text{ g}^{-1}$ shoot mass s^{-1}), d = day of the growing season, or plant age in this case, and τ is the length of the dark period (seconds), calculated as:

$$\tau = 86400 - 3600 \left(\frac{24}{\pi} \cos^{-1}(\tan \phi \tan \delta) \right) \quad (3)$$

with ϕ = latitude (39.5° in Reno, NV), and δ = solar declination, described as:

$$\delta = 23.5 \cos 2\pi \frac{\text{jd} - 172}{365}, \quad (4)$$

where jd = Julian day.

Statistical analysis

Effects of CO₂ partial pressure and N fertilizer on whole-shoot respiration were tested by ANOVA (Data Desk 4.1 statistical software, Data Description Inc., Ithaca, NY, USA). Means separation based on planned comparisons were accomplished with a Scheffe *post hoc* test. Treatment effects and means separation were considered significant when $P \leq 0.05$.

Results

Direct effects

Increasing the CO₂ partial pressure of air entering the cuvette (C_e) consistently reduced the apparent respiration rates of ponderosa pine shoots (Figure 1). The reduction in CO₂ efflux was independent of the time of measurement and the direction of the CO₂ change, and was always fully reversible. To assess the response of apparent respiration to CO₂ partial pressure among the treatment combinations, we compared the percentage reduction between the two end points (0 and 100 Pa), as well as the percentage reduction between the two growth CO₂ partial pressures (35 and 70 Pa). After 80 days of growth at 70 Pa CO₂, the average reduction in apparent respiration for a change in C_e from 0 to 100 Pa was significantly higher than the reduction observed after 80 days of growth at 35 Pa CO₂ (Table 1). The percent reduction in apparent respiration from 35 to 70 Pa (C_e), was statistically indistinguishable between seedlings in the two CO₂ growth treatments.

After 163 days, there were no significant differences in the percent reduction of apparent respiration between 0 and 100 Pa or between 35 and 70 Pa (C_e) for low-N plants grown in 35 or 70 Pa CO₂ (Table 2). However, the addition of N fertilizer to the soil resulted in significant changes in the direct response of apparent respiration to CO₂ partial pressure. High-N plants

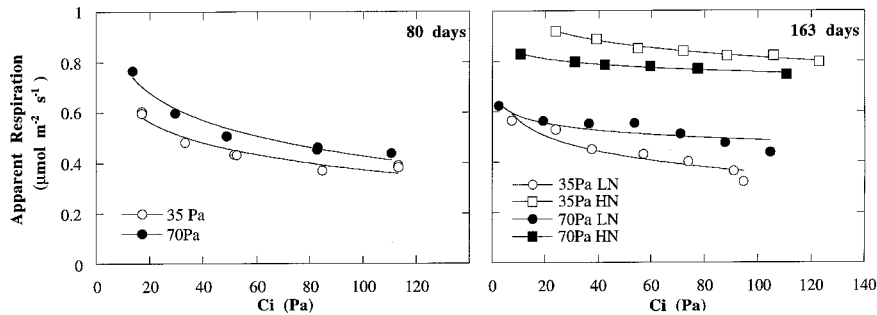


Figure 1. Representative curves of apparent respiration ($\mu\text{mol m}^2 \text{ s}^{-1}$) versus intercellular CO₂ partial pressure (C_i) in ponderosa pine shoots measured either 80 or 163 days after planting ($n = 3$ or 4).

Table 1. Summary of the direct effects of CO₂ partial pressure on apparent respiration of ponderosa pine seedlings grown for 80 days in either 35 or 70 Pa ambient CO₂. (Values within a column followed by the same letter are not different at $P = 0.05$. Values are means ± 1 SE, $n = 3$ or 4.)

Growth CO ₂	Reduction in apparent respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$) with a change in ambient CO ₂ from:			
	0 to 100 Pa	(%)	35 to 70 Pa	(%)
35 Pa	0.156 \pm 0.024a	29 \pm 0.03a	0.032 \pm 0.009a	7 \pm 0.02a
70 Pa	0.332 \pm 0.046b	46 \pm 0.08b	0.055 \pm 0.007a	12 \pm 0.01a

were much less responsive to short-term changes in CO₂ partial pressure, decreasing only 8 to 12% when C_e was increased from 0 to 100 Pa, compared with reductions of 37 to 43% in low-N plants over the same range. For high-N seedlings grown in 35 or 70 Pa CO₂, there were no significant differences in percent reduction of apparent respiration over the range of 0 to 100 Pa, or 35 to 70 Pa. The percent change in apparent respiration from 35 to 70 Pa CO₂ was significantly correlated to leaf C/N ratio (Figure 2).

Indirect effects

A summary of indirect effects of elevated CO₂ on apparent respiration after 80 days of growth on the basis of mass, leaf area, N content and several other parameters is presented in Table 3. Depending on how the data are expressed, the effect of CO₂ on apparent respiration changes from a 20% reduction

Table 2. Summary of the direct effects of CO₂ partial pressure on apparent respiration in ponderosa pine seedlings grown for 163 days in either 35 or 70 Pa ambient CO₂ and 7 or 107 ppm soil N. (Values within a column followed by the same letter are not different at $P = 0.05$, values are means ± 1 SE, $n = 3$.)

Growth treatment	Reduction in apparent respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$) with a change in ambient CO ₂ from:			
	0 to 100 Pa	(%)	35 to 70 Pa	(%)
35 Pa CO ₂ , 7 ppm N	0.195 \pm 0.03b	43 \pm 0.07b	0.043 \pm 0.004a	11 \pm 0.01bc
35 Pa CO ₂ , 107 ppm N	0.118 \pm 0.03ab	12 \pm 0.01a	0.032 \pm 0.002a	4 \pm 0.01ab
70 Pa CO ₂ , 7 ppm N	0.184 \pm 0.01b	37 \pm 0.06b	0.054 \pm 0.02a	13 \pm 0.01c
70 Pa CO ₂ , 107 ppm N	0.070 \pm 0.01a	8 \pm 0.01a	0.022 \pm 0.001a	3 \pm 0.01a

Table 3. Summary of the apparent respiration rates measured at the growth CO₂ partial pressure (35 or 70 Pa) and the indirect effects of CO₂ partial pressure on apparent respiration in 80-day-old ponderosa pine seedlings ($ns =$ not different at $P = 0.05$, values are means ± 1 SE, $n = 4$).

CO ₂ Efflux per unit	Growth CO ₂ partial pressure		Indirect effect 70/35
	35 Pa	70 Pa	
Leaf area ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.408 \pm 0.013	0.407 \pm 0.043	1.00 ns
Leaf mass ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.012 \pm 0.002	0.010 \pm 0.002	0.82 ns
Shoot mass ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.010 \pm 0.001	0.009 \pm 0.001	0.85 ns
Leaf N ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.662 \pm 0.118	0.687 \pm 0.038	1.04 ns
Shoot N ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.594 \pm 0.108	0.617 \pm 0.029	1.04 ns
Leaf C ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.025 \pm 0.004	0.021 \pm 0.002	0.83 ns
Shoot C ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.021 \pm 0.003	0.018 \pm 0.002	0.86 ns
Leaf C/N ($\mu\text{mol g g}^{-1} \text{s}^{-1}$)	8.7 $\times 10^{-5}$ \pm 6.3 $\times 10^{-6}$	7.0 $\times 10^{-5}$ \pm 1.8 $\times 10^{-6}$	0.80 ns
Shoot C/N ($\mu\text{mol g g}^{-1} \text{s}^{-1}$)	7.9 $\times 10^{-5}$ \pm 4.5 $\times 10^{-6}$	6.7 $\times 10^{-5}$ \pm 1.7 $\times 10^{-6}$	0.84 ns

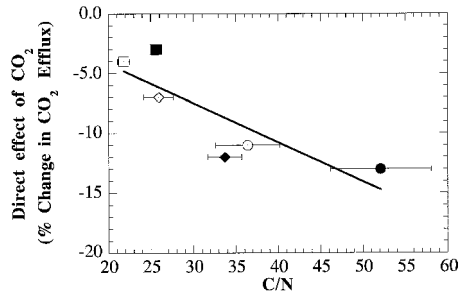


Figure 2. Direct effect of CO₂ partial pressure on the leaf carbon to nitrogen ratio (C/N). Symbols: $\diamond =$ 35 Pa CO₂ (80 days); $\blacklozenge =$ 70 Pa CO₂ (80 days); $\circ =$ 35 Pa CO₂, 7 ppm N (163 days); $\square =$ 35 Pa CO₂, 107 ppm N (163 days); $\bullet =$ 70 Pa CO₂, 7 ppm N (163 days); $\blacksquare =$ 70 Pa CO₂, 107 ppm N (163 days). Treatment means ± 1 standard error of the mean, $n = 3$ to 4 replicates per treatment (y error bars are smaller than printed symbols). Direct effect = $-2.32 + 0.33 \times \text{C/N}$; $R^2 = 0.71$.

to a 4% increase, yet there were no statistically significant changes in the respiration rate of whole shoots measured at the growth CO₂ partial pressure. In general, mass-based measurements showed larger decreases in apparent respiration than area-based or nitrogen-based measurements.

The apparent respiration rates of low-N plants on Day 163 were similar to rates measured on Day 80, when expressed on a leaf area-basis (Table 4). However, apparent respiration at Day 163 was significantly decreased compared to the Day 80 measurements when expressed on a leaf or shoot mass basis, a leaf or shoot nitrogen basis, or a leaf or shoot carbon basis,

Table 4. Summary of apparent respiration rates measured at the growth CO₂ partial pressure (35 or 70 Pa) in ponderosa pine seedlings grown for 163 days in either 7 or 107 ppm soil N. (Values are means ± 1 SE, *n* = 3.)

CO ₂ Efflux per unit	Low-N (7 ppm)		High-N (107 ppm)	
	35 Pa	70 Pa	35 Pa	70 Pa
Leaf area (μmol m ⁻² s ⁻¹)	0.393 ± 0.043	0.371 ± 0.073	0.888 ± 0.169	0.789 ± 0.074
Leaf mass (μmol g ⁻¹ s ⁻¹)	0.007 ± 0.001	0.006 ± 0.001	0.017 ± 0.004	0.013 ± 0.002
Shoot mass (μmol g ⁻¹ s ⁻¹)	0.005 ± 0.001	0.004 ± 0.001	0.013 ± 0.002	0.011 ± 0.001
Leaf N (μmol g ⁻¹ s ⁻¹)	0.516 ± 0.065	0.602 ± 0.101	0.758 ± 0.149	0.708 ± 0.104
Shoot N (μmol g ⁻¹ s ⁻¹)	0.450 ± 0.065	0.522 ± 0.089	0.631 ± 0.099	0.634 ± 0.103
Leaf C (μmol g ⁻¹ s ⁻¹)	0.015 ± 0.002	0.012 ± 0.002	0.035 ± 0.008	0.028 ± 0.004
Shoot C (μmol g ⁻¹ s ⁻¹)	0.001 ± 0.000	0.001 ± 0.000	0.005 ± 0.002	0.005 ± 0.002
Leaf C/N (μmol g g ⁻¹ s ⁻¹)	8.8 × 10 ⁻⁵ ± 2.1 × 10 ⁻⁵	3.9 × 10 ⁻⁵ ± 9.0 × 10 ⁻⁶	5.5 × 10 ⁻⁴ ± 2.0 × 10 ⁻⁴	4.2 × 10 ⁻⁴ ± 7.4 × 10 ⁻⁵
Shoot C/N (μmol g g ⁻¹ s ⁻¹)	7.0 × 10 ⁻⁵ ± 1.6 × 10 ⁻⁵	3.4 × 10 ⁻⁵ ± 7.0 × 10 ⁻⁶	4.9 × 10 ⁻⁴ ± 1.9 × 10 ⁻⁴	3.6 × 10 ⁻⁴ ± 5.0 × 10 ⁻⁵

Table 5. Summary of indirect effects of CO₂ partial pressure and soil N on apparent respiration in ponderosa pine seedlings grown for 163 days in either 35 or 70 Pa ambient CO₂ and 7 or 107 ppm soil N. All respiration measurements were made at the growth CO₂ partial pressure. (ns = not different at *P* = 0.05, * = *P* ≤ 0.05, ** = *P* ≤ 0.01, † = *P* = 0.07.)

CO ₂ efflux per unit	CO ₂ effect (70/35 Pa)		Nitrogen effect (High-N/Low-N)	
	Low-N (7 ppm)	High-N (107 ppm)	35 Pa	70 Pa
Leaf area (μmol m ⁻² s ⁻¹)	0.95 ns	0.89 ns	2.26 **	2.13 *
Leaf mass (μmol g ⁻¹ s ⁻¹)	0.80 ns	0.77 ns	2.38 **	2.32 *
Shoot mass (μmol g ⁻¹ s ⁻¹)	0.82 ns	0.84 ns	2.36 **	2.45 *
Leaf N (μmol g ⁻¹ s ⁻¹)	1.17 ns	0.93 ns	1.47 ns	1.18 *
Shoot N (μmol g ⁻¹ s ⁻¹)	1.16 ns	1.00 ns	1.40 ns	1.21 *
Leaf C (μmol g ⁻¹ s ⁻¹)	0.82 ns	0.79 ns	2.41 **	2.33 *
Shoot C (μmol g ⁻¹ s ⁻¹)	0.43 ns	1.02 ns	5.13 ns	12.20 †
Leaf C/N (μmol g g ⁻¹ s ⁻¹)	0.44 ns	0.76 ns	6.19 **	10.62 *
Shoot C/N (μmol g g ⁻¹ s ⁻¹)	0.49 ns	0.73 ns	7.07 *	10.61 *

probably reflecting an increase in structural material (Table 5). Increased N availability was associated with increased CO₂ efflux, regardless of CO₂ treatment. Specific apparent respiration (μmol CO₂ efflux g⁻¹ shoot mass s⁻¹) was significantly negatively correlated with leaf C/N ratio (Figure 3).

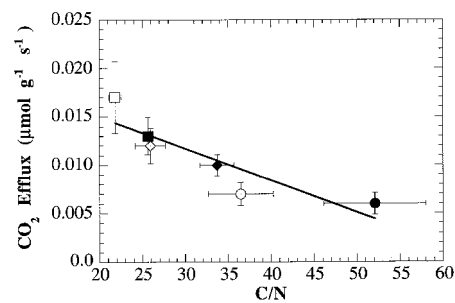


Figure 3. Whole-shoot respiration (μmol CO₂ g⁻¹ shoot mass s⁻¹) versus leaf carbon to nitrogen ratio (C/N). Symbols: ◇ = 35 Pa CO₂ (80 days); ◆ = 70 Pa CO₂ (80 days); ○ = 35 Pa CO₂, 7 ppm N (163 days); □ = 35 Pa CO₂, 107 ppm N (163 days); ● = 70 Pa CO₂, 7 ppm N (163 days); ■ = 70 Pa CO₂, 107 ppm N (163 days), *n* = 3 to 4. CO₂ Efflux = 0.216 - 3.3 × 10⁻⁴ × C/N; *R*² = 0.79.

Carbon flux model

The predicted daily loss of carbon through CO₂ efflux during the natural dark period of the first growing season is given in Figure 4. The model predicted slightly lower daily respiratory losses of CO₂ during the first 100 days of growth for plants grown in elevated CO₂ partial pressures than for plants grown at ambient partial pressures. Plants grown in the low-N + high-CO₂ treatment continued to have lower daily carbon loss throughout the duration of the modeled period, whereas plants grown in the high-N + high-CO₂ treatment had more daily carbon loss than their low-CO₂ counterparts. Integrating these carbon losses over the 160-day modeled period resulted in slightly less total carbon loss from seedlings grown in the low-N + high-CO₂ treatment and slightly more total carbon loss from seedlings grown in the high-N + high-CO₂ treatment than seedlings grown in the corresponding low-CO₂ treatments (19 and 2% respectively, Figure 5). When these data were expressed per unit biomass (μmol g⁻¹), low-N seedlings were not affected by the CO₂ growth treatment (Figure 5), whereas high-N seedlings had reduced mass-specific shoot respiration (22%). If the observed changes in biomass accumulation and allocation resulting from growth in elevated CO₂ were not accompanied by the small changes in apparent respiration rate, total carbon loss from seedlings grown in the low-N + high-

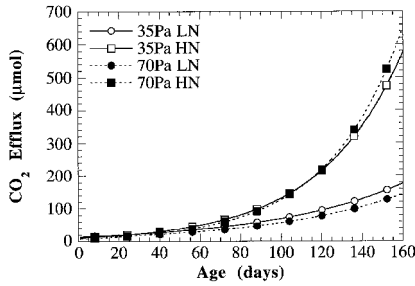


Figure 4. Modeled daily CO₂ efflux (µmol) as a function of plant age for ponderosa pine seedlings grown for 160 days in either 35 or 70 Pa CO₂ and 7 or 107 ppm N.

CO₂ and high-N + high-CO₂ treatments would have been 4 and 12% lower, respectively, than the estimated total CO₂ loss from seedlings grown in elevated CO₂. These differences translate to a 16% reduction in respiration for seedlings grown in the low-N + high-CO₂ treatment compared with seedlings grown in the low-N + low-CO₂ treatment, and a 16% increase in respiration in seedlings grown in the high-N + high-CO₂ treatment compared with seedlings grown in the high-N + low-CO₂-treatment.

Discussion

Our finding of decreased apparent respiration in response to short-term increases in ambient CO₂ partial pressure in ponderosa pine confirms observations made for several other plant species (Amthor 1995). The general shape of the response curves of CO₂ efflux versus intercellular CO₂ partial pressure reported by Amthor et al. (1992) are similar to those reported here despite large differences between the study species in leaf and plant morphology, physiology, and natural history. Unfortunately, interpretation and extrapolation of the observed direct effect of CO₂ on apparent respiration are severely limited by the lack of a mechanistic understanding of the phenomenon. Until a mechanism(s) is identified, understanding of this common response is facilitated by considering respiratory control theory (Amthor 1995). Currently it is believed that mitochondrial respiration rates are primarily regulated by the consumption of respiratory products or their pool size (Møller and Palmer 1984, Palmer 1984, Copeland and Turner 1987, Dry et al. 1987, Lambers 1990, ap Rees 1990).

Respiratory control theory suggests a direct and negative relationship between intercellular CO₂ partial pressure and the use of ATP, NAD(P)H or C-skeleton intermediates. The consumption of these respiratory products is associated with normal plant metabolism such as growth and maintenance processes. Plants are rarely detrimentally affected by elevated CO₂ and commonly grow larger than plants grown under ambient CO₂ conditions. Thus, it is difficult to imagine a reduction in these processes resulting directly from increased intercellular CO₂ partial pressures. We found that plants growing at very different rates (as a result of age, CO₂ or N availability) responded similarly to a change in ambient CO₂ from 35 to 70 Pa, indirectly suggesting that the consumption of respiratory products for growth processes is not responsible for the observed results. Protein turnover is regarded as a major component of maintenance processes (Bouma et al. 1994), and a positive relationship between respiration and protein content has been reported (Lambers et al. 1989, Pons et al. 1989, De Visser et al. 1992). Although we did not measure leaf or shoot protein concentrations, they can be estimated from leaf and shoot N concentrations (but see Näsholm and Ericsson 1990). We found that the direct effect of CO₂ on apparent respiration (percent reduction from 35 to 70 Pa) was sensitive to leaf/shoot N, but in an unexpected direction. Shoot apparent respiration was less sensitive to ambient CO₂ in high-N plants than in low-N plants, suggesting that the direct effect of CO₂ is not the result of decreased consumption of respiratory products resulting from inhibited or reduced maintenance processes.

Respiration can also supply energy to seemingly futile processes such as the cyanide-insensitive alternative oxidase of the mitochondrial electron transport chain (Moore and Siedow 1991), or the cycling of hexose sugars through the vacuole (Huber 1989, Geigenberger and Stütt 1991). It is possible that CO₂ directly affects one of these processes without disrupting normal plant function. On the other hand, dark CO₂ fixation may account for the lack of an observed correlation between metabolic activity and direct CO₂ effects. Amthor (1995) examined the feasibility of a net uptake of CO₂ in the dark and concluded that at least part of the observed decrease in CO₂ efflux may be the result of CO₂ fixation by PEPc.

Although there have been many studies of the long-term effects of CO₂ on respiration, only rarely are both short- and long-term effects examined concurrently (Bunce 1990, Thomas and Griffin 1994). Unlike the reports of Bunce (1990) and Thomas and Griffin (1994), we did not find an interaction

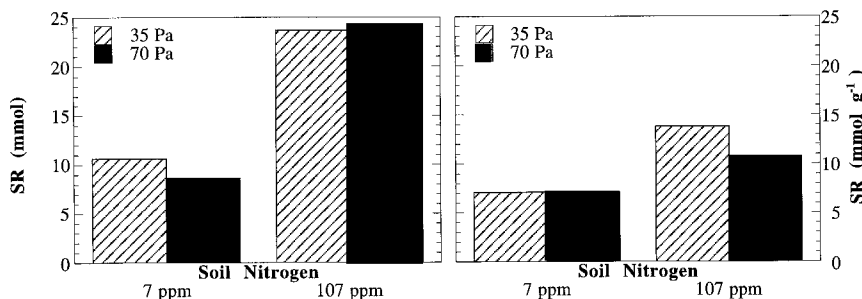


Figure 5. Modeled seasonal shoot respiration (SR, left) and mass-specific seasonal shoot respiration (SR_m, right) from ponderosa pine seedlings grown for 160 days in either 35 or 70 Pa CO₂ and 7 or 107 ppm N.

between the direct and indirect effects; i.e., the growth CO_2 partial pressure did not significantly alter the percent reduction in apparent respiration when C_e was increased from 35 to 70 Pa. However, at the end of the first growing season, the predicted reduction in apparent respiration from the R versus C_i curves did not closely match the observed steady-state rates. Steady-state apparent respiration rates at the growth CO_2 partial pressure of low-N seedlings were reduced less by elevated CO_2 than the predicted response from the R/C_i curves, whereas high-N seedlings had greater reductions than predicted. This suggests that the long-term reduction in CO_2 efflux resulting from growth in elevated CO_2 is not merely a sustained short-term response but is likely a response to a different mechanism(s). Reductions in the content and activity of key respiratory enzymes are possible mechanisms. For example, Van Oosten et al. (1992) found reductions in glucose-6-phosphate dehydrogenase when *Picea abies* (Bong.) Carr. seedlings were grown in elevated CO_2 , and Azcón-Bieto et al. (1994) found reduced cytochrome c oxidase activity in leaves of *Lindera benzoin* (L.) Blume and *Scirpus olneyi* grown in elevated CO_2 .

In addition to the control exerted by the use of respiratory products (demand), there is evidence of control through the supply of respiratory substrates, as exemplified by the positive relationship between nonstructural carbohydrates and respiration (Azcón-Bieto and Osmond 1983). Growth in elevated CO_2 is often associated with the accumulation of nonstructural carbohydrates (Wong 1990), providing a mechanism by which growth in elevated CO_2 could influence dark respiration rates.

Several studies have demonstrated the importance of data expression in the interpretation of indirect effects of CO_2 on apparent respiration (Poorter et al. 1992, Thomas et al. 1993, Thomas and Griffin 1994, Azcón-Bieto et al. 1994). For example, an accumulation of nonstructural carbohydrates can lead to an increase in the leaf weight/leaf area ratio (W , g m^{-2}), decreasing the specific respiration rate, but increasing area-based respiration rates (Thomas and Griffin 1994). Azcón-Bieto et al. (1994) report no change in W and found decreased respiration per unit carbon or nitrogen in *Scirpus olneyi* and *Lindera benzoin*. In our study, the units of data expression had little impact on the observed results. This may be due to the relative lack of accumulation of nonstructural carbohydrates in coniferous species compared with herbaceous species (Griffin, unpublished data). Our results are further complicated by the difficulty of measuring the relative growth rate (RGR) of individual seedlings over short time periods. The correlation between RGR and respiration is a well known positive relationship (Amthor 1989). In the present study, any differences in RGR among the treatment replicates are included in the overall treatment variance and confound the treatment effect.

Although we found no significant indirect effects of CO_2 on apparent respiration, or any effects of the treatments on the direct effects, both the direct and indirect effects were significantly correlated to leaf C/N ratio. In both cases there were only weak correlations between either C or N and the measured response. The C/N ratio may be a good predictor of respiration because it reflects not only growth and tissue qual-

ity, but also may represent a balance between the supply of respiratory substrates (C) and the demand for respiratory products (N).

Seedlings in the high-N treatments had between 1.18 and 12.2 times higher apparent respiration rates than seedlings in the low-N treatments. Increased N availability increases plant growth, protein accumulation and secondary metabolism, all factors that can increase respiration rates (Amthor 1989). The absence of interactions between the effects of N and CO_2 on apparent respiration suggests that N independently alters plant metabolism in a way that leaves the CO_2 response intact, and may indicate that the effects of CO_2 are not mediated through known pathways of respiratory control.

As a first step in exploring how our results might scale through time, we modeled the cumulative effect of the small changes in CO_2 efflux for the duration of a single growing season for individual ponderosa pine seedlings. We found that, despite a 49% increase in total plant biomass, high-N plants grown in elevated CO_2 lost only 2% more carbon during the cumulative dark period than low-N plants grown in 35 Pa CO_2 . Under low-N conditions, the effect of elevated CO_2 on total CO_2 efflux was dominated by effects on total plant mass. Total plant mass was slightly, but not significantly, decreased in low-N + high- CO_2 -grown seedlings compared to low-N + low- CO_2 -grown seedlings, and thus the total CO_2 efflux was slightly less.

Growth in elevated CO_2 often leads to changes in biomass allocation, and the total CO_2 efflux predicted by our model is therefore a function of several factors, including decreased respiration, increased biomass accumulation and altered allocation (i.e., decreased shoot fraction (shoot mass/total mass)). A change in any one of these parameters would lead to altered seasonal shoot respiration. We found that reduced apparent respiration alone would account for a 12% decrease in total seasonal CO_2 efflux of the high-N + high- CO_2 -grown seedlings. The difference between the 12% decrease and the 2% calculated increase is therefore the product of the increased biomass and the decreased shoot fraction. That is, CO_2 -induced differences in plant size alone would account for a 16% increase in carbon efflux from high-N plants.

Although our model is based on a limited data set and contains several simplifying assumptions (i.e., constant CO_2 efflux during the night and the absence of interaction between respiration and growth and maintenance processes), it emphasizes the relative treatment effects, as well as the relative influence, of the three parameters, total biomass, shoot fraction and apparent respiration rate. Furthermore, the model demonstrates that very small decreases in the instantaneous rate of CO_2 efflux can scale to substantial changes in carbon fluxes over the duration of a growing season. If these carbon savings are allocated to the bole wood of relatively long-lived forest trees, they represent a significant increment in the residence time of carbon in the terrestrial biosphere. We conclude that plant apparent respiration is a significant parameter of the global carbon cycle and is sensitive to environmental changes.

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