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Measurement carbon dioxide concentration does not affect root respiration of nine tree species in the field

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Summary Inhibition of respiration has been reported as a short-term response of tree roots to elevated measurement CO_2 concentration ($[CO_2]$), calling into question the validity of root respiration rates determined at CO_2 concentrations that differ from the soil $[CO_2]$ in the rooting zone. Our objectives were to validate previous observations of a direct CO_2 effect on root respiration in sugar maple (*Acer saccharum* Marsh.) and to determine if high $[CO_2]$ also inhibited root respiration in other tree species. Root respiration rates for nine common North American tree species were measured in the field at ambient soil temperature at both 350 and 1000 μl CO_2 l^{-1}. No evidence of direct inhibition of root respiration by elevated measurement $[CO_2]$ was found for any of the species tested. The ratio of respiration rates at 1000 and 350 μl CO_2 l^{-1} ranged from 0.97 to 1.07, and the 95% confidence intervals for this ratio included unity for all species tested. Tests of a respiration cuvette used in earlier experiments suggested that gas leakage from the cuvette/IRGA system created an apparent direct CO_2 effect on respiration of sugar maple roots when none actually existed. Small sample masses used in those experiments exacerbated the error. Careful attention to the possibility of gas leaks and the avoidance of small sample masses should produce data that will allow researchers to accurately assess whether direct effects of measurement $[CO_2]$ exist. Our findings of no direct CO_2 effect on respiration of roots of a wide variety of species suggest that such effects may be less common than previously thought for tree roots.

Keywords: Acer saccharum, direct effect of CO_2, Juniperus monosperma, Picea glauca, Pinus edulis, Pinus elliottii, Pinus resinosa, Populus balsamifera, Quercus alba, Quercus rubra.

Introduction

Direct effects of CO_2 concentration ($[CO_2]$) on respiration, in which the measured respiration rate changes as the $[CO_2]$ at which measurements are made is varied, have been reported for a variety of plant tissues (Gale 1982, Gifford et al. 1985, Bunce 1990, Amthor et al. 1992). Such effects have included inhibition of tree root respiration as measurement $[CO_2]$ is increased (Qi et al. 1994, Ryan et al. 1996, Burton et al. 1997, Clinton and Vose 1999, McDowell et al. 1999), calling into question the validity of root respiration rates determined at concentrations of CO_2 that differ from the soil $[CO_2]$ in the rooting zone. However, recent reports indicate that direct CO_2 effects on respiration do not occur for roots of some tree species (Bouma et al. 1997*a*) and that they may not occur at all for leaves of many tree species (Amthor 2000). There are several possible explanations for why a direct effect of $[CO_2]$ on root respiration has been observed in some studies and not others: (1) sensitivity of respiration to $[CO_2]$ would not be expected to occur in high pH soils where CO_2 exists primarily in the bicarbonate form (Bouma et al. 1997*a*); (2) sensitivity of respiration to $[CO_2]$ may differ among species because of differences among species in the relative contributions of the cytochrome and non-phosphorylating alternative respiration pathways (Bouma et al. 1997*b*); (3) there is tissue- and species-specific regulation of the sensitivity of respiratory enzymes to $[CO_2]$ (Bouma et al. 1997*b*); and (4) gas leakage from the respiration chamber or gas analyzer could create an apparent direct CO_2 effect when none exists (Gonzàlez-Meler and Siedow 1999, Amthor 2000). Because this last possibility explained Amthor's (2000) inability to reproduce previously reported direct CO_2 effects in plant leaves (Amthor et al. 1992, Amthor 1997), it needs to be carefully examined for other species before the true importance of direct CO_2 effects on plant tissue respiration can be assessed.

We previously reported that root respiration of sugar maple (*Acer saccharum* Marsh.) was at least 20% greater at a $[CO_2]$ of 350 μl l^{-1} than at 1000 μl l^{-1} (Burton et al. 1997). The samples used in that study were excised roots, analyzed in the laboratory within several hours of collection, using an infrared gas analyzer (IRGA) and standard leaf cuvette. From 1998 to 2000, we attempted to verify this effect of $[CO_2]$ on tree root respiration by means of measurements of root respiration conducted in the field immediately after root excision. Respiration rates for root samples of nine common tree species were determined at two concentrations of CO_2 (350 and 1000 μl l^{-1}) with an IRGA equipped with a cuvette designed specifically for measuring root respiration at ambient soil temperature.

Objectives were to confirm the existence of a direct CO_2 effect on respiration of sugar maple roots and to determine the degree to which such an effect existed for eight other common North American tree species.

Materials and methods

Forested sites used in the study included balsam poplar (*Populus balsamifera* L.) and white spruce (*Picea glauca* (Moench) Voss) stands at the Bonanza Creek Long Term Ecological Research (LTER) site in interior Alaska, sugar maple forests and a red pine (*Pinus resinosa* Ait.) plantation in Michigan, a mixed-hardwood forest at the Coweeta LTER in North Carolina, a white oak (*Quercus alba* L.) dominated forest in Georgia, a slash pine (*Pinus elliottii* Engelm.) plantation on the coastal plain in northeastern Florida, and a pinyon–juniper woodland at the Sevilleta LTER in New Mexico. In the pinyon–juniper woodland, root samples from individual pinyon pine (*Pinus edulis* Engelm.) and one-seeded juniper (*Juniperus monosperma* (Engelm.) Sarg.) trees were measured. Root samples from the mixed-hardwood forest in North Carolina were collected adjacent to red oak trees (*Quercus rubra* L.) and field observations of the morphological characteristics of the roots suggest that they were predominantly of that species. Respiration measurements were made at all sites during the summer (June, July or August) of 1998, with additional measurements made at the sugar maple and red pine sites in Michigan during the summers of 1999 and 2000.

Samples for respiration measurement consisted of excised fine roots (< 1 mm diameter) collected from the top 5 cm of organic matter and mineral soil at three to four locations within a 30 × 30-m area at a site, and composited. A trowel was used to overturn a small area (about 10 × 10 cm) of surface soil at each location from which root samples were collected. The roots were brushed free of loose soil and organic matter, but were not washed or rinsed. Root samples typically consisted of five to seven intact root mats, each comprising an intact network of root segments containing mainly first-, second- and third-order roots. Detailed examinations of similar root mats indicated that first-order roots contributed about 50% of the root length sampled and second-order roots contributed about 25% (Pregitzer et al. 2002). Immediately following collection, root samples, typically 2–3 g fresh weight, were placed in a root respiration cuvette attached to an IRGA (CIRAS-I portable gas analyzer, PP Systems, Haverhill, MA). The one-piece base of the aluminum root respiration cuvette was 5 cm in diameter, with an internal chamber for roots 5 cm in depth with a volume of 76 cm^3. Beneath the respiration chamber was a solid aluminum plug 12 cm in length. The entire 17-cm-long aluminum base was inserted in the soil, with only the upper 1 cm of the base and the cuvette top above the soil surface. This allowed roots inside the cuvette to be maintained at ambient soil temperature during the measurement period (verified by comparing temperature measured by a thermistor inside the cuvette, in contact with the root sample, to soil temperature adjacent to the cuvette). The root cuvette has only one circular gasket between the base and the top, and the gas inlet and outlet ports are located on the cuvette top. Thus any leakage to or from the cuvette during field root respiration measurements would be from air located just above the soil surface. The $[CO_2]$ just above the soil surface at the sites ranges from 350 to 450 $\mu l\ l^{-1}$, based on CO_2 concentrations recorded during soil respiration measurements at the sites (A.J. Burton, unpublished data).

The IRGA and cuvette were configured in an open system, with respiration rate determined by difference in the concentrations of CO_2 entering and leaving the cuvette. The $[CO_2]$ differential was typically around 40 $\mu l\ l^{-1}$ (range of 25 to 90 $\mu l\ l^{-1}$). The IRGA measured both the outgoing and incoming gas flow rates and adjusted calculated respiration rates for water vapor dilution. Steady respiration rates were achieved within 15 to 20 min after placing a sample in the cuvette. The input $[CO_2]$ for the cuvette was maintained at either 350 or 1000 $\mu l\ l^{-1}$ for this period. After the measurement was completed, the sample was removed and placed in a polyethylene bag so that it would not desiccate between measurements. The input $[CO_2]$ was then adjusted to the concentration not used during the first analysis (either 350 or 1000 $\mu l\ l^{-1}$). The IRGA and cuvette were allowed to equilibrate to the new $[CO_2]$ for about 8 min, after which time the IRGA was adjusted to zero and balanced. The root sample was then returned to the cuvette for measurement of respiration at the new $[CO_2]$ concentration. Root respiration rates were measured first at 350 $\mu l\ l^{-1}$ for 39% of the samples. For the remaining samples, respiration rates were first measured at 1000 $\mu l\ l^{-1}$ and then at 350 $\mu l\ l^{-1}$.

The root cuvette was tested while empty during every period of operation to ensure that proper zero readings were occurring and to avoid possible data errors that would arise from gas leaks. For samples run at a measurement $[CO_2]$ of 1000 $\mu l\ l^{-1}$, leaks, when present, appear as negative respiration rates for the empty cuvette. In the one case where a major differential occurred while running the cuvette empty, an improperly seated gasket was discovered and corrected. Minor differentials (< 2 $\mu l\ l^{-1}$ CO_2) occurred for about one of every 10 samples measured, and we recorded their values to facilitate corrections to the resulting data. In no case were these corrections more than a small fraction of the recorded respiration rates (< 0.2 nmol $g^{-1}\ s^{-1}$ out of > 4.0 nmol $g^{-1}\ s^{-1}$).

Following respiration measurements, root samples were placed in coolers, or frozen, until they could be returned to the laboratory for cleaning of any adhering debris and determination of root dry mass. The adhering soil and organic debris removed in the laboratory accounted for less than 5% of the field sample mass in all cases. Microbial respiration in the adhering soil and organic debris would have been measured as root respiration, but rates of microbial respiration per gram of forest soil material (Zak et al. 1999) are often orders of magnitude less than those we measured per gram of root tissue. Thus, the contribution of these materials to measured

root respiration rates should be much less than 5% of the reported values.

The effect of measurement [CO_2] on root respiration rates was determined by repeated measures analysis of variance, with [CO_2] as a within subject effect. Tree species was included as a categorical variable in the analysis only to determine if there were differences among species in the existence of a possible direct CO_2 effect on respiration, as indicated by a [CO_2] × species interaction. Because ambient soil temperatures differed among sites (from 7 to 26 °C), the between subjects "species" effect also includes the effect of differences among sites in measurement temperature. We did not test statistically for the effects of temperature on root respiration in this data set because it was not relevant in determining if a direct CO_2 effect exists and if there were differences among species in the CO_2 effect.

During May 2000, we tested the possibility that gas leakage from the respiration chamber created some or all of the direct CO_2 effect that we had previously observed in IRGA measurements of sugar maple root respiration. This was done by determining apparent root respiration in two empty cuvettes across a range of CO_2 concentrations from 100 to 2000 μl l^{-1}. The cuvettes tested were the leaf cuvette (PLC broadleaf cuvette, PP Systems, Haverhill, MA) that we had used previously to measure sugar maple root respiration at concentrations of CO_2 ranging from 350 to 2000 μl l^{-1} (Burton et al. 1997) and the root cuvette used in the present study. The leaf cuvette is small: 18 mm in diameter, with a volume of about 3 cm^3 into which samples are placed and a total cuvette volume, including mixing chamber and fan, of about 10 cm^3. The leaf cuvette can only accommodate samples of about 0.1 g dry mass. The root cuvette can accommodate much larger samples; in practice we typically use samples of about 1 g dry mass in this cuvette. The apparent respiration of empty cuvettes at the various CO_2 concentrations was calculated based on these typical sample masses. The laboratory [CO_2] external to the cuvettes during these tests ranged from 380 to 420 μl l^{-1}.

Results

We found no evidence of direct inhibition of root respiration by elevated measurement [CO_2] for any of the species tested (Table 1, Figure 1). Respiration rates at 350 μl l^{-1} (R_{350}) and 1000 μl l^{-1} (R_{1000}) were nearly identical, as indicated by mean values close to 1.0 for the ratio R_{1000}/R_{350} (Table 2). The 95% confidence intervals for R_{1000}/R_{350} of all species for which we made multiple measurements included unity.

Tests of the empty cuvettes showed positive apparent respiration at measurement CO_2 concentrations less than atmospheric and negative apparent respiration at CO_2 concentrations greater than atmospheric (Figures 2 and 3). This is consistent with small amounts of leakage from the cuvette/IRGA system and would create an apparent direct CO_2 effect for samples analyzed in the leaf cuvette. In the empty leaf cuvette, the difference in apparent respiration rates at 350 and 1000 μl l^{-1} was 2.0 nmol CO_2 g^{-1} s^{-1}, which represents a significant proportion of the root respiration rates typically measured (cf. Figure 1). For the empty root cuvette, the potential errors associated with leakage were much less. The difference in apparent respiration rates at 350 and 1000 μl l^{-1} was only 0.17 nmol CO_2 g^{-1} s^{-1}, much smaller than the respiration rates typically measured for tree roots.

Table 1. Repeated measures analysis of variance table for root respiration rates measured at 350 and 1000 μl CO_2 l^{-1} for roots of nine North American tree species. Note that the species effect also includes the effects of differences among the sites in measurement temperature.

Source	df	SS	MS	F	$P > F$
Between subjects					
Species	8	147.2	18.4	2.74	0.034
Error	19	127.8	6.7		
Within subject					
[CO_2]	1	0.002	0.002	0.006	0.942
[CO_2] × Species	8	0.659	0.082	0.262	0.971
Error	19	5.979	0.315		

Discussion

Where gas leaks occur, they will always result in an apparent inhibition of respiration rates at higher measurement CO_2 concentrations, regardless of the [CO_2] external to the measurement cuvette. If gas leakage occurred during our field measurements of root respiration, an external [CO_2] less than 350 μl l^{-1} would cause a smaller decrease in CO_2 differential for the 350 μl l^{-1} treatment than for the 1000 μl l^{-1} treatment; an external [CO_2] greater than 1000 μl l^{-1} would cause a greater increase in the CO_2 differential for the 350 μl l^{-1} treatment than for the 1000 μl l^{-1} treatment; and an external [CO_2] between 350 and 1000 μl l^{-1} would cause an increase in the CO_2 differential for the 350 μl l^{-1} treatment and a decrease in

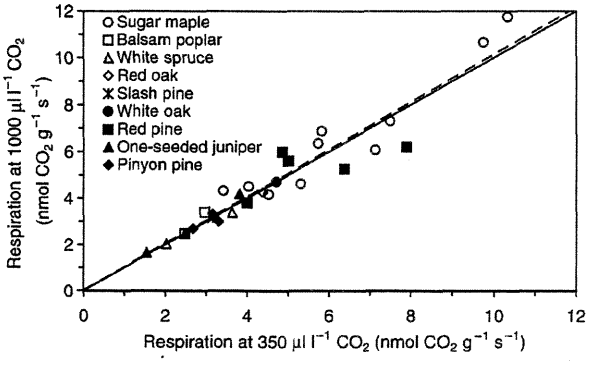

Figure 1. Root respiration at 1000 μl l^{-1} CO_2 versus root respiration at 350 μl l^{-1} CO_2 for nine tree species. The solid line indicates a 1:1 relationship. The dashed line represents regression results for the actual data ($R_{1000} = 1.016R_{350}$) and does not differ significantly from the 1:1 line.

Table 2. Ratios of respiration rates measured at [CO_2] of 1000 μl l^{-1} (R_{1000}) and 350 μl l^{-1} (R_{350}) for roots of North American tree species.

Tree species	Location	R_{1000}/R_{350}	n	SE
Balsam poplar	Alaska	1.07	2	0.07
White spruce	Alaska	0.97	2	0.03
Sugar maple	Michigan	1.05	10	0.04
Red pine	Michigan	0.98	5	0.09
Red oak	North Carolina	0.97	1	ND[1]
White oak	Georgia	1.00	1	ND
Slash pine	Florida	1.00	1	ND
Pinyon pine	New Mexico	0.99	3	0.04
One-seeded juniper	New Mexico	1.06	3	0.03
All samples		1.02	28	0.02

[1] ND = Not determined because $n = 1$.

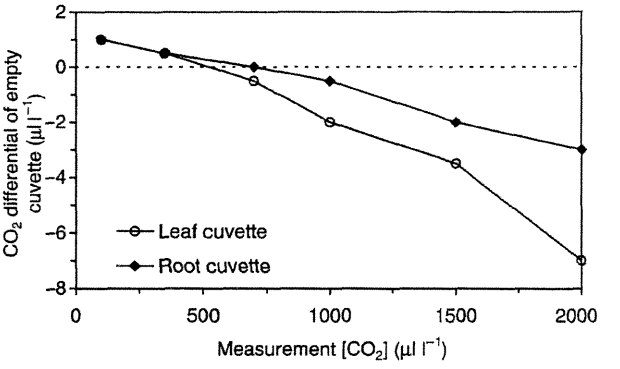

Figure 3. Carbon dioxide concentration differentials in empty cuvettes over the [CO_2] range of 100 to 2000 μl l^{-1}. The gas flow rate during the test was 150 ml min^{-1} for each cuvette.

CO_2 differential for the 1000 μl l^{-1} treatment. In all cases, the CO_2 differential for the 350 μl l^{-1} treatment would increase relative to that for the 1000 μl l^{-1} treatment, resulting in apparent inhibition of respiration at 1000 μl l^{-1} relative to that at 350 μl l^{-1}. If true inhibition were occurring at high measurement [CO_2], then gas leakage would enhance the degree of apparent inhibition. We did not find evidence of inhibition during our field measurements with the root cuvette, indicating that no real inhibition occurred for the species investigated. Any leakage that did occur from the cuvette (see Figure 3) produced changes in [CO_2] that were too small relative to the CO_2 differential created by respiration from the root sample to affect our results.

The finding of no direct effect of measurement [CO_2] on root respiration rates contradicts our previous finding that respiration rates of sugar maple roots were 20% greater at a measurement [CO_2] of 350 than at 1000 μl l^{-1} (Burton et al. 1997). The apparent CO_2 effect associated with gas leakage in the leaf cuvette/IRGA system (Figure 2) is sufficient to account for this difference. In our previous work we also measured slight

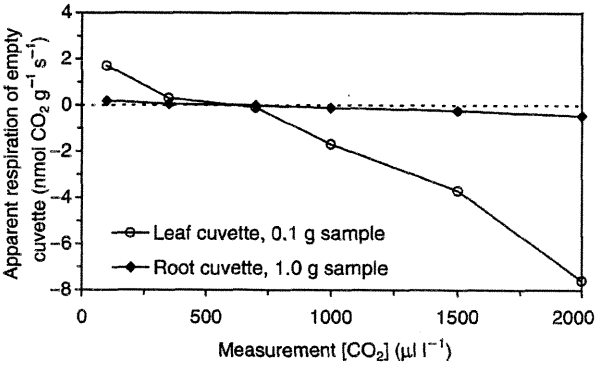

Figure 2. Apparent root respiration in empty cuvettes over the [CO_2] range of 100 to 2000 μl l^{-1}. Root respiration rates were calculated for hypothetical sample sizes of 0.1 g dry mass for the leaf cuvette and 1.0 g dry mass for the root cuvette. These sample masses are representative of those typically measured in each cuvette type.

declines in respiration, measured as O_2 consumption, as measurement [CO_2] increased from 1000 to > 20,000 μl l^{-1} in a sealed O_2 electrode system. This decline in respiration rate occurred over a period of up to 60 min. We had originally concluded that the cause of the decline in O_2 uptake rates over time was probably a CO_2 effect, because the data fit well with the observed CO_2 effect measured at lower [CO_2] (< 2000 μl l^{-1}) using the leaf cuvette/IRGA system (Figure 5 in Burton et al. 1997). However, because the direct effect observed using the leaf cuvette/IRGA system appears to be the result of gas leaks, we must reconsider whether the slight declines over time in the sealed O_2 electrode system were attributable to increases in [CO_2] inside the chamber or to other causes. The slight declines in respiration, as O_2 uptake, could simply be a consequence of decreasing metabolic activity as the time since root excision increased. Also, if the O_2 electrode system used in the previous study were not perfectly sealed, gas leaks could contribute to the slight apparent CO_2 effect observed. As root O_2 uptake drew down the chamber's internal [O_2], the rate of leakage of atmospheric O_2 into the cuvette would increase over time, lessening the observed respiration rate. However, calibration measurements during the experiment, made with sealed, empty cuvettes and a 15,000 μl l^{-1} O_2 standard gas, indicated no leakage was occurring.

The much greater apparent direct effect of measurement [CO_2] associated with gas leakage for the leaf cuvette than for the root cuvette is due in part to less leakage from the root cuvette/IRGA configuration (Figure 3) and in part to the much larger samples that the root cuvette can accommodate. The rates of apparent respiration in Figure 2 were based on the sample mass that was typically placed in the cuvettes: 0.1 g dry mass for the leaf cuvette and 1.0 g dry mass for the root cuvette. The larger samples used in the root cuvette typically created [CO_2] differentials near 40 μl l^{-1}, whereas the smaller samples used in the leaf cuvette never created [CO_2] differentials greater 10 μl l^{-1}, despite higher temperatures and respiration rates during those measurements (Burton et al. 1997). Small changes in [CO_2] differential as a result of leaks (Figure 3) would thus have a much greater effect on respiration

rates determined with the leaf cuvette than with the root cuvette.

Potential locations of minor leaks include gas tubing connectors, mixing fans and foam gaskets. The likelihood that minor leaks from such locations will significantly influence results increases as the cuvette volume decreases, because of an increase in the ratio of cuvette volume to gasket surface area and the small CO_2 differentials, which result from the use of small sample masses. It is essential to ensure that respiration chambers are well sealed, especially where roots or leaf petioles enter the chamber through gaskets. Amthor (2000) used vacuum grease to eliminate leaks occurring where large *Acer rubrum* L. leaf veins crossed chamber gaskets.

Testing empty cuvettes across a range of CO_2 concentrations and ensuring that empty cuvettes show no apparent respiration should help ensure data quality and allow researchers to determine if direct CO_2 effects are real or are artifacts associated with gas leaks. Although these procedures can readily be applied to most cuvette/IRGA systems, they are more difficult to apply to root box studies, which have shown large direct effects of measurement [CO_2] on root respiration of Douglas-fir (Qi et al. 1994) and western hemlock (McDowell et al. 1999). An alternative method to check for the effects of gas leaks on possible CO_2 effects is to change the [CO_2] in the atmosphere external to the experimental unit and then run a measurement CO_2 response curve. If gas leaks are creating a false CO_2 effect, then placing a root box/seedling unit (or IRGA cuvette with sample) in an enclosed area with higher ambient [CO_2] should cause respiration rates at each measurement [CO_2] to increase, whereas placing the entire system in a room with lower ambient [CO_2] should cause respiration rates at each measurement [CO_2] to decrease.

Our results and those of Amthor (2000) do not necessarily mean that direct effects of CO_2 on tissue respiration do not exist in some species, but they indicate that evidence for such effects should be carefully scrutinized. The search for a mechanism to explain direct effects of measurement [CO_2] on tissue respiration has yet to explain the large declines that have been observed at high [CO_2]. In one of the few cases where a possible mechanism has been confirmed, Gonzàlez-Meler et al. (1996) measured a direct inhibition of respiration by elevated [CO_2] in isolated plant mitochondria that was caused by inhibition of cytochrome *c* oxidase and succinate dehydrogenase activities. However Gonzàlez-Meler and Siedow (1999) estimated that inhibition of mitochondrial cytochrome *c* oxidase at high [CO_2], when scaled to the whole-tree level, would have a minimal effect and could not explain the amounts of CO_2 inhibition that have been reported at the tissue- and whole-plant level. They concluded that methodological errors, especially diffusive leaks in gas-exchange systems that are operated at low positive pressures, need to be taken into consideration. Careful attention to this possibility and the avoidance of small sample sizes should produce data that will allow researchers to accurately assess whether direct effects of measurement [CO_2] exist. Our findings of no direct CO_2 effect for respiration of roots of a wide variety of species suggest that such effects may be much less common than previously thought for tree roots.

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