Nocturnal stomatal conductance and implications for modelling $^{18}$O of leaf-respired CO$_2$ in temperate tree species


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Abstract. Variation in the oxygen isotope composition of within-canopy CO$_2$ has potential to allow partitioning of the ecosystem respiratory flux into above- and below-ground components. Recent theoretical work has highlighted the sensitivity of the oxygen isotope composition of leaf-respired CO$_2$ ($\delta_{Rl}$) to nocturnal stomatal conductance. When the one-way flux model was tested on Ricinus communis L. large enrichments in $\delta_{Rl}$ were observed. However, most species for which the isotope flux partitioning technique has been or would be applied (i.e. temperate tree species) are much more conservative users of water than R. communis. So, high stomatal conductance and very high enrichment of $\delta_{Rl}$ observed may not be typical for temperate tree species. Using existing gas-exchange measurements on six temperate tree species, we demonstrate significant water loss through stomata for all species (i.e. statistically significantly greater than cuticular loss alone) at some time for some leaves during the night. $\delta_{Rl}$ values predicted by the one-way flux model revealed that $\delta_{Rl}$ might be very much more enriched than when the net flux alone is considered, particularly close to sunrise and sunset. Incorporation of the one-way flux model into ecosystem respiration partitioning studies will affect model outputs and interpretation of variation in the oxygen isotope composition of atmospheric CO$_2$.

Keywords: leaf respiration, nocturnal stomatal conductance, Quercus rubra, stable oxygen isotope ratio.

Introduction

Variation in the oxygen isotope composition of atmospheric CO$_2$ has been suggested to provide a valuable constraint to carbon cycle models at both global (Francey and Tans 1987; Farquhar et al., 1993; Ciais et al. 1997) and ecosystem scales (Yakir and Wang 1996; Bowling et al. 2003a; b; Ometto et al. 2005). Both respiration and photosynthesis affect the atmospheric isotope composition. CO$_2$ respired by an ecosystem reflects isotopic exchange between oxygen in CO$_2$ and in pools of water, and these pools of water may differ isotopically from each other. For example, leaf water is often significantly more enriched in $^{18}$O than soil water, so that CO$_2$ respired by leaves is expected to be more enriched than soil-respired CO$_2$ (Flanagan et al. 1997; 1999).

Cernusak et al. (2004) recently presented a model describing environmental and physiological influences on the oxygen isotope composition of leaf-respired CO$_2$ ($\delta_{Rl}$), with consideration of the one-way fluxes of CO$_2$ into and out of the leaf. Testing their model with leaves in controlled gas-exchange chambers highlighted the importance of CO$_2$ that diffuses into the leaf, exchanges with leaf water (so reflects leaf water $^{18}$O enrichment), then diffuses out of the leaf, without being involved in the net flux of CO$_2$ from the leaf. This treatment of the one-way fluxes is analogous to the CO$_2$ invasion effect in soils described by Tans (1998; also see Miller et al. 1999; Stern et al. 2001), and to the original treatment of isotopic effects during CO$_2$ assimilation (Farquhar et al. 1993). It explains highly
Table 1. List of mathematical symbols used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value / unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_1$</td>
<td>Discrimination against C\textsuperscript{18}OO during diffusion through stomata</td>
<td>8.8‰</td>
</tr>
<tr>
<td>$a_2$</td>
<td>Discrimination against C\textsuperscript{18}OO during diffusion through the leaf boundary layer</td>
<td>5.8‰</td>
</tr>
<tr>
<td>$a_3$</td>
<td>Summed discriminations against C\textsuperscript{18}OO during liquid phase diffusion and dissolution</td>
<td>0.8‰</td>
</tr>
<tr>
<td>$\bar{a}$</td>
<td>Weighted mean discrimination against C\textsuperscript{18}OO for diffusion from the chloroplast to the atmosphere</td>
<td>%</td>
</tr>
<tr>
<td>$C$</td>
<td>Molar density of water</td>
<td>$55.5 \times 10^3$ mol m\textsuperscript{-3}</td>
</tr>
<tr>
<td>$C_a$</td>
<td>Mole fraction of CO\textsubscript{2} in the atmosphere</td>
<td>µmol mol\textsuperscript{-1}</td>
</tr>
<tr>
<td>$C_c$</td>
<td>Mole fraction of CO\textsubscript{2} in the chloroplast</td>
<td>µmol mol\textsuperscript{-1}</td>
</tr>
<tr>
<td>$C_i$</td>
<td>Mole fraction of CO\textsubscript{2} in leaf intercellular spaces</td>
<td>µmol mol\textsuperscript{-1}</td>
</tr>
<tr>
<td>$C_s$</td>
<td>Mole fraction of CO\textsubscript{2} at the leaf surface</td>
<td>µmol mol\textsuperscript{-1}</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusivity of H\textsubscript{2}\textsuperscript{18}O in water</td>
<td>$2.66 \times 10^{-9}$ m\textsuperscript{2} s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$\delta_a$</td>
<td>$\delta$\textsuperscript{18}O of atmospheric CO\textsubscript{2}</td>
<td>‰</td>
</tr>
<tr>
<td>$\delta_c$</td>
<td>$\delta$\textsuperscript{18}O of chloroplastic CO\textsubscript{2}</td>
<td>‰</td>
</tr>
<tr>
<td>$\delta_{C0}$</td>
<td>$\delta$\textsuperscript{18}O of CO\textsubscript{2} in the chloroplast that has not equilibrated with chloroplast water</td>
<td>‰</td>
</tr>
<tr>
<td>$\delta_{e}$</td>
<td>$\delta$\textsuperscript{18}O of water at the evaporating site in the leaf</td>
<td>‰</td>
</tr>
<tr>
<td>$\delta_{es}$</td>
<td>$\delta$\textsuperscript{18}O of water at the evaporating site in the leaf, at isotopic steady-state</td>
<td>‰</td>
</tr>
<tr>
<td>$\delta_{e0}$</td>
<td>$\delta$\textsuperscript{18}O of ecosystem-respired CO\textsubscript{2}</td>
<td>‰</td>
</tr>
<tr>
<td>$\delta_{ls}$</td>
<td>$\delta$\textsuperscript{18}O of leaf-respired CO\textsubscript{2}</td>
<td>‰</td>
</tr>
<tr>
<td>$\delta_{sl}$</td>
<td>$\delta$\textsuperscript{18}O of soil-respired CO\textsubscript{2}</td>
<td>‰</td>
</tr>
<tr>
<td>$\Delta$</td>
<td>$\delta$\textsuperscript{18}O enrichment at the sites of evaporation within the leaf, compared to source water</td>
<td>‰</td>
</tr>
<tr>
<td>$\Delta_{es}$</td>
<td>$\delta$\textsuperscript{18}O enrichment at the sites of evaporation within the leaf, compared to source water, at isotopic steady-state</td>
<td>‰</td>
</tr>
<tr>
<td>$\Delta_v$</td>
<td>$\delta$\textsuperscript{18}O enrichment of vapour in the atmosphere, compared to source water</td>
<td>‰</td>
</tr>
<tr>
<td>$e(T_l)$</td>
<td>Saturation vapour pressure at leaf temperature</td>
<td>mmol m\textsuperscript{-2} s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$E$</td>
<td>Transpiration rate</td>
<td>mmol m\textsuperscript{-2} s\textsuperscript{-1} or mol m\textsuperscript{-2} s\textsuperscript{-1} in Eqn 1</td>
</tr>
<tr>
<td>$e^*$</td>
<td>Equilibrium $\delta$\textsuperscript{18}O fractionation between liquid water and vapour</td>
<td>‰</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Kinetic fractionation during diffusion of H\textsubscript{2}\textsuperscript{18}O from leaf intercellular air spaces to the atmosphere</td>
<td>‰</td>
</tr>
<tr>
<td>$\varepsilon_a$</td>
<td>Equilibrium $\delta$\textsuperscript{18}O fractionation between CO\textsubscript{2} and water</td>
<td>‰</td>
</tr>
<tr>
<td>$f$</td>
<td>Ratio of cuticular conductance to CO\textsubscript{2} and water</td>
<td>–</td>
</tr>
<tr>
<td>$f_{bl}$</td>
<td>Boundary layer conductance to water vapour</td>
<td>mol m\textsuperscript{-2} s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$f_{cw}$</td>
<td>Cuticular conductance to water vapour</td>
<td>mol m\textsuperscript{-2} s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$g_{cw}$</td>
<td>Stomatal and cuticular conductance to water vapour</td>
<td>mol m\textsuperscript{-2} s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$g_{sw}$</td>
<td>Stomatal conductance to water vapour</td>
<td>mol m\textsuperscript{-2} s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$g_{sw}'$</td>
<td>Stomatal conductance to water vapour</td>
<td>mol m\textsuperscript{-2} s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$g_{sw}$</td>
<td>Total conductance to water vapour</td>
<td>mol m\textsuperscript{-2} s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$K$</td>
<td>Ratio of the number of stomata on one side of the leaf to the number on the other side</td>
<td>–</td>
</tr>
<tr>
<td>$L$</td>
<td>The effective length over which the Péclet effect occurs</td>
<td>m</td>
</tr>
<tr>
<td>$P$</td>
<td>Total atmospheric pressure</td>
<td>Pa</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Péclet number</td>
<td>dimensionless</td>
</tr>
<tr>
<td>$s$</td>
<td>Leaf area within the chamber</td>
<td>m\textsuperscript{2}</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Standard deviation</td>
<td>–</td>
</tr>
<tr>
<td>$T_l$</td>
<td>Leaf temperature</td>
<td>K</td>
</tr>
<tr>
<td>$\Theta$</td>
<td>Proportion of chloroplast CO\textsubscript{2} isotopically equilibrated with chloroplast water</td>
<td>–</td>
</tr>
<tr>
<td>$w$</td>
<td>Chamber flow rate</td>
<td>mol s\textsuperscript{-1}</td>
</tr>
<tr>
<td>$W$</td>
<td>Leaf water content</td>
<td>mol m\textsuperscript{-1}</td>
</tr>
<tr>
<td>$w_c$</td>
<td>Water mole fraction of air leaving the chamber</td>
<td>mmol mol\textsuperscript{-1} and mol mol\textsuperscript{-1} in Eqn 1</td>
</tr>
<tr>
<td>$w_i$</td>
<td>Water mole fraction inside the leaf</td>
<td>mmol mol\textsuperscript{-1} and mol mol\textsuperscript{-1} in Eqn 2</td>
</tr>
<tr>
<td>$w_o$</td>
<td>Water mole fraction of air entering the chamber</td>
<td>mmol mol\textsuperscript{-1} and mol mol\textsuperscript{-1} in Eqn 1</td>
</tr>
</tbody>
</table>
enriched leaf-respired CO₂ observed under some conditions (Seib et al. 2005) that are not predicted by simpler models considering just the net respiratory flux (e.g. Flanagan et al. 1997, 1999; Bowling et al. 2003a, b). The one-way flux model presented by Cernusak et al. (2004) predicts that CO₂ respired by leaves should deviate from that predicted by a net flux model when the difference in isotopic composition of CO₂ in the chloroplast (δ₁₈O) and the ambient air (δ₀₂) is large, and when stomatal conductance is high. Both these conditions were met in the model evaluation experiment conducted by Cernusak et al. (2004), and large differences between δ₁₈O and δ₀₂ are expected in natural ecosystems if leaf water is more enriched than soil water. However, it is unclear whether high stomatal conductance is common in respiring leaves.

Stomata are thought to regulate water loss from leaves in relation to rates of photosynthetic carbon fixation and water supply (Cowan and Farquhar 1977), suggesting that non-CAM plants should close their stomata at night when there is no opportunity for carbon gain. However, there is increasing evidence of significant loss of water through stomata in the dark in many species from a range of environments (Muchow et al. 1980, Benuy 1999, Musselman and Munnick 2000; Gruulke et al. 2004). Recent work suggests that plants with inherently high daytime stomatal conductance tend to have high nocturnal stomatal conductance (gₑₑₑₑ) (Snyder et al. 2003), and that stomata respond to air saturation deficit in the dark (Bucci et al. 2004). However, stomatal conductance has been measured so infrequently in the dark (Bucci et al. 2003), and that stomata respond to air saturation deficit in the dark (Bucci et al. 2004). However, stomatal conductance has been measured so infrequently in the dark (Bucci et al. 2003), and that stomata respond to air saturation deficit in the dark (Bucci et al. 2004).

In this paper we address three questions for temperate tree species: (1) is stomatal conductance significant in the dark in a range of species; (2) what regulates nocturnal stomatal conductance; and (3) are significant differences in δ₁₈O of leaf-respired CO₂ predicted between the net flux and the one-way flux models at typical values of nocturnal stomatal conductances?

Materials and methods

CO₂ and water vapour exchange measurements

Measurements of the rate of exchange of CO₂ and water vapour between Quercus rubra L. leaves and the atmosphere were made during four 24-h periods in midsummer. Eight leaves spread over two Q. rubra trees at Black Rock Forest (New York, USA, latitude 41.4° N, longitude 74.0° W, 270 m above sea level) for each of three leaf classes (upper canopy leaves, Black Rock Forest has a 10–16-m-tall canopy of Q. rubra during the measurement period was 5.8 m² m⁻². Mean annual precipitation at the site is 1190 mm, and mean air temperature in July is 23.4 °C.

As the LI-6400 gas-exchange system was operating near detection limits during the night (i.e. CO₂ and water fluxes were very small), the chamber was carefully leak tested, and the infrared gas analysers matched between every third measurement. Leaves were equilibrated in the chamber for 5 min (i.e. the coefficient of variation for the CO₂ partial pressure difference between the sample and the reference analysers was below 1%, and leaves were assumed to be at steady-state) before three measurements were made at −5-s intervals. The averages of the three measurements of CO₂ and water vapour exchange were used for each leaf. Measurements of incident irradiance, air temperature, and relative humidity 2 m above the forest canopy were also made throughout the measurement period at 5-s intervals and stored on a datalogger as hourly averages.

Nighttime gₑₑₑₑ was also extracted from archived raw gas-exchange data from measurements made at night for several temperate forest species [the respiration rates from these measurements were published by Turnbull et al. (2001), Whitehead et al. (2002) and Griffin et al. (2004)]. These measurements were all made with a LI-6400 system as described above, and included 12 independent leak tests with an empty chamber, in which the average apparent gₑₑₑₑ was 0 ± 3.7 mmol m⁻² s⁻¹.

Determination of significant nocturnal stomatal conductance

Transpiration rate (E) of a leaf in an open system, such as an LI-6400, is given by the difference in water mole fractions between air entering and leaving the chamber (wₑₑₑₑ and wₑₑₑₑ, respectively), the flow rate of ambient air entering the chamber (wᵊ), and area of leaf within the chamber (a) by (von Caemmerer and Farquhar 1981; Li-Cor 6400 manual Eqn 1–4):

\[
E = \frac{a(wₑₑₑₑ - wₑₑₑₑ)}{a(wᵊ - wₑₑₑₑ)} \tag{1}
\]

Total conductance to water vapour through the stomata and the leaf boundary layer (gₑₑₑₑ) when stomata are present on both sides of the leaf is given by (von Caemmerer and Farquhar 1981; Li-Cor 6400 manual Eqn 1–7):

\[
gₑₑₑₑ = E \left(1 - \frac{wᵊ}{wₑₑₑₑ} \right) \tag{2}
\]

where wᵊ is the vapour mole fraction inside the leaf, and is calculated from leaf temperature (Tₑₑₑₑ) and total atmospheric pressure (Pₑₑₑₑ) by (Li-Cor 6400 manual Eqn 1–8):

\[
wᵊ = \frac{tₑₑₑₑ}{Pₑₑₑₑ} \tag{3}
\]

In Eqn 3 the function (Tₑₑₑₑ/Pₑₑₑₑ) is the saturation vapour pressure at leaf temperature. Boundary layer conductance to water vapour from one side of the leaf (gₑₑₑₑ) is estimated from the ratio of stomatal frequency on one side of the leaf to the other (gₛₛ) leaf area, and fan speed using the standard Li-Cor 6400 lookup table. Stomatal and cuticular conductance to water vapour (gₛₛ) is then given by (Li-Cor 6400 manual Eqs 1–9 and 1–10):

\[
gₛₛ = \frac{1}{gₑₑₑₑ} \frac{1}{\frac{K + 1}{K} - \frac{1}{K}} \tag{4}
\]

 Clearly, errors in wᵊ, wₑₑₑₑ, Tₑₑₑₑ, Pₑₑₑₑ, and fan speed could generate errors in gₑₑₑₑ, which may lead to incorrect conclusions regarding significance (or non-significance) of nocturnal stomatal conductance. It has been recognised for some time that accurate measurement of leaf temperature is imperative for E and gₑₑₑₑ calculation (e.g. Tyree and Wulff 1990). Assuming the junction of the leaf temperature...
thermocouple is correctly positioned against the leaf, the IRGAs have been calibrated and frequently matched, and the chamber is leak-free, measurement error can largely be attributed to instrument noise. To assess the effect of instrument noise on calculation of \( g_w \), we conducted a Monte Carlo analysis (10,000 individual calculations) on \( g_w \) estimated under average conditions for the \( Q \) \( \text{rubus} \) experiment and including typical instrument error, as outlined in the LI-6400 manual (Li-Cor Bioscience 2002). Table 2 summarises the average value and instrument noise associated with each measurement.

As expected, the coefficient of variation (cv) increased as the error in leaf temperature increased, and as \( w_i - w_o \) decreased (Fig. 1). The cv did not differ markedly as \( w_i \) varied, so long as air temperature influences on \( w_o \) were reflected in \( T_i \) (data not shown). A coefficient of variation as high as 37%, at \( w_i - w_o = 0.1 \text{ mmol m}^{-2} \text{s}^{-1} \) and \( T_i = 19^\circ \text{C} \), suggests that under these conditions calculated \( g_w \) should be interpreted with extreme caution. If correctly positioned, the leaf thermocouple should be expected to measure \( T_i \) within 0.1°C, and the coefficient of variation in \( g_w \) depends to a large extent on \( w_i - w_o \).

Cuticular conductance to water vapour (\( g_{cw} \)) is thought to be in the order of 0.07 mmol m\(^{-2}\) s\(^{-1}\) for stomatous cuticles and 0.05 mmol m\(^{-2}\) s\(^{-1}\) for astomatous cuticles (Hantrück et al. 2004). We determine significant water loss through stomata to occur when \( g_w \) minus one standard deviation (σ) is greater than 0.1 mmol m\(^{-2}\) s\(^{-1}\), where σ is estimated from Fig. 1 (\( cv = \sigma / \text{mean value} \)) at the measured \( w_i - w_o \).

Differences between air temperatures inside and outside the leaf cuvette are known to introduce large errors when calculating stomatal conductance (Tyree and Wulff 1998). Air temperature within the chamber when upper sunlit leaves were measured was consistently warmer than that measured by the sensor placed 2m above the forest canopy. This difference was as great as 10.5°C during warm periods, but averaged 6.6±0.3°C during day light and 3.7±0.1°C in the dark. The elevated temperature of within-chamber air with zero errors probably is due to the operation of pumps and fans within the photosynthesis system. By allowing leaves to equilibrate within the chamber for at least 5 min before taking measurements and waiting until the coefficient of variation of the difference in CO\(_2\) partial pressures between sample and reference analysers was below 1%, we assume that leaf temperature adjusted to the warmer air temperature within the chamber.

### Isotope theory

Cernusak et al. (2004) recently presented a model that considered both one-way fluxes of CO\(_2\) into and out of a leaf, and the possibility that CO\(_2\) respired by leaves may not be in complete equilibrium with leaf water. We call this the ‘one-way flux model’. The \( ^{18}\text{O} \) of leaf-respired CO\(_2\) (\( \delta_{\text{lw}} \)) may be described by (Cernusak et al. 2004)

\[
\delta_{\text{lw}} = \left( \frac{1 + \delta_{\text{ai}} + \delta_{\text{ei}}}{1 - \theta} - 1 \right) \left( \frac{1 + \delta_{\text{ai}} - \delta_{\text{ai}}}{1 - \theta} \right) - \bar{\delta}_{\text{lw}}.
\]

where \( \theta \) is the proportion of chloroplast CO\(_2\) that is isotopically equilibrated with chloroplast water, \( \delta_{\text{ai}} \) and \( \delta_{\text{ei}} \) are the \( ^{18}\text{O} \) of chloroplastic water, the \( ^{18}\text{O} \) of CO\(_2\) in the chloroplast that has not equilibrated with local water, and the \( ^{18}\text{O} \) of CO\(_2\) in the atmosphere, respectively. \( \delta_{\text{ai}} \) and \( \delta_{\text{ei}} \) are the CO\(_2\) mole fractions in the ambient atmosphere and in the chloroplasts, respectively, and \( \bar{\delta}_{\text{lw}} \) is the equilibrium \( ^{18}\text{O} \) fractionation between CO\(_2\) and water. The isotopic equilibrium between CO\(_2\) and water is dependent on temperature by (Brememkeijer et al. 1983):

\[
\epsilon_{\text{lw}} (\%o) = \left( \frac{17064}{T_i} - 1793 \right) + 10,000.
\]

where \( T_i \) is in K. In Eqn 5, \( \bar{\delta} \) is the weighted mean discrimination against C\(_{18}\)OO for diffusion from the chloroplast to the atmosphere, and is given by (Farquhar and Lloyd 1993):

\[
\bar{\delta} = \frac{\delta_{\text{gi}} - \delta_{\text{ai}} + \delta_{\text{ei}} - \delta_{\text{oi}}}{\epsilon_{\text{gi}} - \epsilon_{\text{ei}} - \epsilon_{\text{oi}}}
\]

where \( \epsilon_{\text{gi}} \) is the summed discriminations against C\(_{18}\)OO during liquid phase diffusion and dissolution (0.8‰), \( \epsilon_{\text{ei}} \) and \( \epsilon_{\text{oi}} \) are the discriminations against C\(_{18}\)OO during diffusion through the stomata and the boundary layer (8.8 and 5.8‰, respectively), and \( \epsilon_{\text{gi}} \) and \( \epsilon_{\text{ei}} \) are CO\(_2\) mole fractions in the leaf intercellular spaces and at the leaf surface, respectively.

### Table 2. Variables, default values and instrument measurement noise used in the Monte Carlo analysis to calculate errors in stomatal conductance

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Units</th>
<th>Default value</th>
<th>Instrument error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water vapour mole fraction entering the chamber</td>
<td>( w_i )</td>
<td>mmol m(^{-2}) s(^{-1})</td>
<td>17.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Water vapour mole fraction leaving the chamber</td>
<td>( u_i )</td>
<td>mmol m(^{-2}) s(^{-1})</td>
<td>17.4-18.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Flow rate through the chamber</td>
<td>( u )</td>
<td>mmol m(^{-2}) s(^{-1})</td>
<td>300</td>
<td>1</td>
</tr>
<tr>
<td>Leaf area</td>
<td>( A )</td>
<td>m(^{2})</td>
<td>0.0006</td>
<td>0.00005</td>
</tr>
<tr>
<td>Leaf temperature</td>
<td>( T_l )</td>
<td>°C</td>
<td>19</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>Total atmospheric pressure</td>
<td>( P_{tot} )</td>
<td>kPa</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Boundary layer conductance to water vapour</td>
<td>( g_{cw} )</td>
<td>mol m(^{-2}) s(^{-1})</td>
<td>2.84</td>
<td>0.10</td>
</tr>
</tbody>
</table>
of mitochondria between chloroplasts, and sometimes pressed against the cellular membrane (Griffith et al. 2004). Functional carbonic anhydrase-like proteins have been found in mitochondria (Parris et al. 2004), suggesting that some respiratory CO₂ may equilibrate with mitochondrial water before entering the intercellular air spaces. If mitochondria are immediately adjacent to chloroplasts, and at similar distances from evaporative sites, mitochondrial water is likely to have the same isotopic composition as chloroplastic water. Thus, CO₂ that has exchanged with mitochondrial water may be isotopically indistinguishable from CO₂ that has exchanged with leaves and ambient water vapour.

The oxygen isotopic composition of water at the evaporating sites of leaves and ambient water vapour are expressed relative to the source water (Farquhar et al. 1997). 813 The steady-state leaf water model (Eqn 8) is unlikely to give accurate predictions of δ₁₈O under field conditions, particularly at night (Flanagan and Ehleringer 1991; Harwood et al. 1998). A non-steady-state model has been developed (Fan and Cernusak 2005), and shows good agreement with measured leaf water δ₁₈O (Cernusak et al. 2002, 2005). The non-steady-state enrichment of water at the sites of evaporation (δe) is given by:

\[
\Delta_e = \Delta_0 - \frac{\omega}{\delta_{es}} \left( \frac{1 - \varepsilon^-}{} \right),
\]

where δ₀ = 1 + ε₀ = 1 + ε₁, ε is the leaf water content in mmol m⁻³, and the Péclet number, ρ, is the ratio of convective of uncharged water to the sites of evaporation to the backward diffusion of H₂¹⁸O in water (2.66 × 10⁻¹⁰ m² s⁻¹).

Prior to the publication of the one-way flux model describing δ₀, earlier researchers (e.g. Flanagan et al. 1997, 1999; Bowling et al. 2003) made several assumptions to apply a simplified version of Eqn 5. By assuming that δ = 1, stomata are tightly closed (i.e. C₃ / C₄ approaches zero), and that δ = δ₀, δ = 1 and δ₀ = 0 are equal to unity (these terms are –1.04 and 1.007), oxygen isotope ratio of leaf-respired CO₂ may be described simply as an isotopic equilibrium between oxygen atoms in CO₂ and in leaf chloroplastic water:

\[
\delta_{0} = \bar{\delta}_s + \varepsilon_0 - \bar{\delta}.
\]

We call this the "net flux model," as it does not consider the CO₂ that diffuses into the leaf, exchanges with water, and diffuses out again.

Even if all stomata are tightly closed, diffusion of CO₂ and water vapour still occurs through the cuticle. The ratio of ambient to chloroplastic CO₂ partial pressures, an important parameter in Eqns 5 and 7, is particularly sensitive to respiration rate when diffusion occurs only through the cuticle. If cuticular conductance to water vapour is 0.1 mmol m⁻² s⁻¹ (Santrück et al. 2004) and cuticular conductance to CO₂ is similar, then when the leaf respiration rate is 1 µmol m⁻² s⁻¹, C₃ / C₄ is 0.03 (assuming C₃ = 370 µmol mol⁻¹ and mesophyll conductance is 140 mmol m⁻² s⁻¹). But if the respiration rate is as high as 3 µmol m⁻² s⁻¹ then C₃ / C₄ is 0.01 in the example above.

Boyce et al. (1997) demonstrated that cuticular conductance to CO₂ is only ~6% of that for water vapour at low irradiance, despite the ratio of diffusivities of CO₂ and water vapour in air being 1.6 (Nobel 1983). This would further increase the CO₂ partial pressure inside the leaves when stomata are closed. To account for the possibility that cuticular conductance to CO₂ is lower than to water vapour in the one-way flux model of δ₀, conductance to CO₂ is calculated separately for stomatal and cuticular components. Values of gₛₑₗ₃ measured by the photosynthesis system are the result of both cuticular (gₛₑₗ₃) and stomatal conductances (gₛₑₗ₃) to water vapour. Stomatal conductance to water vapour is assumed to be zero when gₛₑₗ₃ < 0.1 mmol m⁻² s⁻¹. Conductance of CO₂ through the stomata and cuticle (gₛₑₗ₃) is then given by:

\[
gₛₑₗ₃ (\text{mmol m}⁻² \text{s}⁻¹) = 1.6 (gₛₑₗ₃ − 0.1) + gₛₑₗ₃.
\]

The fractionation factor for diffusion through air (and stomata) was recently determined to be 32% by Cappa et al. (2003) and, assuming a 2 / 3 power effect according to Pohlhausen analysis (Kay 1966), fractionation during diffusion through the boundary layer will be 21% (Cernusak et al. 2003). The steady-state leaf water model (Eqn 8) is unlikely to give accurate predictions of δ₁₈O under field conditions, particularly at night (Flanagan and Ehleringer 1991; Harwood et al. 1998). A non-steady-state model has been developed (Fan and Cernusak 2005), and shows good agreement with measured leaf water δ₁₈O (Cernusak et al. 2002, 2005). The non-steady-state enrichment of water at the sites of evaporation (δe) is given by:

\[
\Delta_e = \Delta_0 - \frac{\omega}{\delta_{es}} \left( \frac{1 - \varepsilon^-}{} \right),
\]

where δ₀ = 1 + ε₀ = 1 + ε₁, ε is the leaf water content in mmol m⁻³, and the Péclet number, ρ, is the ratio of convective of uncharged water to the sites of evaporation to the backward diffusion of H₂¹⁸O in water (2.66 × 10⁻¹⁰ m² s⁻¹).
where \( f \) is the ratio of cuticular conductances to CO\(_2\) and water vapour (either 0.06 or 1), \( g_{sw} = g_{sw}^{\infty} \) when \( g_{sw} < 0.1 \text{ mmol m}^{-2} \text{ s}^{-1} \), and \( g_{sw} = 0.1 \text{ mmol m}^{-2} \text{ s}^{-1} \) when \( g_{sw} > 0.1 \text{ mmol m}^{-2} \text{ s}^{-1} \). The factor of 1.6 describes the ratio of diffusivities of CO\(_2\) and water vapour through open stomata, i.e. when the stomatal pore area is greater than the mean free paths of the diffusing molecules. When pores are very narrow and interactions between molecules and the pore walls are more frequent than those between molecules, the diffusivities of H\(_2\)O and CO\(_2\) are thought to be equal (Farquhar and Lloyd 1993). However, it seems likely that rather than all stomata being very slightly open, a few stomata are unable to close. In this case the factor of 1.6 is more appropriate. The model predicting \( \delta^{18}O \) of leaf-respired CO\(_2\) is not particularly sensitive to diffusivities at very low \( g_{sw} \), so we assume the factor of 1.6 is appropriate for all measured values of \( g_{sw} \).

The leaf isoflux (in \( \text{ mmol m}^{-2} \text{ s}^{-1} \)) is calculated as \( \delta_{sw} \), multiplied by the leaf respiration rate. To scale the leaf isoflux to the canopy level for the \( Q. \ rubra \) measurements, isofluxes for each of the three leaf positional classes are multiplied by the leaf class LAI (leaf area per unit ground area for the individual class) and summed. This gives the total leaf isoflux in \( \text{ mmol m}^{-2} \text{ s}^{-1} \). LAI for each leaf class was estimated from measurements of relative foliage area density and fraction of sunlit foliage (Whitehead et al. 2004a, b), as 1.60, 2.28 and 1.91 \text{ m}^{2} \text{ m}^{-2} \) for upper sunlit, upper shaded and lower leaves, respectively.

An objective of the current paper is to assess the affect of the invasion term in Eqn 5 on \( \delta_{sw} \) under typical field conditions for a temperate broad-leaved tree species. If \( \theta = 1 \), and stomata are tightly closed, then \( C_1 / C_0 \) should approach zero and the net flux model (Eqn 14) should give similar values to the one-way flux model (Eqn 5).

We have assumed that \( \delta^{18}O \) of source water is \(-8.0 \text{‰}\), \( \delta_{sw} = -\varepsilon \) (about \(-9.5 \text{‰}\)), \( \delta_{sw} = 14.3 \text{‰} \), \( \delta_{sw} \), and \( \delta_{sw} \) (\(-32 \text{‰}\)), that mesophyll conductance to CO\(_2\) (needed to calculate \( C_1 \)) is \( 0.1 \text{ mmol m}^{-2} \text{ s}^{-1} \) (Ether and Livingston 2004), \( \theta = 17.7 \text{ mmol mol}^{-1} \) and \( L = 30 \text{ mm} \) (an average value for broad-leaved tree species, Barbour et al. 2004). The value for \( \delta_{sw} \) is taken from Cernusak et al. (2004), from controlled environment laboratory experiments, and \( \theta \) from the average water content of lupin leaves (Cernusak et al. 2002). Assuming a constant value for these parameters throughout the 4-d measurement period is unlikely to be realistic. Cernusak et al. (2004) found that respired CO\(_2\) was not in equilibrium with chloroplastic water in leaves respiring in the dark at 350 \text{ mmol mol}^{-1} \text{ CO}_2, rather that \( \theta = 0.79 \). To test the effects of incomplete equilibration of respired CO\(_2\), we also applied Eqn 5 with \( \theta = 0.79 \). A further assumption is that Eqns 5 and 14 apply when CO\(_2\) and water vapour exchange occur only through the cuticle, i.e. when stomata are closed and \( g_{sw} < 0.1 \text{ mmol m}^{-2} \text{ s}^{-1} \).

### Results

**Weather conditions during \( Q. \ rubra \) experiment**

Conditions were clear and sunny during the 4 d of continuous measurements of leaf CO\(_2\) and water vapour exchange. Both day and night air saturation deficit (\( D \)) declined during the measurement period, so that maximum \( D \) (19.5 mmol mol\(^{-1} \)) was recorded on the afternoon of the first day, and minimum \( D \) (1.0 mmol mol\(^{-1} \)) at sunrise on day five (Fig. 2). During a single 24-h period, \( D \) varied with air temperature.

**Stomatal conductance of \( Q. \ rubra \)**

During the day, upper sunlit leaves tended to have higher photosynthetic rates and higher stomatal conductance than either shaded upper leaves or leaves lower in the canopy. Stomatal conductance rapidly declined as net photosynthetic rate approached zero close to sunset (Fig. 3). Minimum values of conductance were 2.0, 3.0 and 0.2 mmol m\(^{-2} \text{ s}^{-1} \) for upper sunlit, upper shaded and lower leaves, respectively. The highest stomatal conductance recorded for respiring

![Fig. 2. Weather variables for the four days when Quercus rubra stomatal conductance measurements were made. Five days separate day four and day six. Values for irradiance are shown for the 400–700 nm range (Whitehead et al. 2004a).](image-url)
leaves (39.0 mmol m$^{-2}$ s$^{-1}$, when the respiration rate was 0.47 µmol m$^{-2}$ s$^{-1}$) was from lower leaves when irradiance above the canopy was 150 µmol m$^{-2}$ s$^{-1}$ (but little light reached the lower leaves: irradiance inside the leaf chamber was just 1 µmol m$^{-2}$ s$^{-1}$) as the sun set on the first day. Stomata closed completely each night only in the lower leaves. If cuticular conductance is assumed to be 0.1 mmol m$^{-2}$ s$^{-1}$, then stomata were open (i.e. $g_{sw} > 0.1$ mmol m$^{-2}$ s$^{-1}$, where $g_{sw}$ is estimated at the measured $w_{i} - w_{o}$ from error analysis described in the Materials and methods section) when leaves were respiring for 91, 97 and 48% of the night for sunlit upper leaves, shaded upper leaves and lower leaves, respectively.

No significant relationship was found between $g_{sw}$ and the saturation deficit of air entering the leaf chamber, suggesting that stomata did not respond to changes in $D$ during the night.

**Fig. 3.** Variation in leaf net CO$_2$ exchange and stomatal conductance ($g_{sw}$) over four 24-h periods for *Quercus rubra* leaves in three positions within the canopy. Sunlit and shaded upper canopy positions refer to exposure during daylight hours. CO$_2$ exchange values from Whitehead et al. (2004a).
night (Fig. 4). Highest stomatal conductances tended to occur near sunset or sunrise, when air temperature and \( D \) were either maximum (sunset) or minimum (sunrise). Further, no significant relationship was found between average midnight \( g_{sw} \) (an average between 2300 and 0100 h) and \( D \) at the same time (Fig. 5A). However, amongst lower and shaded upper leaves, average midnight \( g_{sw} \) was positively related to average midday \( g_{sw} \) (an average between 1100 and 1300 h) of the previous day \((P=0.014; \text{Fig} \ 5B)\).

### Fig. 4. Relationship between air saturation deficit (\( D \)) and stomatal conductance in the dark for Quercus rubra leaves at three positions within the canopy. Sunlit and shaded upper canopy positions refer to exposure during daylight hours.

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**Modelling \( \Delta_\lambda \) and \( \delta_{RL} \) for Q. rubra**

The non-steady-state model predicted leaf water enrichment above source water (\( \Delta_\lambda \)) between 12.3 and 24.6‰, tending to be more enriched during the afternoon and less enriched at night (Fig. 6). Leaf water was predicted to be less enriched and less variable in the lower leaves, due to lower and less variable \( D \). The ratio of cuticular conductance to CO\(_2\) and water did not significantly affect the \( \delta^{18}\text{O} \) of respired CO\(_2\) with modelled values always within 1% and usually within 0.3% when \( f \) was set at 1 or 0.06. Accordingly, we only present modelled values and interpretation when \( f=1 \). The one-way flux model predicted widely variable \( \delta_{RL} \) between 38 and 1310‰ and between 32 and 776‰ for \( \theta=1 \) and \( \theta=0.79 \), respectively. In contrast, the net flux approximation predicted relatively constant \( \delta^{18}\text{O} \) of respired CO\(_2\), between 42 and 47‰. Both the one-way flux model and the net flux simplification predicted generally lower \( \delta_{RL} \) for leaves lower in the canopy, compared to upper shaded and sunlit leaves. The one-way flux model predicted a slightly more enriched \( \delta_{RL} \) than the net flux model at low \( g_{sw} \) both when \( \theta=1 \) and when \( \theta=0.79 \).

Plotting the difference between values modelled by the one-way flux and net flux simplification against \( g_{sw} \) shows that the estimates of \( \delta_{RL} \) differ markedly when \( g_{sw} \) is greater than \( \sim5.0 \text{mmol m}^{-2} \text{s}^{-1} \) (Fig. 7). The difference in the modelled estimates was as great as 1265 and 731‰ when \( \theta=1 \) and \( \theta=0.79 \), respectively. Large differences typically occurred close to sunset and sunrise, and most often in lower and upper shaded leaves when net CO\(_2\) exchange changed from positive (photosynthesis) to negative (respiration) at sunset.

Leaf isofluxes varied from \(-6\) to \(390 \mu\text{mol m}^{-2} \text{s}^{-1} \)‰ and from \(-4\) to \(758 \mu\text{mol m}^{-2} \text{s}^{-1} \)‰ for the one-way flux model.

---

**Fig. 5. Relationships between average stomatal conductance at midnight and (A) average air saturation deficit at midnight, and (B) average stomatal conductance at midday the previous day for Quercus rubra leaves at three positions within the canopy. Sunlit and shaded upper canopy positions refer to exposure during daylight hours.**
Fig. 6. Modelled $^{18}$O enrichment at the sites of evaporation in leaves and modelled nocturnal $^{18}$O of leaf-respired CO$_2$ ($\delta_{RL}$) for Quercus rubra leaves at three positions within the canopy. Sunlit and shaded upper canopy positions refer to exposure during daylight hours. $\delta_{RL}$ is modelled using the net flux model (○), the one-way model assuming full equilibration (●) and the one-way model assuming incomplete equilibration (■) for each leaf class. Values that are off scale (6) have been excluded.

with $\theta = 1$ and $\theta = 0.79$, respectively, and from $-203$ to $113 \mu$mol m$^{-2}$ s$^{-1}$‰ for the net flux simplification (Fig. 8). Lower leaves had both lower $\delta_{RL}$ and lower respiration rates, so the leaf isofluxes were typically ranked: lower < upper shaded < upper sunlit. The canopy-level leaf isoflux varied from 584 to 1724 $\mu$mol m$^{-2}$ ground$^{-1}$ s$^{-1}$‰ and from 392 to 919 $\mu$mol m$^{-2}$ ground$^{-1}$ s$^{-1}$‰ for the one-way flux model with $\theta = 1$ and $\theta = 0.79$, respectively, and from $-415$ to 478 $\mu$mol m$^{-2}$ ground$^{-1}$ s$^{-1}$‰ for the net flux simplification. Values of the canopy-level leaf isoflux predicted by the net flux simplification were always lower than the one-way flux modelled values, both with full equilibration and with $\theta = 0.79$, as $\delta_{RL}$ predicted by the one-way flux models was nearly always more enriched for the three leaf
class. As expected from leaf δ18O values, the net flux and one-way flux models were most different around sunset and sunrise when stomatal conductances were highest.

Nocturnal stomatal conductance in other temperate tree species

Of the other temperate tree species assessed, significant stomatal conductance (i.e. \( g_{\text{sw}} \)) varied between 20 and 85% as average \( g_{\text{sw}} \) increased from 4.0 to 15.0 mmol m\(^{-2}\) s\(^{-1}\), from *Pinus ponderosa* to *Quintinia acutifolia*. The three conifers assessed had lower average \( g_{\text{sw}} \) and a lower percentage of measurements recording open stomata. No significant relationship between \( D \) and nocturnal \( g_{\text{sw}} \) was found for any species (data not shown).

Discussion

Is stomatal conductance of temperate tree species at night significantly higher than cuticular conductance?

The one-way flux model describing δ\(^{18}\)O of leaf-respired CO\(_2\) (δ\(\Delta\)) developed by Cernusak *et al.* (2004) highlights the sensitivity of δ\(\Delta\) to stomatal conductance at night, e.g. an increase in \( g_{\text{sw}} \) from 10 to 20 mmol m\(^{-2}\) s\(^{-1}\) increases predicted δ\(\Delta\) by ~20% under typical conditions during the *Q. rubra* measurements. The model was developed under controlled-environment conditions with the weed species *Ricinus communis*. However, the high values for \( g_{\text{sw}} \) in the dark in the Cernusak *et al.* (2004) experiment will not be realistic for typical species in which the model would be applied to partition ecosystem respiration using isotopes of CO\(_2\), as measurements were made on darkened *R. communis* leaves (a species with inherently high stomatal conductance) during the day when circadian rhythms probably resulted in higher stomatal conductance than would be observed during the night. Most attempts to isotopically partition ecosystem respiration have been made for temperate forest species (*e.g.* *Pinus ponderosa*, Bowling *et al.* 2003a, b; *P. pinaster*, Ogée *et al.* 2004; mixed conifer–broadleaved forests in Japan, Kato *et al.* 2004), so the first aim of the current paper was to assess nocturnal stomatal conductance in several temperate tree species.

Of the six species assessed, all recorded significant nocturnal \( g_{\text{sw}} \) for individual leaves at some measurement times, suggesting that stomata are not completely closed in all leaves at all times in the dark. Overall average \( g_{\text{sw}} \) in the dark was significantly higher than would be expected from cuticular conductance and instrument noise in any species, and individual measurements of \( g_{\text{sw}} \) minus one standard deviation were greater than would be expected if stomata had been fully closed between 20 and 83% of the time (Table 3). The three angiosperm species tended to have higher nocturnal stomatal conductance and higher percentage of measurements indicating open stomata than the three coniferous species assessed. However, \( g_{\text{sw}} \) in all species was considerably lower than in *R. communis* (Cernusak *et al.* 2004).

Observations of significant \( g_{\text{sw}} \) in the dark have been made in several species (Snyder *et al.* 2003) particularly in response to ozone damage (*e.g.* Gruilke *et al.* 2004 and

![Fig. 7. Relationship between nocturnal stomatal conductance (\( g_{\text{sw}} \)) and difference between \( \delta^{18}\)O of leaf-respired CO\(_2\) modelled by (A) the one-way and net flux models assuming full equilibration (net flux model minus one-way flux model) and (B) the one-way model assuming incomplete equilibration and the net flux model (net flux minus one-way flux model) for *Quercus rubra* leaves at three positions within the canopy. Sunlit and shaded upper canopy positions refer to exposure during daylight hours. Values that are off scale are indicated with an arrow at the appropriate value of \( g_{\text{sw}} \).](image-url)
Nocturnal stomatal conductance and $\delta^{18}$O of leaf-respired CO$_2$

**Fig. 8.** Modelled nocturnal C$^{18}$O leaf isofluxes (i.e. modelled $\delta^{18}$O of leaf-respired CO$_2$ multiplied by measured respiration rate) for *Quercus rubra* leaves at three positions within the canopy (A–L), and total canopy-scale leaf isoflux expressed per unit ground area (M–P). Sunlit and shaded upper canopy positions refer to exposure during daylight hours. $\delta^{18}$O of leaf-respired CO$_2$ is estimated using the net flux model (□), the one-way flux model assuming complete equilibration (△) or the one-way flux model assuming incomplete equilibration (□) for each leaf class. The value that is off scale is indicated.

**Table 3.** Range, average and percentage of measurements over 0.1 mmol m$^{-2}$ s$^{-1}$ of nocturnal stomatal conductance in six temperate tree species

<table>
<thead>
<tr>
<th>Species</th>
<th>Range in nocturnal $g_{sw}$ (mmol m$^{-2}$ s$^{-1}$)</th>
<th>Average nocturnal $g_{sw}$ (mmol m$^{-2}$ s$^{-1}$)</th>
<th>Significant $g_{sw}$ (% of measurements)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinus ponderosa</td>
<td>1.4–11.0</td>
<td>3.9 ± 1.7</td>
<td>20</td>
</tr>
<tr>
<td>Pinus radiata</td>
<td>2.4–27.5</td>
<td>8.9 ± 0.9</td>
<td>54</td>
</tr>
<tr>
<td>Dacrydium cupressinum</td>
<td>0.0–21.5</td>
<td>4.8 ± 0.2</td>
<td>63</td>
</tr>
<tr>
<td>Weinmannia racemosa</td>
<td>0.0–70.1</td>
<td>11.2 ± 0.6</td>
<td>70</td>
</tr>
<tr>
<td>Quinina acutifolia</td>
<td>0.0–41.9</td>
<td>15.1 ± 0.6</td>
<td>83</td>
</tr>
<tr>
<td>Quercus rubra</td>
<td>0.0–39.0</td>
<td>10.6 ± 0.2</td>
<td>87</td>
</tr>
</tbody>
</table>

Further, Bucci et al. (2004) recorded high nocturnal stomatal conductance (up to 140 mmol m$^{-2}$ s$^{-1}$) in three tropical tree species, also demonstrating significant sap flow throughout the night. The measurements reported in the current paper indicating significant stomatal conductance during the night, at least in some leaves for part of the time, combined with previous work suggest that stomata may be naturally slightly leaky in many species. In environments with high soil water availability and low air saturation deficits at night, the cost of leaky stomata is small and may even be
offset by benefits associated with continued water movement through the plant. These benefits may include improved nutrient acquisition by higher total water flux through the plant (Mason et al. 1992; McDonald et al. 2002; Snyder et al. 2003), nocturnal recovery from xylem cavitation during the day (Donovan et al. 2003), prevention of excess leaf turgor at night (Dinonov et al. 2001), and continuation of O₂ delivery to xylem parenchyma in the stems of larger trees (Gansert 2003).

What regulates nocturnal stomatal conductance in temperate tree species?

We found little evidence of regulation of stomatal conductance in the dark in the six temperate tree species assessed, with no significant relationships between D and gᵦ in all three tropical tree species. For the Q. rubra leaves studied here, significant nocturnal gᵦ tended to be more common in leaves switching from net positive to negative photosynthesis around sunset, and as gᵦ increased in a 'pre-emptive' response to sunrise. That is, gᵦ decreased after local light levels resulted in net respiration, and increased before net photosynthesis started the following day. Stomatal aperture is known to be under a degree of control by a circadian rhythm (Raschke 1979), and observations of stomata starting to open before sunrise have been recorded in at least two other species (Zeiger et al. 1981). Stomatal opening before sunrise is also evident in the Bucci et al. (2004) data, and as the lowest air temperatures and D were measured before dawn, the negative relationship between D and gᵦ found in that study may simply be explained by a circadian rhythm in stomatal aperture, or by stomata opening in response to extremely low levels of blue light present before dawn (e.g. Zeiger et al. 1981).

Among upper shaded Q. rubra leaves and those lower in the canopy, much more variability (66%, P=0.014: Fig. 4) in average gᵦ at midnight was explained by the average gᵦ at midday on the previous day than by D. Interestingly, Snyder et al. (2003) found that species with inherently high daytime stomatal conductance also tended to have high nocturnal gᵦ. This is further evidence of limited stomatal regulation at night, as gᵦ in the dark seems to be largely a function of stomatal conductance during the day. Conifers tend to have lower maximum gᵦ than broadleaved trees (Wang et al. 1998, Tissue et al. 2005), and we report here that nocturnal gᵦ tends to be higher among angiosperms than conifers.

Does nocturnal stomatal conductance result in differences between one-way and net flux models of the δ¹⁸O of respired CO₂?

In the Q. rubra example given in the current paper, values of δ₁₈O predicted by the net flux simplification were mostly within 50% of the one-way flux model (assuming full equilibration) when gᵦ < 10.0 mmol m⁻² s⁻¹ (Figs 6, 7). Differences between the one-way flux model with full equilibration and the net flux simplification at low gᵦ are due to Cᵦ / Cᵦ being greater than zero. The lowest modelled value for Cᵦ / Cᵦ is 0.35, when gᵦ = 2.2 mmol m⁻² s⁻¹. Under these conditions the net flux approximation predicts δ₁₈O to be 9.6% lower than the one-way flux model.

Interestingly, when incomplete equilibration of chloroplastic CO₂ is assumed in the one-way flux model, the effects of Cᵦ / Cᵦ > 0 are offset by the effect of 0 < 1, resulting in the difference between the net flux approximation and one-way flux modelled δ₁₈O being smaller when 0 = 0.79 than when 0 = 1. Cernusak et al. (2004) estimated 0 to be 0.79 in R. communis leaves respiring in the dark. 0 is thought to be very close to unity when leaves are photosynthesising (Farquhar et al. 1993; Giffon and Yakir 2001; Cernusak et al. 2004). Given the requirement for accurate predictions of δ₁₈O for ecosystem respiration partitioning, further investigation of variation in 0 among leaves respiring in the dark is warranted. The assumption of 0 less than one also requires knowledge of the value of δ₁₈O, for which few estimates have been attempted (see Cernusak et al. 2004 for discussion). For the current model application we have used the value for δ₁₈O estimated during dark respiration in R. communis leaves by Cernusak et al. (2004). More detailed measurements are required to determine the value and/or extent of variability in δ₁₈O.

Another assumption made in the current application of the one-way flux model is that Eqn 5 accurately describes the δ¹⁸O of leaf-respired CO₂ when stomata are closed and CO₂ and water exchange occur exclusively through the cuticle. Cernusak et al. (2004) demonstrated that the one-way fluxes of CO₂ into and out of the leaf were important in determining δ₁₈O for respiring leaves with stomatal conductances between 30 and 280 mmol m⁻² s⁻¹. Further model testing is required at very low stomatal conductance and when gas exchange occurs primarily through the cuticle.

Enrichment in δ₁₈O predicted by the one-way flux model was very high (up to 1130‰) in the Q. rubra example when gᵦ was high near sunset and sunrise. Values for δ₁₈O as high as 324‰ have been measured in laboratory conditions (Cernusak et al. 2004), so the enrichments predicted by the one-way flux model are not inconceivable. The very large differences between δ₁₈O predicted by the one-way flux model and the net flux approximation point to the importance of in situ sampling for model validation, and suggest that great care must be taken in measurement and modelling of δ¹⁸O of respiratory CO₂ for flux partitioning near sunset and sunrise.

To assess the effects of assuming Cᵦ / Cᵦ = 0 on partitioning the ecosystem respiratory flux into above- and below-ground components using δ¹⁸O of ecosystem-respired CO₂ (δ₁₈O) (ignoring stem and branch respiration), we assume that the soil respiration rate is constant at 3 0 μmol m⁻² s⁻¹.
(an average value for temperate forests in mid-summer; Litton et al. 2003; Fisk et al. 2004; Killiell et al. 2004; Eklblad et al. 2005). Further, we assume that δ18O of soil-respired CO2 (δR) is constant through time at 35‰, i.e. in full equilibrium with source water at 16°C and ignoring soil invasion fluxes for this simple demonstration, and that the one-way flux model (Eqn 5) with full equilibration accurately predicts δR. A soil respiration rate of 3.0 μmol m−2 s−1 gives a proportionately smaller contribution of soil respiration to ecosystem respiration of ~0.27 over the four nights on which O. rubra leaf respiration rates were measured. If the values of δR are generated using the one-way flux model with θ = 1, then δR is always more enriched than either δB or δB predicted by the net flux approximation. Source partitioning when the isotope composition of the mixture lies outside the bounds of the source is meaningless. If we assume incomplete equilibration of leaf respired CO2 within the one-way flux model and constant δR, to predict values of δB, then δB lies within the bounds of δB and net flux-predicted δB just one-third of the time over the four measurement nights (but note the inconsistency of assuming θ = 1 with C2/ C1 > 0 and θ = 1 with C2/ C1 = 0). Even when partitioning is possible, the net flux model both under- and over-estimates the proportionional contribution of below-ground respiration to ecosystem respiration, by up to 34%. We believe the one-way fluxes into and out of the leaf and the soil must be considered when modelling leaf- and soil-respired CO2 to partition the ecosystem isoflux, particularly close to sunset and sunrise. New online methods of isotopic analysis of CO2 (e.g. online mass spectrometers). Schnyder et al. 2003; Klumpp et al. 2005; tunable diode laser absorption spectroscopy: Bowling et al. 2003) will allow rigorous model testing at temporal resolutions orders of magnitude higher than previously attempted. We recommend applying Eqn 5 and the soil CO2 invasion model (Tans 1998; Boyer et al. 1999) to predict the δ13C of CO2 from the two main sources before attempting to partition the ecosystem respiratory flux.

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Nocturnal stomatal conductance and $^\delta$18O of leaf-respired CO2


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