The effect of aqueous transport of CO$_2$ in xylem sap on gas exchange in woody plants

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Summary The influence of CO$_2$ transported in the transpiration stream on measurements of leaf photosynthesis and stem respiration was investigated. Measurements were made on trees in a temperate forest in Scotland and in a tropical rain forest in Cameroon, and on shrubs in the Sahelian zone in Niger. A chamber was designed to measure the CO$_2$ partial pressure in the gas phase within the woody stems of trees. High CO$_2$ partial pressures were found, ranging from 3000 to 9200 Pa. Henry’s Law was used to estimate the CO$_2$ concentration of xylem sap, assuming that it was in equilibrium with the measured gas phase partial pressures. The transport of CO$_2$ in the xylem sap was calculated by multiplying sap CO$_2$ concentration by transpiration rate. The magnitude of aqueous transport in the studied species ranged from 0.03 to 0.35 $\mu$mol m$^{-2}$ s$^{-1}$, representing 0.5 to 7.1% of typical leaf photosynthetic rates. These values strongly depend on sap pH. To examine the influence of aqueous transport of CO$_2$ on stem gas exchange, we made simultaneous measurements of stem CO$_2$ efflux and sap flow on the same stem. After removing the effect of temperature, stem CO$_2$ efflux was positively related to sap flow. The apparent effect on measurements of stem respiration was up to 0.7 $\mu$mol m$^{-2}$ s$^{-1}$, representing ~12% of peak stem respiration rates.

Keywords: dissolved carbon dioxide, internal circulation, photosynthesis, refixation, respiration, sap flow.

Introduction

In physiological studies of gas exchange, both respiration and photosynthetic rates are inferred from measurements of the partial pressure of CO$_2$ in air ($p$CO$_2$) enclosed within a chamber. It is assumed that the rate of the biochemical process is equivalent to the CO$_2$ flux between the plant and the chamber air. However, it is possible that CO$_2$ produced by respiration is transported in the transpiration stream and consumed by photosynthesis in the leaves, representing an aqueous transport of CO$_2$. This would cause gas exchange measurements to underestimate photosynthesis. It is less clear how the aqueous transport of CO$_2$ would affect woody-tissue respiration measurements, as little is known about the distribution of CO$_2$ gradients within plant stems. However, there will generally be a vertical gradient in sap CO$_2$ concentration, as $p$CO$_2$ will be high in the air spaces within the soil and wood because of releases by microbial, root and stem respiration, whereas the intercellular $p$CO$_2$ in leaves is low, typically 25 Pa. An attempt to quantify aqueous transport of CO$_2$ was made by Hari et al. (1991) in Scots pine. They inferred sap CO$_2$ concentration from measurements of $p$CO$_2$ in the air in bore holes drilled in the tree. Samples were taken with a syringe through a rubber septum covering the hole and measured with an infra-red gas analyzer. Results ranged from 300 to 2000 Pa. The upper value was converted to an estimate of xylem sap CO$_2$ concentration, taken to be the concentration of all products of CO$_2$ dissolved in water, $[CO_2] = [CO_2]+[HCO_3^-]+[CO_3^{2-}]$, using Henry’s Law (Equation 2). This value was multiplied by a typical transpiration rate to estimate the magnitude of aqueous transport. The amount of CO$_2$ carried in the sap flow to the leaves was estimated to be equivalent to 2 to 9% of the photosynthetic rate. However, the air samples extracted were larger than the volume of the bore holes (20 cm$^3$ from a 15.3 cm$^2$ hole), creating a negative pressure that could induce a leak into the sample. Also, wound respiration at the cut surface of the hole may have influenced results. Unfortunately, few other estimates of $p$CO$_2$ within plant stems are available. Chase (1934, cited by Kramer and Kozlowski 1960) measured $p$CO$_2$ between 2000 and 25,000 Pa (2000 Pa = 20,000 $\mu$mol mol$^{-1}$ = 2%) within stems of poplar, oak, elm and pine. More recently, Raven and Farquhar (1989) measured $p$CO$_2$ of air in equilibrium with xylem exudate from Phaseolus vulgaris L. seedlings, and obtained mean values of 2180 and 3100 Pa. There is some evidence for an effect of sap flow on stem CO$_2$ efflux (Negisi 1981, Ryan 1990). Negisi (1979) measured CO$_2$ efflux on detached stems of young Pinus densiflora Siebold & Zucc. trees through which the flow rate of water was controlled using a pump. In the range 15 to 25 cm$^2$ h$^{-1}$, typical of the rates of sap flow in these trees on clear summer days, the CO$_2$ efflux rate was 80 to 40% of the rate at zero sap flow. Martin et al. (1994) found that stem CO$_2$ efflux rates in loblolly pine seedlings were reduced by a mean of 0.18 $\mu$mol m$^{-2}$ s$^{-1}$ during periods of high transpiration (1.18 mmol
m\(^{-2} \text{s}^{-1}\)) compared with periods of low transpiration (0.52 mmol m\(^{-2} \text{s}^{-1}\)).

In this study, we aimed to: (i) confirm previous measurements of high pCO\(_2\) within woody tissues using an improved methodology, and (ii) quantify the effect of aqueous transport of CO\(_2\) on actual measurements of leaf photosynthesis and stem respiration.

**Methods**

The concentration of CO\(_2\) in sap was measured using a method similar to that of Hari et al. (1991), but with a chamber designed to overcome the pressure and wounding problems. Measurements were carried out on trees in Scotland and in Cameroon, and thereby span a large environmental range.

Two small cylindrical chambers (80 mm high × 35 mm diameter) were constructed for the collection of samples of gas from the trees. These were sealed onto the tree surface and left until pCO\(_2\) inside the chamber had stopped increasing. The chambers were fitted with two outlets. To one outlet was attached a short piece of butyl rubber, which could be opened and sealed with an adjustable clip, through which samples could be removed by attaching a syringe (without a needle). The other outlet was fitted with a balloon, which in effect formed an impermeable lung within the chamber, with a mouth open to the atmosphere. This allowed atmospheric pressure to be maintained within the chamber even after removing several gas samples, as the balloon was deflated before attachment of the chamber to the tree and inflated slightly as each sample was withdrawn.

Chambers were sealed onto the tree using “Blue-tac” adhesive and elastics, and completely shaded by black cloth to prevent corticular photosynthesis (except in one case, where the chamber was left unshaded). The drilling of a bore hole was unnecessary. Chambers were left in position for up to three weeks and samples (2 to 5 cm diameter) were constructed for the collection of samples of gas from the trees. These were sealed onto the tree surface and left for at least 24 hours, and up to nine days.

Results

Figure 1 shows that the chamber pCO\(_2\) reached a value of around 3000 Pa within a day. The value was lower in the unshaded chamber, probably as a result of cortical photosynthesis. The value was temporarily higher when a hole was drilled in the wood inside the chamber, presumably as a result of wound respiration. In the measurements in Cameroon, the chamber was left for at least 24 hours, and up to nine days. There was no obvious trend in pCO\(_2\) with time after 24 hours. We conclude that values of pCO\(_2\) attained after 24 hours are reasonable estimates of values that occur naturally. Measured values of pCO\(_2\) were between 3000 and 9200 Pa, i.e., around 85 to 250 times ambient (Table 1). These values were used in Equation 2 to calculate [CO\(_2\)*]. The estimate of [CO\(_2\)*] is sensitive to the pH of the sap (Figure 2), and so other estimates

\[
\text{[CO}_2^*\text{]} = \text{pCO}_2 K_H(T) \left(1 + \frac{K_1(T)}{[\text{H}^+]^2} + \frac{K_1(T) K_2(T)}{[\text{H}^+]^3}\right),
\]

where [CO\(_2^*\)] is the concentration of all products of CO\(_2\) dissolved in water, i.e., [CO\(_2^*\)] = [CO\(_2\)aq] + [H\(_2\)CO\(_3\)] + [HCO\(_3\)] + [CO\(_3^2\)] ; pCO\(_2\) is the partial pressure of CO\(_2\) in air; \(K_H(T)\) is Henry’s constant for CO\(_2\); and \(K_1(T)\) and \(K_2(T)\) are dissociation constants for bicarbonate and carbonate ions.

Measurements were made on three silver birch trees (Betula pendula Roth.) on campus at the University of Edinburgh (55°55′ N, 3°12′ W) in September 1993, and on four Musanga ceropioioides Br. R. trees and four Distemonanthus benthamianus Baill. trees in the Mbalmayo Reserve (3°23′ N, 11°30′ E), Cameroon in February–May 1994. Musanga ceropioioides is a pioneer species, whereas D. benthamianus is a climax species. The pH of the xylem sap was estimated in two ways. In the field, pH paper (BDH Ltd., Lutterworth, U.K.; accuracy of ±0.2 pH) was pressed against freshly revealed sapwood. In the laboratory, wood samples, which had been taken using a drill, were made into a suspension with distilled water, and this was then measured with a pH meter (Jencons, Leighton Buzzard, U.K.; accuracy of ±0.02 pH). Wood surface temperatures were measured with a Cu-constantan thermocouple referenced to a data logger.

**Results**

Figure 1. Increase in chamber pCO\(_2\) with time in the experiment on B. pendula. A value of around 3000 Pa was reached after 24 hours. This equilibrium value was temporarily increased by wounding and lowered when the chamber was unshaded.
from the literature were obtained (Table 2). Taking the midpoint of the whole range in Table 2, (pH 6.4) and a conservative estimate of $p\text{CO}_2$ of 3000 Pa, Equation 2 gives a value of 0.054 mmol CO$_2$ mmol$^{-1}$ H$_2$O or 3.02 mmol CO$_2$ dm$^{-3}$ H$_2$O for [CO$_2^*$] at 10 °C.

**Discussion**

*Effect on leaf photosynthesis measurements*

Leaf photosynthesis measurements were made on the same trees as the [CO$_2^*$] estimates were made, or on similar trees nearby (Meir 1996, Rey and Jarvis 1998). The effect of aqueous transport on these measurements was calculated by multiplying sap [CO$_2^*$] by the transpiration rate (assumed to be equal to the rate at which water enters a leaf) and expressing this as a percentage of the photosynthetic rate of the leaf (Table 3). The calculated magnitude of aqueous transport in the species studied here ranged from 0.03 to 0.35 μmol m$^{-2}$ s$^{-1}$, at mean transpiration rates, representing 0.5 to 7.1% of mean leaf photosynthetic rates.

Estimates of xylem sap [CO$_2^*$] are also available from studies on parasitic mistletoe growing on tree hosts. Carbon in sap has a different isotopic signal from atmospheric carbon, which can be used to calculate how much carbon mistletoe gains heterotrophically from sap, and how much is assimilated in the normal way from the atmosphere. Xylem sap [CO$_2^*$] can then be calculated from the degree of heterotrophy and measured rates of photosynthesis and transpiration. These studies give values of 3 to 16 mmol CO$_2$ dm$^{-3}$ for eight Australian tree species (Marshall et al. 1994), and up to 22 mmol CO$_2$ dm$^{-3}$ in Juniperus osteosperma (Torr.) Little (Marshall and Ehleringer 1990) and 26 mmol CO$_2$ dm$^{-3}$ for Quercus rubra L. (van Die and Willlemse 1975, cited in Marshall and Ehleringer 1990).

The potential effect of aqueous transport on leaf photosynthesis measurements is shown graphically in Figure 3. As the estimation of [CO$_2^*$] is sensitive to pH, aqueous transport is plotted over the likely range of pH and $p\text{CO}_2$. No measurements of sap pH were made in the isotope studies, so only the range of values is indicated. The low end of this range is close to the lower values found in this study. The upper end of this range corresponds with the highest values found in this study when extrapolated to high pH. This suggests that the considerable variation in [CO$_2^*$] estimates is real, rather than arising from experimental error. Variation may be caused by differences in sap pH, which varies widely, as well as differences in respiration rate and diffusivity.

In the lower half of the pH range and with a $p\text{CO}_2$ of 3000 Pa, the effect is small in absolute terms, and will be less than 0.15 μmol m$^{-2}$ s$^{-1}$ at a typical transpiration rate of 3 mmol m$^{-2}$ s$^{-1}$. Note, however, that this is an error of several percent at a low photosynthetic rate (e.g., 3% at 5 μmol m$^{-2}$ s$^{-1}$). However, with pH of 6.4 to 7.2 and $p\text{CO}_2$ of 2000 to 9000 Pa,

![Figure 2](https://example.com/figure2.png) **Figure 2.** Effects of temperature and pH on [CO$_2^*$], the concentration of all products of CO$_2$ dissolved in water. The curves are given by Henry’s Law (Equation 2), assuming the water is in equilibrium with air with a CO$_2$ partial pressure of 3000 Pa.
Table 3. Aqueous transport rate (µmol m⁻² s⁻¹) of CO₂ based on the values of [CO₂⁎] in Table 1. Values of $A_1$ (µmol m⁻² s⁻¹) and $E$ (mmol m⁻² s⁻¹) are mean leaf photosynthesis rate and mean transpiration rate, respectively, from the studies of Meir (1996) and Rey and Jarvis (1998) (ambient treatment). The data in the last row are based on isotope studies on eight Australian tree species (Marshall et al. 1994). No measurements of pH or temperature were reported in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>$A_1$</th>
<th>$E$</th>
<th>pH</th>
<th>$T$ (°C)</th>
<th>Aqueous transport rate</th>
<th>Aqueous transport as % of $A_1$</th>
</tr>
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<tr>
<td>B. pendula</td>
<td>11.4</td>
<td>2.4</td>
<td>6.4</td>
<td>10.0</td>
<td>0.13</td>
<td>1.1</td>
</tr>
<tr>
<td>C. cecropiodes</td>
<td>4.9</td>
<td>1.7</td>
<td>6.8</td>
<td>25.9</td>
<td>0.35</td>
<td>7.1</td>
</tr>
<tr>
<td>D. benthamianus</td>
<td>6.2</td>
<td>1.5</td>
<td>5.2</td>
<td>25.2</td>
<td>0.03</td>
<td>0.5</td>
</tr>
<tr>
<td>Various</td>
<td>4.8</td>
<td>2.0</td>
<td>–</td>
<td>–</td>
<td>0.36</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Figure 3. Potential effect of aqueous transport on leaf photosynthesis measurements. Points represent means for the three species studied here. Curves represent theoretical values given by Equation 2 over the likely range of pH and $pCO_2$. Values are expressed as µmol CO₂ per mmol of water transpired, and so can be multiplied by a given leaf transpiration rate in mmol m⁻² s⁻¹ to give aqueous transport of CO₂ in µmol m⁻² s⁻¹. Dotted lines show the range found in isotope studies (Marshall and Ehleringer 1990, Marshall et al. 1994). No pH measurements were made in these studies.

The effect is in the range of 0.3 to 1.5 µmol m⁻² s⁻¹, and may represent a serious error. Indirect evidence for the importance of the aqueous transport of carbon comes from the studies of Vapaavouri and Pelkonen (1985) and Vuorinen et al. (1989). In their experiments on willow cuttings growing hydroponically, growth was increased by up to 31% by adding sodium bicarbonate (NaHCO₃) to the water bathing the roots. Experiments using radiocarbon (in the form of NaH¹⁴CO₃) strongly suggested that either the dissolved CO₂ or HCO₃⁻ in the transpiration stream was assimilated in photosynthesis.

Effect of sap flow on stem CO₂ flux

To examine the influence of sap flow on stem CO₂ efflux, we reanalyzed data from a previous experiment where both processes were measured simultaneously on the same stem. The experiment was carried out on the shrub Combretum micranthum G. Don at the tiger bush sub-site in the HAPEx-Sahel southern super-site (13°12' N, 2°14' E) (Goutorbe et al. 1997, Levy et al. 1997) in Niger. In situ measurements of CO₂ flux from a stem were made using an open gas-exchange system with a purpose-built chamber sealed onto the stem, approximately 1 m above ground (Levy and Jarvis 1998). A constant power sap flow gauge (Dynamax Inc., Houston, TX) (Baker and van Bavel 1987) was installed on the same stem, approximately 20 cm below the chamber. Further details are given by Allen and Grime (1995). In the raw data, stem CO₂ efflux, sap flow and stem temperature were correlated (Figure 4). The effect of temperature was quantified by fitting data to the exponential equation:

$$ R(T) = R_o \exp(kT), $$

where $R_o$ is the respiration rate at 0 °C, $k$ is a temperature coefficient and $T$ is temperature in °C. Equation 3 accounted for 89% of variation in the data. The influence of temperature was then removed from the data by calculating the residuals, $R'$:

$$ R' = R_{obs} - R(T). $$

Values of $R'$ were plotted against sap flow with a range of time lags. The highest correspondence was found when a time lag of 150 mins was applied to sap flow data, which is close to the estimated value of ~160 mins for the time taken for xylem sap to reach the chamber from the soil surface (sap flow velocities were ca. 0.1 mm s⁻¹ at a flow rate of 200 g h⁻¹).

An increase in $R'$ with sap flow can be seen in Figure 4b. The magnitude of the effect was up to ~0.7 µmol m⁻² s⁻¹ at 300 g h⁻¹, or 12% of peak efflux rates. As well as the effect of sap flow, the values of $R'$ contain all the experimental error and variation in respiration arising from other sources, which may be why the relationship is not clearer. Because of the time lag in these data, this influence is attributed to high values of [CO₂⁎] in sap arising from the roots. As the transpiration stream moves upwards, the water from the roots, in equilibrium with a high $pCO_2$, is drawn into the stem, where $pCO_2$ is much lower. This results in dissolved CO₂ coming out of solution and being released as a gas inside the stem, and an efflux of CO₂ from the stem to the air.
These results are in contrast to those from previous experiments that reported a reduction in stem CO$_2$ efflux with sap flow. The difference may be related to the conditions in which the experiments were done. In Negisi's (1979) experiment, the water that passed through the stem was initially in equilibrium with laboratory air and relatively low in dissolved CO$_2$, producing a diffusion gradient within the stem opposite to that expected in natural conditions. Martin et al. (1994) used potted seedlings, in which the build-up of CO$_2$ within the soil and stem may be much less. Also, their chamber was placed immediately below the crown, where CO$_2$ produced by stem respiration may move along a concentration gradient to the leaves.

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References


