

Effect of measurement CO₂ concentration on sugar maple root respiration

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Summary Accurate estimates of root respiration are crucial to predicting belowground C cycling in forest ecosystems. Inhibition of respiration has been reported as a short-term response of plant tissue to elevated measurement [CO₂]. We sought to determine if measurement [CO₂] affected root respiration in samples from mature sugar maple (*Acer saccharum* Marsh.) forests and to assess possible errors associated with root respiration measurements made at [CO₂]s lower than that typical of the soil atmosphere. Root respiration was measured as both CO₂ production and O₂ consumption on excised fine roots (≤ 1.0 mm) at [CO₂]s ranging from 350 to $> 20,000 \mu\text{l l}^{-1}$. Root respiration was significantly affected by the [CO₂] at which measurements were made for both CO₂ production and O₂ consumption. Root respiration was most sensitive to [CO₂] near and below normal soil concentrations ($< 1500 \mu\text{l l}^{-1}$). Respiration rates changed little at [CO₂]s above $3000 \mu\text{l l}^{-1}$ and were essentially constant above $6000 \mu\text{l l}^{-1}$ CO₂. These findings call into question estimates of root respiration made at or near atmospheric [CO₂], suggesting that they overestimate actual rates in the soil. Our results indicate that sugar maple root respiration at atmospheric [CO₂] ($350 \mu\text{l l}^{-1}$) is about 139% of that at soil [CO₂]. Although the causal mechanism remains unknown, the increase in root respiration at low measurement [CO₂] is significant and should be accounted for when estimating or modeling root respiration. Until the direct effect of [CO₂] on root respiration is fully understood, we recommend making measurements at a [CO₂] representative of, or higher than, soil [CO₂]. In all cases, the [CO₂] at which measurements are made and the [CO₂] typical of the soil atmosphere should be reported.

Keywords: *Acer saccharum*, CO₂ production, direct effect of CO₂, oxygen consumption, root respiration.

Introduction

Plant respiration is an important component of terrestrial C cycles (Landsberg 1986, Running and Gower 1991, Ryan 1991, Vogt 1991). In forest ecosystems, root respiration can represent 30 to 50% of total belowground C allocation (Ryan et al. 1996) and contributes from 30 to 60% of total soil CO₂ efflux (Edwards and Harris 1977, Ewel et al. 1987, Bowden

et al. 1993, Haynes and Gower 1995, Ryan et al. 1996). Therefore, accurate estimates of root respiration are crucial to predicting responses of forest C cycling and productivity to climate change or elevated [CO₂].

Inhibition of respiration has been reported as both a long-term and short-term response of plant tissue to elevated [CO₂] (Amthor 1991). Altered respiration following long-term exposure to elevated [CO₂] can result from changes in growth rate, growth efficiency or tissue chemical composition (Amthor 1991, Bunce and Caulfield 1991, Wullschleger et al. 1992, 1994), and such changes may have important impacts on C cycling in a future, higher-[CO₂] world. Of more immediate concern, however, may be the short-term reversible effects of elevated [CO₂], in which reductions in measured tissue respiration occur as the [CO₂] at which measurements are made is increased (Gale 1982, Gifford et al. 1985, Bunce 1990, Amthor et al. 1992). Such effects have recently been reported for roots of Douglas-fir seedlings (Qi et al. 1994). Whether this CO₂ effect also occurs for roots from mature trees and how universal it is across tree species is unknown. Nevertheless, these findings call into question estimates of belowground C allocation that are based in part on root respiration measured at or near atmospheric [CO₂] ($350 \mu\text{l l}^{-1}$), which is much lower than that typical of the soil environment.

We determined if measurement [CO₂] inhibited the respiration of fine roots (≤ 1.0 mm) from mature sugar maple forests. Objectives were: (1) to determine the degree, if any, to which sugar maple root respiration declines as [CO₂] is increased; (2) to determine the [CO₂] range over which the effect occurs; (3) to determine if the effect is similar for both CO₂ production and O₂ consumption; and (4) to assess possible errors associated with root respiration measurements made at atmospheric [CO₂].

Materials and methods

Study sites and experimental design

To investigate the effects of measurement [CO₂] on fine root respiration, a series of experiments examining CO₂ production and O₂ consumption was conducted. Fine root samples used in the experiments were collected from four sugar maple forests

in Michigan (Table 1). The forests are second-growth northern hardwoods, approximately 85 years in age, dominated by sugar maple and occurring on sandy, well-drained Spodosols (Burton et al. 1991). Six 30 × 30 m study plots are located at each site (Zogg et al. 1996). Samples for root respiration measurements were collected from 10-m-wide buffer strips surrounding each plot. For all experiments, soil cores (10 cm deep, 5.4 cm diameter) were collected from three random locations in each buffer. The cores were transported to nearby field laboratories (less than 1 h travel time per site), and composited on a plot basis. All fine (≤ 1.0 mm), non-woody, live roots were sorted from each core and rinsed free of soil and organic matter with deionized water. Live roots were distinguished by white, cream, tan or brown coloration and a smooth appearance. Dead roots were dark brown or black in color, were brittle, and had frayed, rough edges. Root samples consisted primarily of intact small root mats consisting of many attached root segments. These small root mats typically contained two to five root orders and had total root lengths of < 50 to > 500 mm (V. Lessard, Michigan Technological University, Houghton, MI, unpublished data). Care was taken to minimize damage to the roots, and fine roots were severed only when necessary for separation from coarse roots. Excess water was blotted from the root samples and 0.5 g (fresh weight) subsamples were wrapped in moistened tissue paper and used for respiration measurements. All respiration measurements reported in this paper were made at 24 °C and were completed within 3 h of sample collection. Following respiration measurements, root subsamples were oven-dried (65 °C, 24 h) for determination of dry weights.

Respiration as CO₂ production

The effect of measurement [CO₂] on fine root CO₂ production was investigated by: (1) measuring CO₂ production in an open

Table 1. Climatic and overstory characteristics of four sugar maple forests in Michigan, USA. Overstory data are for the year 1995.

	Site A	Site B	Site C	Site D
Latitude (N)	46°52'	45°33'	44°23'	43°40'
Longitude (W)	88°53'	84°51'	85°50'	86°09'
Mean annual precipitation ¹ (mm)	870	830	810	850
Mean annual temperature ¹ (°C)	4.2	5.2	5.8	7.6
Total basal area (m ² ha ⁻¹)	34	31	32	33
Sugar maple basal area (%)	86	86	83	75
Overstory age	88	82	83	87

¹ Thirty-year means from National Oceanic and Atmospheric Administration (1983) records.

system using input [CO₂]s of 350, 700, 1000 and 2000 $\mu\text{l l}^{-1}$; and (2) monitoring CO₂ production over time in a closed respiration system as [CO₂] increased from near atmospheric to $> 5,000 \mu\text{l l}^{-1}$. Carbon dioxide concentrations in these experiments were measured with an infrared gas analyzer (IRGA; CIRAS-I portable gas analyzer, PP Systems, Haverhill, MA).

Measurement of CO₂ production at [CO₂]s of 350, 700, 1000, and 2000 $\mu\text{l l}^{-1}$ was performed in September 1995 using samples from all plots at each site. The IRGA was operated in an open-system configuration and programmed to provide the desired input [CO₂] to the sample chamber. At each concentration, respiration was allowed to achieve steady state before rates were recorded (approximately 20 min). For all samples, the 350 and 2000 $\mu\text{l l}^{-1}$ concentrations were the first two tested. The order in which these two concentrations were used was alternated from one sample to the next in order to assess reversibility of any observed CO₂ effect. Appropriate sample temperature was achieved by maintaining laboratory temperature at 24 °C.

The highest input [CO₂] that the IRGA could be programmed to provide was 2,000 $\mu\text{l l}^{-1}$. The effects on CO₂ production of measurement [CO₂] greater than 2,000 $\mu\text{l l}^{-1}$ were determined by monitoring CO₂ production in a closed system as [CO₂] increased from < 1000 to $> 5,000 \mu\text{l l}^{-1}$. This experiment was conducted at Site B in May of 1995. Respiration subsamples were placed in a water-jacketed cuvette in a closed loop configuration with the IRGA. Samples were allowed to equilibrate to cuvette temperature (24 °C) for 20 min, after which the system was closed and respiration was monitored for 50 to 60 min. Carbon dioxide concentrations were recorded every 5 min, allowing the effects of [CO₂] from < 1000 to $> 5,000 \mu\text{l l}^{-1}$ to be determined. Initial [CO₂] was typically around 700 $\mu\text{l l}^{-1}$. This was a result of room [CO₂] of about 500 $\mu\text{l l}^{-1}$ in the field laboratory and respiration that occurred between the time the system was closed and the initial reading was recorded.

Respiration as O₂ consumption

The effect of measurement [CO₂] on fine root O₂ consumption was investigated by: (1) monitoring O₂ consumption over time in a closed respiration system as [CO₂] increased from < 1000 to $> 20,000 \mu\text{l l}^{-1}$, and (2) measuring O₂ consumption in a closed system at mean [CO₂]s of 14,000, 29,000 and 44,000 $\mu\text{l l}^{-1}$. Oxygen consumption was measured with temperature-controlled O₂ electrode cuvettes (Model LD 2/2 oxygen electrode, Hansatech Instruments Ltd., Norfolk, England) connected to constant temperature circulating water baths (Burton et al. 1996, Zogg et al. 1996).

In September 1995, respiration as O₂ consumption was measured in a closed cuvette as [CO₂] increased from < 1000 to $> 20,000 \mu\text{l l}^{-1}$. This was done for all plots at all sites. Temperature in the field laboratories was maintained at 24 °C to minimize the time needed for samples to equilibrate to measurement temperature. After root samples were placed in the cuvette, they were allowed to equilibrate for 5 min. The cuvettes were then purged with air and closed, and root respi-

ration was followed for 50 to 60 min, during which cuvette $[\text{CO}_2]$ was allowed to increase, with O_2 concentrations recorded every five minutes. Initial $[\text{CO}_2]$ concentration in the cuvettes was between 700 and 1000 $\mu\text{l l}^{-1}$ because of injection of laboratory air at approximately 500 $\mu\text{l l}^{-1}$ CO_2 , and respiration that occurred between the injection of the air and taking of the initial reading. The increase in $[\text{CO}_2]$ during this brief period was calculated by multiplying measured reduction in $[\text{O}_2]$ concentration (from atmospheric) by a respiration quotient of 0.8 (A.J. Burton and G.P. Zogg, unpublished data). The $[\text{CO}_2]$ in the cuvette at the beginning of each subsequent 5-min interval was estimated by adding a value equal to 0.8 times the reduction in $[\text{O}_2]$ that occurred during the previous 5 min.

The experiment in which O_2 consumption was measured in a closed system at mean $[\text{CO}_2]$ s of 14,000, 29,000 and 44,000 $\mu\text{l l}^{-1}$ was conducted to investigate the effects of $[\text{CO}_2]$ concentration near or above the highest $[\text{CO}_2]$ that might occur in the soil (Amundson and Davidson 1990, Yavitt et al. 1995) or when using O_2 electrodes. The experiment was conducted at Site B in early October 1994, and repeated at Site B in May 1995. Fine root samples were placed in cuvettes that were then purged with ten cuvette volumes of certified gas standards containing 0, 15,000 or 30,000 $\mu\text{l l}^{-1}$ CO_2 and 21% O_2 . After purging, the cuvettes were immediately closed. Samples were allowed to equilibrate to temperature (24 °C) for 20 min after which respiration was monitored for 40 to 50 min. Carbon dioxide accumulation during the equilibration and measurement periods resulted in mean measurement $[\text{CO}_2]$ in the closed cuvettes of 14,000, 29,000, and 44,000 $\mu\text{l l}^{-1}$ for the three treatments (values are based on initial $[\text{CO}_2]$, measured O_2 consumption, and a respiration quotient of 0.8).

Statistical analyses

Analysis of variance (ANOVA) and repeated measures ANOVA (Wilkinson 1990) were used to compare root respiration rates recorded at different measurement $[\text{CO}_2]$ s. Where repeated measures ANOVA was used, the response functions for respiration were analyzed for linear, quadratic and higher order effects of $[\text{CO}_2]$ using orthogonal polynomials (Wilkinson 1990). In experiments where $[\text{CO}_2]$ was allowed to increase over time for individual samples, data points were assigned to CO_2 concentration classes prior to repeated measures ANOVA. The concentration classes used were every 500 $\mu\text{l l}^{-1}$ for the experiment in which $[\text{CO}_2]$ was allowed to increase from < 1000 to > 5000 $\mu\text{l l}^{-1}$, and every 1000 $\mu\text{l l}^{-1}$ for the experiment in which $[\text{CO}_2]$ in the oxygen electrode cuvette was allowed to increase from < 1000 to > 20,000 $\mu\text{l l}^{-1}$. The reversibility of the CO_2 effect was tested by a *t*-test in which the mean ratio of respiration at 350 $\mu\text{l l}^{-1}$ CO_2 to that at 2000 $\mu\text{l l}^{-1}$ was compared between samples that received the 350 $\mu\text{l l}^{-1}$ concentration first and those that received the 2000 $\mu\text{l l}^{-1}$ concentration first. To facilitate comparison of the CO_2 production and O_2 consumption data, results were also expressed as rates relative to the rate of respiration measured at 1000 $\mu\text{l l}^{-1}$ CO_2 . Linear and nonlinear regression (Wilkinson 1990) were used to examine the effect of $[\text{CO}_2]$ on respiration for the resulting combined data set.

Results

Root respiration rate measured as CO_2 production was significantly affected by the $[\text{CO}_2]$ at which measurements were made (Figures 1 and 2). Respiration rate measured at 350 $\mu\text{l l}^{-1}$ CO_2 was about 1.2 times the rate measured at 1000 $\mu\text{l l}^{-1}$ and 1.6 times greater than the rate measured at 2000 $\mu\text{l l}^{-1}$ (Figure 1). As $[\text{CO}_2]$ was further increased, the decline in respiration rate was less noticeable, with rates changing little above 3000 $\mu\text{l l}^{-1}$ (Figure 2). The effect of CO_2 on respiration rate was the same with increasing and decreasing $[\text{CO}_2]$. The ratio of respiration rate at 350 $\mu\text{l l}^{-1}$ to that at 2000 $\mu\text{l l}^{-1}$ was 1.57 with increasing $[\text{CO}_2]$ and 1.60 with decreasing $[\text{CO}_2]$ (no significant difference, $P = 0.74$).

Respiration rate measured as O_2 consumption also decreased with increasing measurement $[\text{CO}_2]$ (Figure 3). Respiration rates declined as CO_2 concentration increased to about 6000 $\mu\text{l l}^{-1}$, with most of the decline in respiration rate occurring at $[\text{CO}_2]$ s ≤ 3000 $\mu\text{l l}^{-1}$. Above 6000 $\mu\text{l l}^{-1}$, oxygen consumption rate was essentially constant (Figure 3). Increases in $[\text{CO}_2]$ to very high values (up to 44,000 $\mu\text{l l}^{-1}$) did not further reduce O_2 consumption rates (Figure 4).

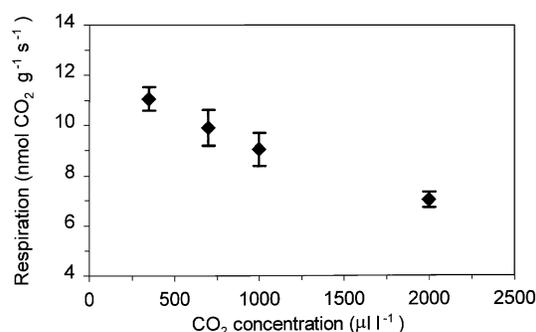


Figure 1. Respiration as CO_2 production at 350, 700, 1000 and 2000 $\mu\text{l l}^{-1}$ CO_2 (all sites, September 1995). Error bars indicate one standard error of the mean. The linear and quadratic effects of $[\text{CO}_2]$ are significant at $P < 0.01$ (repeated measures ANOVA).

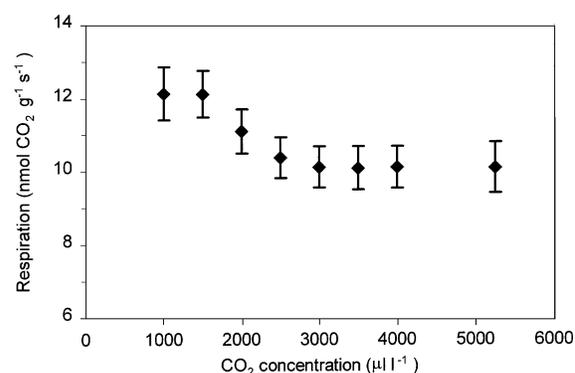


Figure 2. Respiration as CO_2 production at $[\text{CO}_2]$ s from 1000 to > 5000 $\mu\text{l l}^{-1}$ (Site B, May 1995). Error bars indicate one standard error of the mean. The linear, quadratic and cubic effects of $[\text{CO}_2]$ are significant at $P < 0.01$ (repeated measures ANOVA).

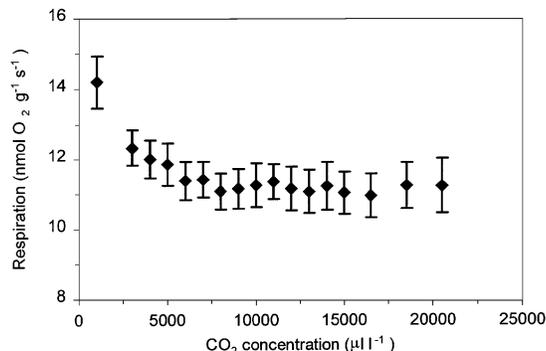


Figure 3. Respiration as O_2 consumption at $[CO_2]$ s from 1,000 to > 20,000 $\mu l l^{-1}$ (all sites, September 1995). Error bars indicate one standard error of the mean. The linear, quadratic and cubic effects of $[CO_2]$ are significant at $P < 0.01$ (repeated measures ANOVA).

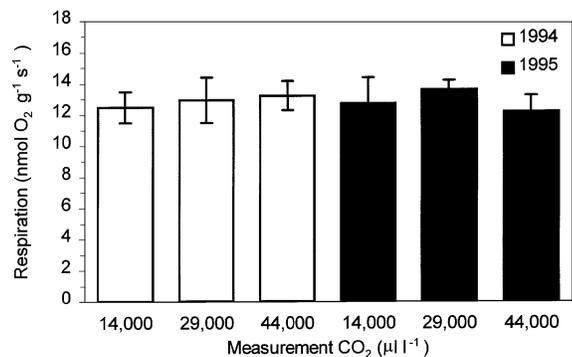


Figure 4. Mean root respiration at average measurement cuvette $[CO_2]$ s of 14,000, 29,000 and 44,000 $\mu l l^{-1}$ (Site B). Error bars indicate one standard error of the mean. Respiration means within years are not significantly different ($P = 0.89$ in 1994; $P = 0.67$ in 1995).

To compare the effects of measurement $[CO_2]$ on rates of O_2 consumption and CO_2 production directly, we expressed the observed respiration rates as percentages relative to rates at 1000 $\mu l l^{-1}$ (Figure 5). Rates of O_2 consumption and CO_2 evolution at 5000 $\mu l l^{-1}$ were both 83% of those observed at 1000 $\mu l l^{-1}$. As CO_2 concentrations were further increased to > 20,000 $\mu l l^{-1}$, O_2 consumption rate declined to approximately 79% of the rate observed at 1000 $\mu l l^{-1}$. The data shown in Figure 5 can be modeled by the relationship:

$$R_{1000} = 78.5 + (17714/[CO_2]), r^2 = 0.89, \quad (1)$$

where R_{1000} is respiration rate relative to that at 1000 $\mu l l^{-1}$, and $[CO_2]$ is measurement concentration in $\mu l l^{-1}$. Models with exponential functions had similar predictive ability to Equation 1 for the range of data shown in Figure 5, but Equation 1 was preferred because it predicts minimal additional decline in respiration at very high $[CO_2]$ (> 20,000 $\mu l l^{-1}$), in agreement with the results illustrated in Figure 4. Equation 1 can be rearranged to the following form which allows respiration rate

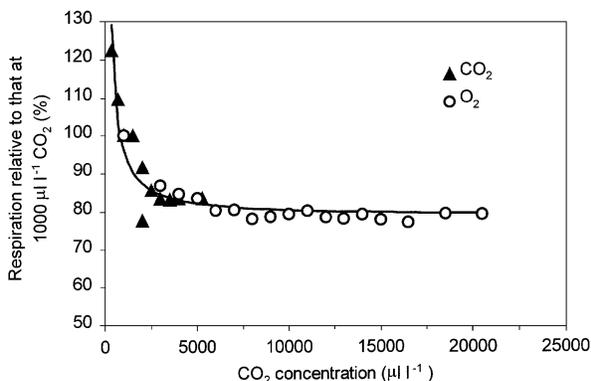


Figure 5. Oxygen consumption and CO_2 production expressed as rates relative to those occurring at a measurement $[CO_2]$ of 1000 $\mu l l^{-1}$. The line displayed is that represented by Equation 1.

(R_B) at a specified $[CO_2]$ to be estimated from the respiration rate (R_A) measured at a different $[CO_2]$:

$$R_B = \frac{R_A(0.785 + 177/[CO_2]_B)}{0.785 + 177/[CO_2]_A} \quad (2)$$

Discussion

The reduction in respiration rate that we observed at high $[CO_2]$ is an example of what Amthor (1991) termed a direct effect—one that results from $[CO_2]$ at the time of respiration measurement and is readily reversible. Such reductions have been reported previously for aboveground plant tissues (Reuveni and Gale 1985, Bunce 1990, Amthor 1991, Amthor et al. 1992) and for roots (Reuveni and Gale 1985, Palta and Nobel 1989, Nobel 1990), including the roots of trees (Qi et al. 1994, Ryan et al. 1996). The degree of direct reduction in respiration rate at high $[CO_2]$ varies greatly among plants and tissues. Amthor et al. (1992) measured respiration rates in leaves of *Rumex crispus* L. at $[CO_2]$ s from 50 to 950 $\mu l l^{-1}$, and found that for every doubling of $[CO_2]$, respiration rate declined by 25 to 30%. Respiration rates at 350 $\mu l l^{-1}$ CO_2 in leaves and plant shoots have been reported to be 1.1 to 2.0 times those at 700 $\mu l l^{-1}$ (Bunce 1990, El-Kohen et al. 1991, cited by Bunce 1994, Wullschlegel et al. 1994). In contrast, Palta and Nobel (1989) found measurement $[CO_2]$ between 350 and 2000 $\mu l l^{-1}$ did not affect respiration in *Agave deserti* Engelm. Lvs. roots, but that respiration was significantly reduced at $[CO_2]$ s above 2000 $\mu l l^{-1}$ and completely inhibited at 20,000 $\mu l l^{-1}$. Pheloung and Barlow (1981) found respiration in wheat apices was insensitive to $[CO_2]$ s from 300 to 5000 $\mu l l^{-1}$. Such findings have led to speculation that organs such as roots might be adapted to the range of $[CO_2]$ that they normally experience and might be insensitive to $[CO_2]$ at or below normal concentrations (Amthor 1991, Amthor et al. 1992). Our findings and those of Qi et al. (1994) suggest that for tree species, the opposite is true—root respiration is most sensitive to $[CO_2]$ at and below normal soil values.

We have measured a median soil $[\text{CO}_2]$ value of $1200 \mu\text{l l}^{-1}$ at our sites (G.P. Zogg, unpublished data from soil gas samples taken with a needle and syringe at 5- and 10-cm depths periodically during the 1995 growing season), similar to the value of $1350 \mu\text{l l}^{-1}$ reported by Yavitt et al. (1995) for northern hardwoods in New York. We found much greater changes in respiration rate per unit change in $[\text{CO}_2]$ at concentrations below these values than above them (Figure 5). Above $3000 \mu\text{l l}^{-1}$, further increases in $[\text{CO}_2]$ resulted in very little change in respiration rate (Figure 5), suggesting it is probably better to view respiration as being artificially enhanced by low measurement $[\text{CO}_2]$ rather than inhibited by high $[\text{CO}_2]$.

Our results are for excised roots and it is possible that sample excision and cleaning affected respiration rates to some degree. It is doubtful that this had much impact on the observed $[\text{CO}_2]$ effect, because Qi et al. (1994) observed similar results for intact, undisturbed roots of Douglas-fir seedlings. In their experiment, very large increases in respiration rate occurred at $[\text{CO}_2]$ less than atmospheric and smaller changes in respiration rate occurred at $[\text{CO}_2]$ above $1500 \mu\text{l l}^{-1}$. These results are of concern because root respiration measurements are often made at or near the atmospheric $[\text{CO}_2]$ of approximately $350 \mu\text{l l}^{-1}$ (Lawrence and Oechel 1983, Cropper and Gholz 1991, Ryan et al. 1996), potentially leading to errors in reported values. Ryan et al. (1996) estimated *Pinus radiata* D. Don root respiration at soil $[\text{CO}_2]$ (about $1500 \mu\text{l l}^{-1}$) to be only 23% of that measured at $400 \mu\text{l l}^{-1}$. Our results suggest that sugar maple root respiration at soil $[\text{CO}_2]$ is about 72% of that at atmospheric $[\text{CO}_2]$ ($350 \mu\text{l l}^{-1}$). These estimates may oversimplify the real world situation, because both root biomass and soil $[\text{CO}_2]$ also vary with depth and time (Yavitt et al. 1995, Hendrick and Pregitzer 1996); however, it is clear that respiration rates made near atmospheric $[\text{CO}_2]$ overestimate actual field values.

The cause of lower plant tissue respiration rates at high $[\text{CO}_2]$ is not well understood, but several theories have been proposed. Amthor (1991) suggested that direct inhibition of respiration by high $[\text{CO}_2]$ might be caused by alteration of intracellular pH, inhibition of enzyme activity through the formation of carbamates, increased fixation of CO_2 , or reduced consumption of respiratory products possibly caused by modification of membrane function. Our data suggest that CO_2 fixation is not the cause. Increased fixation of CO_2 into organic acids at high $[\text{CO}_2]$ without a change in actual respiration would lead to a decrease in apparent CO_2 production, but this cannot explain the co-occurring decrease in O_2 consumption observed for our samples. Griffin et al. (1996) and Gifford et al. (1985) similarly found that rates of O_2 consumption and CO_2 production were both reduced by high $[\text{CO}_2]$ in leaves.

In our experiments, changes in $[\text{CO}_2]$ had a much greater influence on respiration at low $[\text{CO}_2]$ than at high $[\text{CO}_2]$ (Figure 5). This is expressed by the inverse relationship in Equation 1, and is consistent with the type of relationship that might occur if pH changes in an unbuffered system or simple reactions such as carbamate formation were the cause. However, plant cytoplasmic pH appears to be well regulated (Smith and Raven 1979), making it unlikely that CO_2 could affect

respiration through altered intracellular pH unless $[\text{CO}_2]$ changes were very large (Bown 1985). The pH reduction of 0.35 units observed by Nobel (1990) in macerated root tissue exposed to 2% CO_2 for 10 h appears to be consistent with this prediction. Nobel (1990) found little change in root tissue pH after 2 h of exposure to 2% CO_2 , thus his results do not explain the reductions in respiration associated with much smaller changes in $[\text{CO}_2]$ over relatively brief periods of time. In addition, the effects of high CO_2 on respiration have been observed in experiments in which pH was controlled (Amthor 1991).

Inhibition of enzyme activity and altered membrane function both remain as possible explanations for the observed effects of $[\text{CO}_2]$ on respiration. Gonzalez-Meler et al. (1996) suggest that inhibition of respiratory enzymes, especially cytochrome *c* oxidase, is the basis for respiratory inhibition by high $[\text{CO}_2]$. Lorimer (1983) has argued that $[\text{CO}_2]$ may regulate metabolism through carbamate formation (i.e., the reversible reaction between CO_2 and the amine group of an enzyme). For example, inhibited enzyme activity during the latter stages of oxidative decarboxylation could lead to a feedback mechanism in which respiration is reduced until cellular $[\text{CO}_2]$ is lowered by diffusion. Membrane functions such as transport and maintenance of ion gradients could be affected by $[\text{CO}_2]$ through interference with lipid function (localized CO_2 tension can make membranes "thinner") or through formation of carbamates with free amino groups of membrane proteins (Mitz 1979). Such changes could affect consumption of end products (e.g., ATP), reducing respiration through feedback control mechanisms (Amthor 1991).

Decreased activity of the alternative (cyanide-resistant) pathway provides another possible explanation for reduced respiration at high $[\text{CO}_2]$ (Amthor 1991, Qi et al. 1994). This non-phosphorylating pathway serves as a secondary pathway for excess respiratory substrate (Lambers 1980) and also may be important in the coarse control of carbohydrate metabolism (Day and Lambers 1983). When the alternative pathway is engaged, more carbohydrate is expended per unit ATP and respiratory rates are potentially higher. If alternative pathway activity is reduced at elevated $[\text{CO}_2]$, then reduced respiration could occur without a decrease in energy made available (Bunce 1990). Mechanisms through which $[\text{CO}_2]$ might influence electron flow between the two pathways have not been definitively determined, but enzyme inhibition resulting from altered intracellular pH or carbamate formation has been suggested (Palet et al. 1991). Decreased respiration in plant tissues grown at elevated $[\text{CO}_2]$ has been shown, in some cases, to be a result of decreased activity of the alternative pathway (Gifford et al. 1985). Such effects are a consequence of the $[\text{CO}_2]$ history of a plant and are termed indirect effects (Amthor 1991). The mechanisms responsible for these indirect effects are not necessarily the same as those responsible for direct effects associated with $[\text{CO}_2]$ at the time of respiration measurement (Amthor 1991). Qi et al. (1994) felt reduced alternative pathway activity was the most likely mechanism for the direct reduction in respiration they observed in Douglas-fir roots exposed to high $[\text{CO}_2]$. However, Reuveni et al. (1995)

found direct inhibition of respiration by high $[\text{CO}_2]$ in duckweed (*Lemna gibba* L.) fronds was due primarily to reduced activity of the cytochrome pathway and found no support for the hypothesis that the alternative pathway was specifically suppressed. We are not aware of any studies to date that have tested for decreased alternative pathway activity in association with direct reductions of root respiration by high measurement $[\text{CO}_2]$, but such experiments might prove illuminating.

Although the causal mechanism remains unknown, the significance of the reduction in root respiration as measurement $[\text{CO}_2]$ increases cannot be denied. Based on our results, we agree with the conclusion of Qi et al. (1994) that soil CO_2 should be accounted for when estimating respiration and that many earlier estimates of root respiration may require revision. The shape and magnitude of the $[\text{CO}_2]$ effect that we observed for excised sugar maple roots from mature forests generally appears similar to that reported by Qi et al. (1994) for intact root systems of Douglas-fir seedlings. How universal the effect is across all tree species remains to be seen. Until the direct effect of $[\text{CO}_2]$ on root respiration is fully understood, we recommend making measurements at $[\text{CO}_2]$ representative of or higher than soil $[\text{CO}_2]$ where possible. Infrared gas analyzers in which the input $[\text{CO}_2]$ can be pre-programmed provide one method of achieving this and show promise as a means of taking respiration measurements directly in the field. Such field measurements are needed to verify the applicability of results from controlled laboratory experiments to actual field conditions. Unfortunately, input CO_2 of such IRGAs is typically limited to less than $2000 \mu\text{l l}^{-1}$ at present, and, if an open system is used, the respiratory increase in $[\text{CO}_2]$ is often small relative to the concentration of the input gas. Also, gas flow across the sample can potentially lead to desiccation. Oxygen electrodes can operate at much higher $[\text{CO}_2]$ and require no gas flow across the sample, but the resulting data require conversion with an appropriate respiration quotient if they are to be used in constructing carbon budgets. For sugar maple, we have measured a respiration quotient of 0.8 (A.J. Burton and G.P. Zogg, unpublished data). In all cases, the use of CO_2 -free air for respiration measurement should be avoided, and both the $[\text{CO}_2]$ at which measurements were made and the $[\text{CO}_2]$ typical of the soil atmosphere of the species studied should be reported.

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