CSIRO PUBLISHING
www.publish.csiro.au/journals/fpb *Functional Plant Biology*, 2005, **32**, 1107–1121

Nocturnal stomatal conductance and implications for modelling $\delta^{18}O$ of leaf-respired CO_2 in temperate tree species

Margaret M. Barbour[A,G], *Lucas A. Cernusak*[B,C], *David Whitehead*[A], *Kevin L. Griffin*[D], *Matthew H. Turnbull*[E], *David T. Tissue*[F] *and Graham D. Farquhar*[B]

[A]Landcare Research, PO Box 69, Lincoln 8152, New Zealand.
[B]Research School of Biological Sciences, Australian National University, GPO Box 475, ACT 2601, Australia.
[C]Current address: Smithsonian Tropical Research Institute, PO Box 2072, Balboa, Ancon, Republic of Panama.
[D]Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964-8000, USA.
[E]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand.
[F]Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131, USA.
[G]Corresponding author. Email: barbourm@landcareresearch.co.nz

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 (δ_{Rl}) to nocturnal stomatal conductance. When the one-way flux model was tested on *Ricinus communis* L. large enrichments in δ_{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 δ_{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. δ_{Rl} values predicted by the one-way flux model revealed that δ_{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*. 2003*a*, *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 (δ_{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

Abbreviations used: See Table 1 for a complete list of abbreviations and symbols used.

 10.1071/FP05118 1445-4408/05/121107

Table 1. List of mathematical symbols used

Symbol	Definition	Value / unit
a	Discrimination against $C^{18}OO$ during diffusion through stomata	8.8‰
a_b	Discrimination against $C^{18}OO$ during diffusion through the leaf boundary layer	5.8‰
a_w	Summed discriminations against $C^{18}OO$ during liquid phase diffusion and dissolution	0.8‰
$\bar{a}$	Weighted mean discrimination against $C^{18}OO$ for diffusion from the chloroplast to the atmosphere	‰
C	Molar density of water	55.5×10^3 mol m^{-3}
C_a	Mole fraction of CO_2 in the atmosphere	μmol mol^{-1}
C_c	Mole fraction of CO_2 in the chloroplast	μmol mol^{-1}
C_i	Mole fraction of CO_2 in leaf intercellular spaces	μmol mol^{-1}
C_s	Mole fraction of CO_2 at the leaf surface	μmol mol^{-1}
D	Air saturation deficit	mmol mol^{-1}
$\boldsymbol{D}$	Diffusivity of $H_2{}^{18}O$ in water	2.66×10^{-9} m^2 s^{-1}
δ_a	$\delta^{18}O$ of atmospheric CO_2	‰
δ_c	$\delta^{18}O$ of chloroplastic CO_2	‰
δ_{c0}	$\delta^{18}O$ of CO_2 in the chloroplast that has not equilibrated with chloroplast water	‰
δ_e	$\delta^{18}O$ of water at the evaporating site in the leaf	‰
δ_{es}	$\delta^{18}O$ of water at evaporating site in the leaf, at isotopic steady-state	‰
δ_R	$\delta^{18}O$ of ecosystem-respired CO_2	‰
δ_{Rl}	$\delta^{18}O$ of leaf-respired CO_2	‰
δ_{Rs}	$\delta^{18}O$ of soil-respired CO_2	‰
δ_s	$\delta^{18}O$ of source water	‰
Δ_e	^{18}O enrichment at the sites of evaporation within the leaf, compared to source water	‰
Δ_{es}	^{18}O enrichment at the sites of evaporation within the leaf, compared to source water, at isotopic steady-state	‰
Δ_v	^{18}O enrichment of vapour in the atmosphere, compared to source water	‰
$e(T_l)$	Saturation vapour pressure at leaf temperature	–
E	Transpiration rate	mmol m^{-2} s^{-1}, or mol m^{-2} s^{-1} in Eqn 1
ε^+	Equilibrium ^{18}O fractionation between liquid water and vapour	‰
ε_k	Kinetic fractionation during diffusion of $H_2{}^{18}O$ from leaf intercellular air spaces to the atmosphere	‰
ε_w	Equilibrium ^{18}O fractionation between CO_2 and water	‰
f	Ratio of cuticular conductance to CO_2 and water	
g_{bw}	Boundary layer conductance to water vapour	mol m^{-2} s^{-1}
g_{cw}	Cuticular conductance to water vapour	mol m^{-2} s^{-1}
g_{sc}	Stomatal and cuticular conductance to CO_2	mol m^{-2} s^{-1}
g_{sw}	Stomatal and cuticular conductance to water vapour	mol m^{-2} s^{-1}
$g_{sw}{}'$	Stomatal conductance to water vapour	mol m^{-2} s^{-1}
g_{tw}	Total conductance to water vapour	mol m^{-2} s^{-1}
K	Ratio of the number of stomata on one side of the leaf to the number on the other side	–
L	The effective length over which the Péclet effect occurs	m
P	Total atmospheric pressure	Pa
$\wp$	Péclet number	dimensionless
s	Leaf area within the chamber	m^2
σ	Standard deviation	
T_l	Leaf temperature	K
θ	Proportion of chloroplast CO_2 isotopically equilibrated with chloroplast water	
u	Chamber flow rate	mol s^{-1}
W	Leaf water content	mol m^{-2}
w_i	Water mole fraction of air leaving the chamber	mmol mol^{-1}, and mol mol^{-1} in Eqn 1
w_l	Water vapour mole fraction inside the leaf	mmol mol^{-1}, and mol mol^{-1} in Eqn 2
w_o	Water mole fraction of air entering the chamber	mmol mol^{-1}, and mol mol^{-1} in Eqn 1

enriched leaf-respired CO_2 observed under some conditions (Seibt *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*. 2003*a*, *b*). The one-way flux model presented by Cernusak *et al*. (2004) predicts that CO_2 respired by leaves should deviate from that predicted by a net flux model when the difference in isotopic composition of CO_2 in the chloroplast (δ_c) and the ambient air (δ_a) 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 δ_c and δ_a 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; Benyon 1999; Musselman and Minnick 2000; Grulke *et al*. 2004). Recent work suggests that plants with inherently high daytime stomatal conductance tend to have high nocturnal stomatal conductance (g_{sw}) (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 that mechanistic understanding is weak.

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 ^{18}O of leaf-respired CO_2 predicted between the net flux and the one-way flux models at typical values of nocturnal stomatal conductances?

Materials and methods

CO_2 and water vapour exchange measurements

Measurements of the rate of exchange of CO_2 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°E, 270 m above sea level) for each of three leaf classes (upper canopy leaves in full sun and artificially shaded with 50% shade cloth, and naturally shaded leaves lower in the canopy) were measured with a LI-6400 gas-exchange system (Li-Cor Inc., Lincoln, NE) with a clear top chamber, and chamber inlet CO_2 controlled at 370 μmol mol^{-1}. No attempt was made to control leaf temperature or chamber vapour pressure. Photosynthesis and respiration rates are presented in Whitehead *et al*. (2004*b*). Black Rock Forest has a 10–16-m-tall canopy of *Q. rubra*, *Q. prinus* and *Acer rubrum* trees with ~760 stems ha^{-1}. Total leaf index (*LAI*) during the measurement period was 5.8 m^2 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. CO_2 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 CO_2 partial pressure differential 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 CO_2 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 g_{sw} 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 g_{sw} 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 (w_o and w_i, respectively), the flow rate of ambient air entering the chamber (u), and area of leaf within the chamber (s) by (von Caemmerer and Farquhar 1981; Li-Cor 6400 manual Eqn 1–4):

$$E = \frac{u(w_o - w_i)}{s(1 - w_o)}. \quad (1)$$

Total conductance to water vapour through the stomata and the leaf boundary layer (g_{tw}) 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):

$$g_{tw} = \frac{E\left(1 - \frac{w_l + w_o}{2}\right)}{w_l - w_o}, \quad (2)$$

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

$$w_l = \frac{e(T_l)}{P}. \quad (3)$$

In Eqn 3 the function $e(T_l)$ is the saturation vapour pressure at leaf temperature. Boundary layer conductance to water vapour from one side of the leaf (g_{bw}) is estimated from the ratio of stomatal frequency on one side of the leaf to the other (K), leaf area, and fan speed using the standard Li-Cor 6400 lookup table. Stomatal and cuticular conductance to water vapour (g_{sw}) is then given by (Li-Cor 6400 manual Eqns 1–9 and 1–10):

$$g_{sw} = 1 \Big/ \left(\frac{1}{g_{tw}} - \frac{\left(\frac{K^2 + 1}{(K+1)^2}\right)}{g_{bw}} \right). \quad (4)$$

Clearly, errors in w_o, w_i, u, s, T_l, P, and fan speed could generate errors in g_{sw}, 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 g_{sw} calculation (e.g. Tyree and Wilmot 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_{sw}, we conducted a Monte Carlo analysis (10 000 individual calculations) on g_{sw} estimated under average conditions for the *Q. rubra* 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_o varied, so long as air temperature influences on w_o were reflected in T_l (data not shown). A coefficient of variation as high as 37%, at $w_i - w_o = 0.1$ mmol mol^{-1} and $T_l = 19$°C, suggests that under these conditions calculated g_{sw} should be interpreted with extreme caution. If correctly positioned, the leaf thermocouple should be expected to measure T_l within 0.1°C, and the coefficient of variation in g_{sw} 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 (Šantrůček *et al*. 2004). We determine significant water loss through stomata to occur when g_{sw} minus one standard deviation (σ) is greater than 0.1 mmol m^{-2} s^{-1}, where σ is estimated from Fig. 1 ($cv = \sigma$ / 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 Wilmot 1990). Air temperature within the chamber when upper sunlit leaves were measured was consistently warmer than that measured by the sensor placed 2 m above the forest canopy. This difference was as great as 10.5°C under high irradiance, but averaged 6.6 ± 0.3°C during daylight and 3.7 ± 0.1°C in the dark. The elevated temperature of within-chamber air with zero irradiance is probably 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 $\delta^{18}O$ of leaf-respired CO_2 (δ_{Rl}) may be described by (Cernusak *et al*. 2004):

$$\delta_{Rl} = \frac{\theta[\delta_e(1+\varepsilon_w)+\varepsilon_w] + (1-\theta)\delta_{c0} - \frac{C_a}{C_c}(\delta_a - \bar{a}) - \bar{a}}{(1+\bar{a})\left(1-\frac{C_a}{C_c}\right)}, \quad (5)$$

where θ is the proportion of chloroplast CO_2 that is isotopically equilibrated with chloroplast water, δ_e, δ_{c0} and δ_a are the $\delta^{18}O$ of chloroplastic water, the $\delta^{18}O$ of CO_2 in the chloroplast that has not equilibrated with local water, and the $\delta^{18}O$ of CO_2 in the atmosphere, respectively. C_a and C_c are the CO_2 mole fractions in the ambient atmosphere and in the chloroplasts, respectively, and ε_w is the equilibrium ^{18}O fractionation between CO_2 and water. The isotopic equilibrium between CO_2 and water is dependent on temperature by (Brenninkmeijer *et al*. 1983):

$$\varepsilon_w\ (‰) = \frac{17\,604}{T_l} - 17.93, \quad (6)$$

where T_l is in K. In Eqn 5, $\bar{a}$ 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):

$$\bar{a} = \frac{(C_c - C_i)a_w + (C_i - C_s)a + (C_s - C_a)a_b}{C_c - C_a}, \quad (7)$$

where a_w is the summed discriminations against $C^{18}OO$ during liquid phase diffusion and dissolution (0.8‰), a and a_b are the discriminations against $C^{18}OO$ during diffusion through the stomata and the boundary layer (8.8 and 5.8‰, respectively), and C_i, and C_s are CO_2 mole fractions in the leaf intercellular spaces and at the leaf surface, respectively.

The steady-state enrichment above source water of leaf water at the sites of evaporation (and, to a close approximation, the chloroplast; Δ_{es}) has been modelled by Farquhar and Lloyd (1993), by modification of earlier models (Craig and Gordon 1965; Dongmann *et al*. 1974) as:

$$\Delta_{es} = \varepsilon^+ + \varepsilon_k + (\Delta_v - \varepsilon_k)\frac{w_o}{w_l}, \quad (8)$$

where ε^+ is the equilibrium fractionation during the phase change from liquid to vapour, ε_k is the kinetic fractionation during diffusion through stomata and the leaf boundary layer, and Δ_v is the ^{18}O enrichment above source water of atmospheric vapour. We consider respiratory CO_2 to exchange with chloroplastic water, as most of the carbonic anhydrase in leaves resides in chloroplasts (Moroney *et al*. 2001), and chloroplasts are usually found lining the membrane adjacent to intercellular air spaces (i.e. CO_2 produced in the mitochondria must pass through chloroplasts before entering intercellular air spaces). However, electron micrographs have revealed the presence

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

Variable	Symbol	Units	Default value	Instrument error
Water vapour mole fraction entering the chamber	w_o	mmol mol^{-1}	17.3	0.04
Water vapour mole fraction leaving the chamber	w_i	mmol mol^{-1}	17.4–18.4	0.04
Flow rate through the chamber	u	µmol s^{-1}	300	1
Leaf area	s	m^{-2}	0.0006	0.00005
Leaf temperature	T_l	°C	19	0.1–1.0
Total atmospheric pressure	P	kPa	100	1
Boundary layer conductance to water vapour	g_{bw}	mol m^{-2} s^{-1}	2.84	0.10

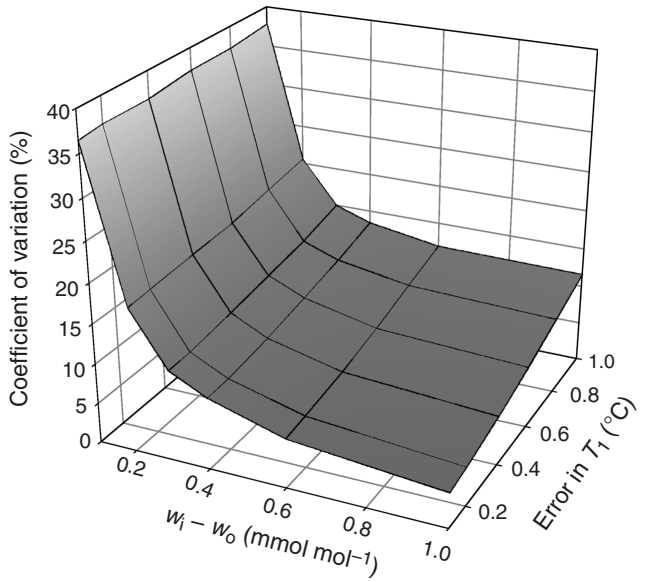

Fig. 1. Variation in the coefficient of variation from 10 000 Monte Carlo estimates of stomatal conductance calculated assuming instrument noise as outlined in Table 2, with leaf thermocouple noise varying between 0.1 and 1.0°C (leaf temperature is constant at 19°C), and with the difference between the water mole fraction of air leaving and entering the leaf chamber ($w_i - w_o$) varying between 0.1 and 1.0 mmol mol^{-1}. $w_i - w_o$ ranged from 0.03 to 1.06 mmol mol^{-1} among the *Quercus rubra* measurements at night.

of mitochondria between chloroplasts, and sometimes pressed against the cellular membrane (Griffin *et al*. 2004). Functional carbonic anhydrase-like proteins have been found in mitochondria (Parisi *et al*. 2004), suggesting that some respiratory CO_2 may equilibrate with mitochondrial water alone 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, CO_2 that has exchanged with mitochondrial water may be isotopically indistinguishable from CO_2 that has exchanged with chloroplastic water.

The oxygen isotopic composition of water at the evaporating sites in leaves and ambient water vapour are expressed relative to the source water, such that $\Delta_{es} = R_e / R_s - 1$ and $\Delta_v = R_v / R_s - 1$, where R is the $^{18}O/^{16}O$, and subscripts e, v and s are water at the evaporating sites, water vapour and source water, respectively. δ_{es} may be calculated from Δ_{es} by:

$$\delta_{es} \approx \Delta_{es} + \delta_s. \tag{9}$$

The equilibrium fractionation factor, ε^+, is dependent on temperature by (Bottinga and Craig 1969):

$$\varepsilon^+ \text{ (‰)} = 2.644 - 3.206\left(\frac{10^3}{T_l}\right) + 1.534\left(\frac{10^6}{T_l^2}\right), \tag{10}$$

where T_l is in K. The total kinetic fractionation factor, ε_k, may be calculated from stomatal and boundary layer conductances by (Farquhar *et al*. 1989):

$$\varepsilon_k = \frac{32{g_{sw}}^{-1} + 21{g_{bw}}^{-1}}{{g_{sw}}^{-1} + {g_{bw}}^{-1}}. \tag{11}$$

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 (Kays 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 Δ_{es} under field conditions, particularly at night (Flanagan and Ehleringer 1991; Harwood *et al*. 1998). A non-steady-state model has been developed (Farquhar and Cernusak 2005), and shows good agreement with measured leaf water $\delta^{18}O$ (Cernusak *et al*. 2002, 2005). The non-steady-state enrichment of water at the sites of evaporation (Δ_e) is given by:

$$\Delta_e = \Delta_{es} - \frac{\alpha_k \alpha^+}{g_{sw} w_l} \frac{d\left(W \times \dfrac{1 - e^{-\wp}}{\wp} \times \Delta_e\right)}{dt}, \tag{12}$$

where $\alpha_k = 1 + \varepsilon_k$, $\alpha^+ = 1 + \varepsilon^+$, W is the leaf water content in mol m^{-2}, and the Péclet number, $\wp$, is the ratio of convection of unenriched water to the sites of evaporation to the backward diffusion of $H_2{}^{18}O$. $\wp$ is given by (Farquhar and Lloyd 1993):

$$\wp = \frac{EL}{C\boldsymbol{D}}, \tag{13}$$

where E is the transpiration rate (mol m^{-2} s^{-1}), L is the effective length over which the Péclet effect occurs (0.001–0.1 m; Wang *et al*. 1998), C is the molar density of water (55.5×10^3 mol m^{-3}) and $\boldsymbol{D}$ is the diffusivity of $H_2{}^{18}O$ in water (2.66×10^{-9} m^2 s^{-1}).

Prior to the publication of the one-way flux model describing δ_{Rl}, earlier researchers (e.g. Flanagan *et al*. 1997, 1999; Bowling *et al*. 2003*b*) made several assumptions to apply a simplified version of Eqn 5. By assuming that $\theta = 1$, stomata are tightly closed (i.e. C_a / C_c approaches zero), and that $1 + \varepsilon_w$ and $1 + \bar{a}$ are equal to unity (these terms are ~1.04 and 1.007), oxygen isotope ratio of leaf-respired CO_2 may be described simply as an isotopic equilibrium between oxygen atoms in CO_2 and in leaf chloroplastic water:

$$\delta_{Rl} = \delta_e + \varepsilon_w - \bar{a}. \tag{14}$$

We call this the 'net flux model', as it does not consider the CO_2 that diffuses into the leaf, exchanges with chloroplastic water, and diffuses out again.

Even if all stomata are tightly closed, diffusion of CO_2 and water vapour still occurs through the cuticle. The ratio of ambient to chloroplastic CO_2 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} (Šantrůček *et al*. 2004) and cuticular conductance to CO_2 is similar, then when the leaf respiration rate is 1 μmol m^{-2} s^{-1}, C_a / C_c is 0.03 (assuming $C_a = 370$ 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 C_a / C_c is 0.01 in the example above.

Boyer *et al*. (1997) demonstrated that cuticular conductance to CO_2 is only ~6% of that for water vapour at low irradiance, despite the ratio of diffusivities of CO_2 and water vapour in air being 1.6 (Nobel 1983). This would further increase the CO_2 partial pressure inside the leaves when stomata are closed. To account for the possibility that cuticular conductance to CO_2 is lower than to water vapour in the one-way flux model of δ_{Rl}, conductance to CO_2 is calculated separately for stomatal and cuticular components. Values of g_{sw} measured by the photosynthesis system are the result of both cuticular (g_{cw}) and stomatal conductances (g_{sw}') to water vapour. Stomatal conductance to water vapour is assumed to be zero when $g_{sw} < 0.1$ mmol m^{-2} s^{-1}. Conductance of CO_2 through the stomata and cuticle (g_{sc}) is then given by:

$$g_{sc} \text{ (mmol m}^{-2}\text{s}^{-1}) = 1.6(g_{sw}' - 0.1) + f g_{cw}, \tag{15}$$

where f is the ratio of cuticular conductances to CO_2 and water vapour (either 0.06 or 1), $g_{cw} = g_{sw}$ when $g_{sw} < 0.1\,mmol\,m^{-2}\,s^{-1}$, and $g_{cw} = 0.1\,mmol\,m^{-2}\,s^{-1}$ when $g_{sw} > 0.1\,mmol\,m^{-2}\,s^{-1}$. The factor of 1.6 describes to 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_2O 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 $\mu mol\,m^{-2}\,s^{-1}$ ‰) is calculated as δ_{Rl} 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 $\mu mol\,m^{-2}$ ground s^{-1} ‰. *LAI* for each leaf class was estimated from measurements of relative foliage area density and fraction of sunlit foliage (Whitehead *et al*. 2004*a*, *b*), as 1.60, 2.28 and 1.91 $m^2\,m^{-2}$ 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 δ_{Rl} under typical field conditions for a temperate broad-leaved tree species. If $\theta = 1$, and stomata are tightly closed, then C_a / C_c should approach zero and the net flux model (Eqn 14) should give similar values to the one-way flux model (Eqn 5). To apply these models we necessarily make several assumptions. We have assumed that $\delta^{18}O$ of source water is -8.0‰, $\Delta_v = -\varepsilon^+$ (about -9‰), $\delta_{c0} = 14.3$‰, $\delta_a = \delta_s + \varepsilon_w$ (~32‰), that mesophyll conductance to CO_2 (needed to calculate C_c) $= 0.14\,mol\,m^{-2}\,s^{-1}$ (Ethier and Livingston 2004), $W = 17.7\,mol\,m^{-2}$ and $L = 30\,mm$ (an average value for broad-leaved tree species; Barbour *et al*. 2004). The value for δ_{c0} is taken from Cernusak *et al*. (2004), from controlled environment laboratory experiments, and W 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 $350\,\mu mol\,mol^{-1}$ 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 $g_{sw} < 0.1\,mmol\,m^{-2}\,s^{-1}$.

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 $0.2\,mmol\,m^{-2}\,s^{-1}$ for upper sunlit, upper shaded and lower leaves, respectively. The highest stomatal conductance recorded for respiring

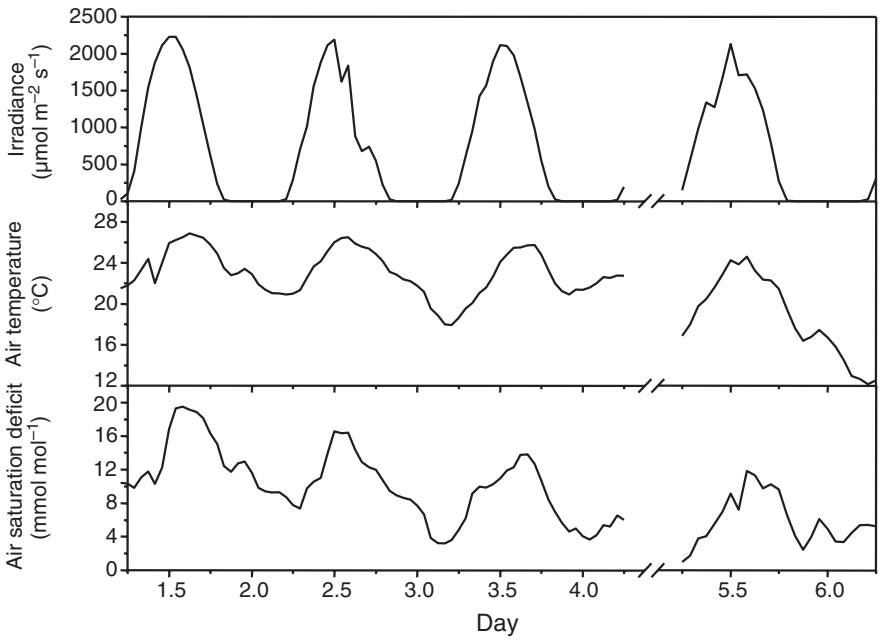

Fig. 2. Weather variables for the four days when *Quercus rubra* stomatal conductance measurements were made. Five days separate day four and day six. Values for irradiance are shown for the 400–700 nm range (Whitehead *et al*. 2004*a*).

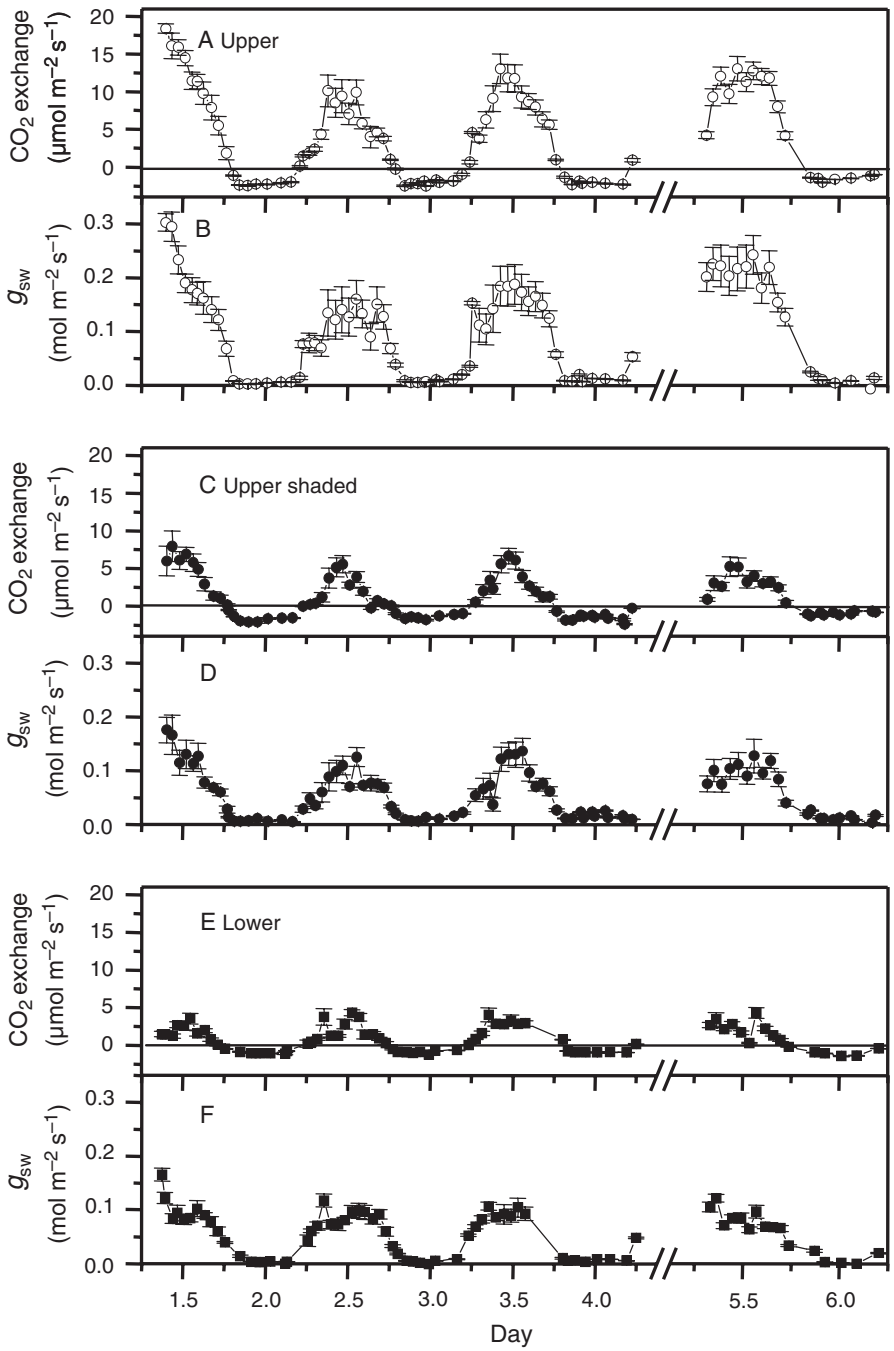

Fig. 3. Variation in leaf net CO_2 exchange and stomatal conductance (g_{sw}) over four 24-h periods for *Quercus rubra* leaves in three positions within the canopy. Sunlit and shaded upper canopy positions refer to exposure during daylight hours. CO_2 exchange values from Whitehead *et al*. (2004*a*).

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. $g_{sw} - 1\sigma > 0.1$ mmol m^{-2} s^{-1}, where σ 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. 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. 5*A*). 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; Fig. 5*B*).

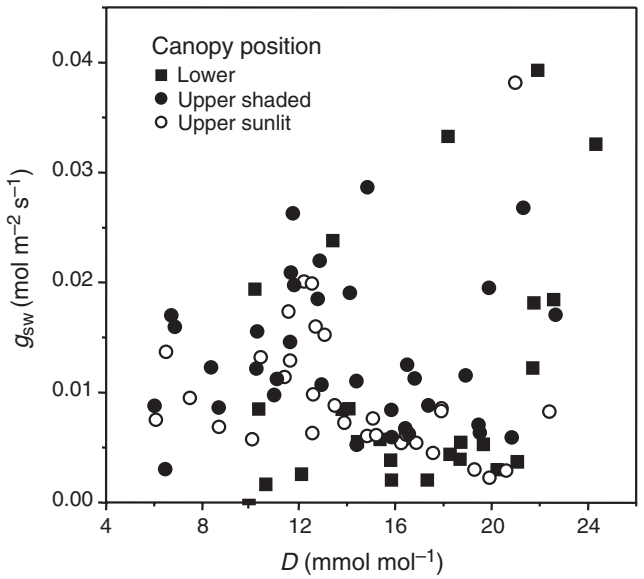

Fig. 4. Relationship between air saturation deficit (D) and stomatal conductance in the dark for *Quercus rubra* leaves at three positions within the canopy. Sunlit and shaded upper canopy positions refer to exposure during daylight hours.

Modelled Δ_e *and* δ_{Rl} *for* Q. rubra

The non-steady-state model predicted leaf water enrichment above source water (Δ_e) 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 $\delta^{18}O$ 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 δ_{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 $\delta^{18}O$ of respired CO_2, between 42 and 47‰. Both the one-way flux model and the net flux simplification predicted generally lower δ_{Rl} for leaves lower in the canopy, compared to upper shaded and sunlit leaves. The one-way flux model predicted a slightly more enriched δ_{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 δ_{Rl} differ markedly when g_{sw} is greater than $\sim$5.0 mmol m^{-2} 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 μmol m^{-2} s^{-1} ‰ and from -4 to 758 μmol m^{-2} s^{-1} ‰ for the one-way flux model

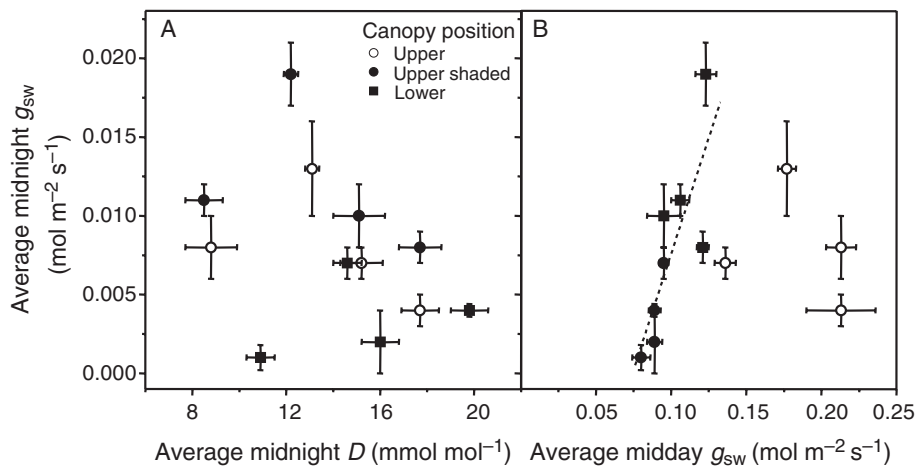

Fig. 5. Relationships between average stomatal conductance at midnight and (*A*) average air saturation deficit at midnight, and (*B*) average stomatal conductance at midday the previous day for *Quercus rubra* leaves at three positions within the canopy. Sunlit and shaded upper canopy positions refer to exposure during daylight hours.

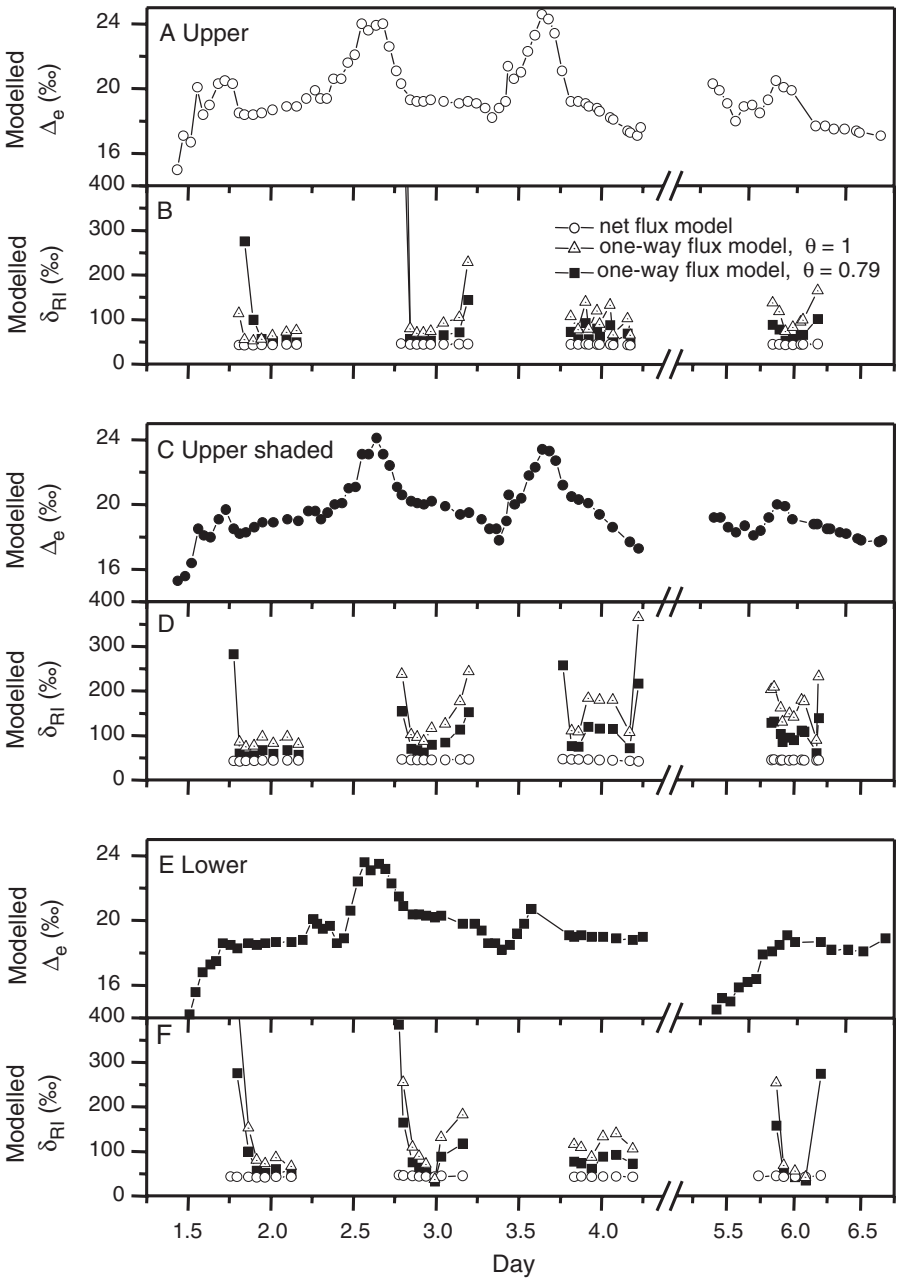

Fig. 6. Modelled ^{18}O enrichment at the sites of evaporation in leaves and modelled nocturnal $\delta^{18}O$ of leaf-respired CO_2 (δ_{Rl}) for *Quercus rubra* leaves at three positions within the canopy. Sunlit and shaded upper canopy positions refer to exposure during daylight hours. δ_{Rl} is modelled 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 δ_{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 $1724\,\mu mol\,m^{-2}\,ground\,s^{-1}$ ‰ and from 392 to $919\,\mu mol\,m^{-2}\,ground\,s^{-1}$ ‰ for the one-way flux model with $\theta = 1$ and $\theta = 0.79$, respectively, and from -415 to $478\,\mu mol\,m^{-2}\,ground\,s^{-1}$ ‰ 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 δ_{Rl} predicted by the one-way flux models was nearly always more enriched for the three leaf

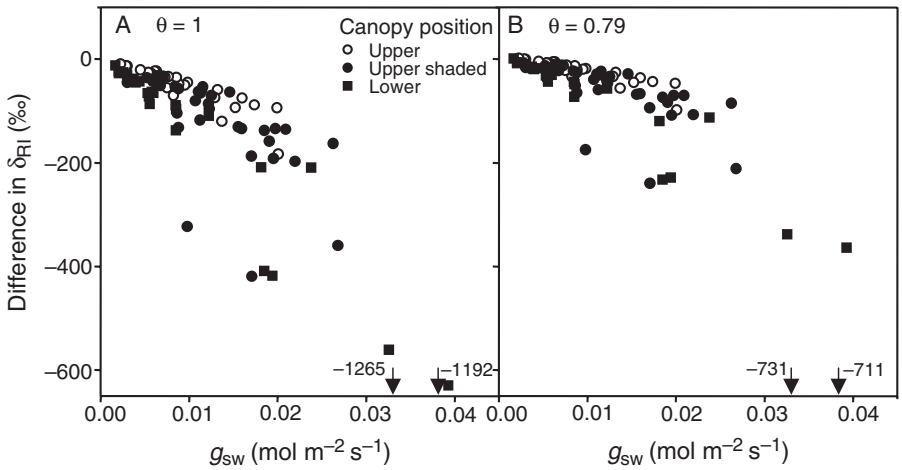

Fig. 7. Relationship between nocturnal stomatal conductance (g_{sw}) and difference between $\delta^{18}O$ of leaf-respired CO_2 modelled by (*A*) the one-way and net flux models assuming full equilibration (net flux model minus one-way flux model) and (*B*) the one-way model assuming incomplete equilibration 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_{sw}.

classes. As expected from leaf δ_{Rl} 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. $g_{sw} - 1\sigma > 0.1$ mmol m^{-2} s^{-1}, where σ for each measurement was estimated from $w_i - w_o$, as described in the Materials and methods) was recorded during the middle of the night in all five species (Table 3). The percentage of measurements recording significant g_{sw} varied between 20 and 83% as average g_{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_{sw} and a lower percentage of measurements recording open stomata. No significant relationship between *D* and nocturnal g_{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 $\delta^{18}O$ of leaf-respired CO_2 (δ_{Rl}) developed by Cernusak *et al*. (2004) highlights the sensitivity of δ_{Rl} to stomatal conductance at night, e.g. an increase in g_{sw} from 10 to 20 mmol m^{-2} s^{-1} increases predicted δ_{Rl} 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_{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*. 2003*a*, *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_{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_{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_{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_{sw} in all species was considerably lower than in *R. communis* (Cernusak *et al*. 2004).

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

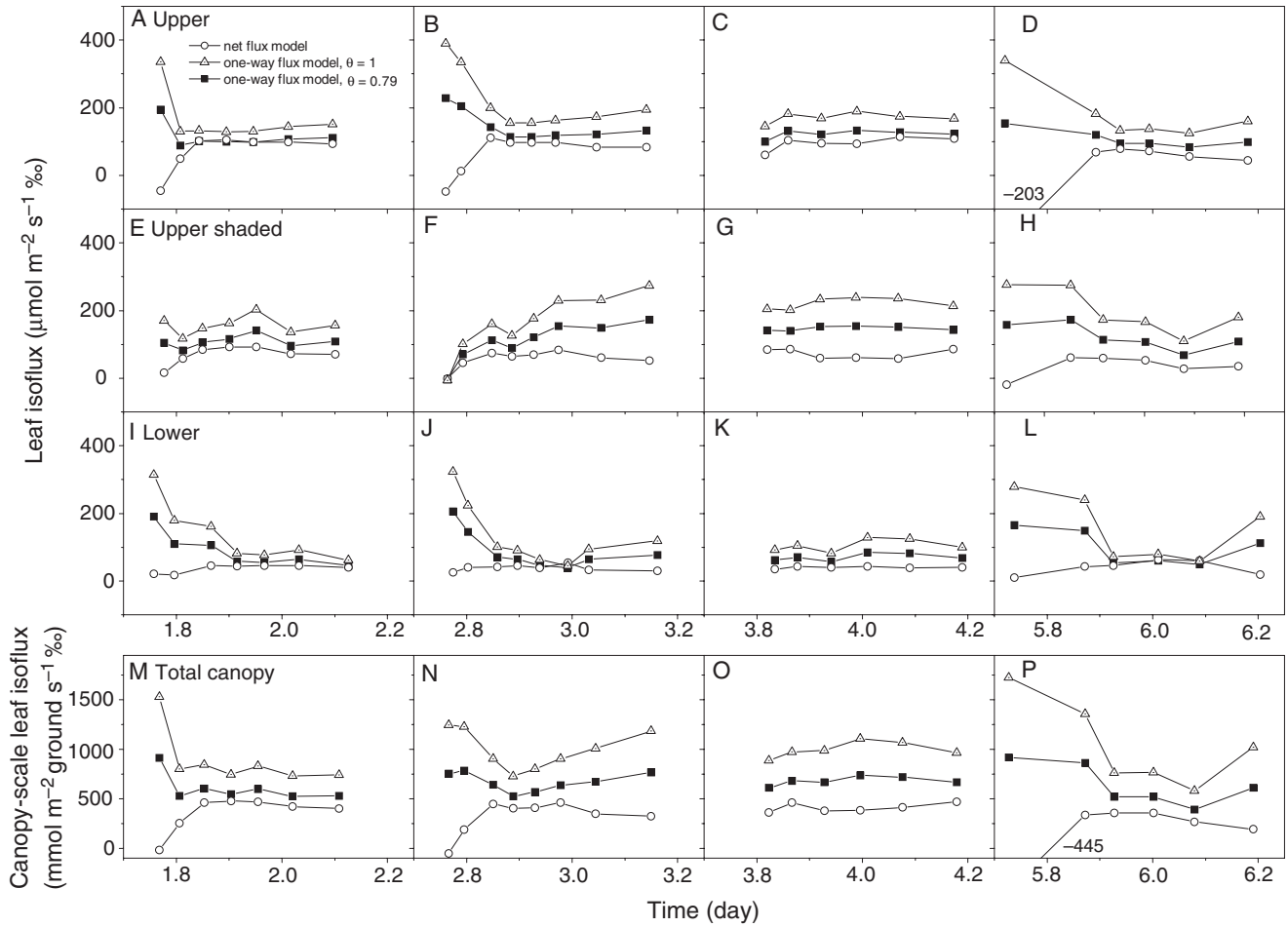

Fig. 8. Modelled nocturnal $C^{18}OO$ leaf isofluxes (i.e. modelled $\delta^{18}O$ of leaf-respired CO_2 multiplied by measured respiration rate) for *Quercus rubra* leaves at three positions within the canopy (*A–L*), and total canopy-scale leaf isoflux expressed per unit ground area (*M–P*). Sunlit 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

Species	Range in nocturnal g_{sw} (mmol m^{-2} s^{-1})	Average nocturnal g_{sw} (mmol m^{-2} s^{-1})	Significant g_{sw} (% of measurements)
Pinus ponderosa	1.4–11.0	3.9 ± 1.7	20
Pinus radiata	2.4–27.5	8.9 ± 0.9	54
Dacrydium cupressinum	0.0–21.5	4.8 ± 0.2	63
Weinmannia racemosa	0.0–70.1	11.2 ± 0.6	70
Quintinia acutifolia	0.0–81.9	15.1 ± 0.6	83
Quercus rubra	0.0–39.0	10.6 ± 0.2	87

references therein). 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 (Masle *et al*. 1992; McDonald *et al*. 2002; Snyder *et al*. 2003), nocturnal recovery from xylem cavitation during the day (Snyder *et al*. 2003), prevention of excess leaf turgor at night (Donovan *et al*. 2001), and continuation of O_2 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 g_{sw} and air saturation deficit for any species. This differs from observations by Bucci *et al*. (2004) of strong negative relationships between D and g_{sw} in three tropical tree species. For the *Q. rubra* leaves studied here, significant nocturnal g_{sw} tended to be more common in leaves switching from net positive to negative photosynthesis around sunset, and as g_{sw} increased in a 'pre-emptive' response to sunrise. That is, g_{sw} 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 g_{sw} 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 g_{sw} at midnight was explained by the average g_{sw} 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 g_{sw}. This is further evidence of limited stomatal regulation at night, as g_{sw} in the dark seems to be largely a function of stomatal conductance during the day. Conifers tend to have lower maximum g_{sw} than broadleaved trees (Wang *et al*. 1998; Tissue *et al*. 2005), and we report here that nocturnal g_{sw} tends to be higher among angiosperms than conifers.

Does nocturnal stomatal conductance result in differences between one-way and net flux models of the $\delta^{18}O$ of respired CO_2?

In the *Q. rubra* example given in the current paper, values of δ_{Rl} predicted by the net flux simplification were mostly within 50‰ of the one-way flux model (assuming full equilibration) when $g_{sw} < 10.0\,\text{mmol}\,\text{m}^{-2}\,\text{s}^{-1}$ (Figs 6, 7). Differences between the one-way flux model with full equilibration and the net flux simplification at low g_{sw} are due to C_a / C_c being greater than zero. The lowest modelled value for C_a / C_c is 0.35, when $g_{sw} = 2.2\,\text{mmol}\,\text{m}^{-2}\,\text{s}^{-1}$. Under these conditions the net flux approximation predicts δ_{Rl} to be 9.6‰ lower than the one-way flux model.

Interestingly, when incomplete equilibration of chloroplastic CO_2 is assumed in the one-way flux model, the effects of $C_a / C_c > 0$ are offset by the effect of $\theta < 1$, resulting in the difference between the net flux approximation and one-way flux modelled δ_{Rl} being smaller when $\theta = 0.79$ than when $\theta = 1$. Cernusak *et al*. (2004) estimated θ to be 0.79 in *R. communis* leaves respiring in the dark. θ is thought to be very close to unity when leaves are photosynthesising (Farquhar *et al*. 1993; Gillon and Yakir 2001; Cernusak *et al*. 2004). Given the requirement for accurate predictions of δ_{Rl} for ecosystem respiration partitioning, further investigation of variation in θ among leaves respiring in the dark is warranted. The assumption of θ less than one also requires knowledge of the value of δ_{c0}, 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 δ_{c0} 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 δ_{c0}.

Another assumption made in the current application of the one-way flux model is that Eqn 5 accurately describes the $\delta^{18}O$ of leaf-respired CO_2 when stomata are closed and CO_2 and water exchange occur exclusively through the cuticle. Cernusak *et al*. (2004) demonstrated that the one-way fluxes of CO_2 into and out of the leaf were important in determining δ_{Rl} for respiring leaves with stomatal conductances between 30 and 280 $\text{mmol}\,\text{m}^{-2}\,\text{s}^{-1}$. Further model testing is required at very low stomatal conductance and when gas exchange occurs primarily through the cuticle.

Enrichment in δ_{Rl} predicted by the one-way flux model was very high (up to 1310‰) in the *Q. rubra* example when g_{sw} was high near sunset and sunrise. Values for δ_{Rl} 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 δ_{Rl} 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 $\delta^{18}O$ of respiratory CO_2 for flux partitioning near sunset and sunrise.

To assess the effects of assuming $C_a / C_c = 0$ on partitioning the ecosystem respiratory flux into above- and below-ground components using $\delta^{18}O$ of ecosystem-respired CO_2 (δ_R) (ignoring stem and branch respiration), we assume that the soil respiration rate is constant at 3.0 $\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$

(an average value for temperate forests in mid-summer; Litton *et al*. 2003; Fisk *et al*. 2004; Kelliher *et al*. 2004; Ekblad *et al*. 2005). Further, we assume that $\delta^{18}O$ of soil-respired CO_2 (δ_{Rs}) 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 δ_{Rl}. A soil respiration rate of 3.0 μmol m^{-2} s^{-1} gives a proportional contribution of soil respiration to ecosystem respiration of ~0.27 over the four nights on which *Q. rubra* leaf respiration rates were measured. If the values of δ_R are generated using the one-way flux model with $\theta = 1$, then δ_R is always more enriched than either δ_{Rs} or δ_{Rl} predicted by the net flux approximation. Source partitioning when the isotope composition of the mixture lies outside the bounds of the sources is meaningless. If we assume incomplete equilibration of leaf respired CO_2 within the one-way flux model and constant δ_{Rs} to predict values of δ_R, then δ_R lies within the bounds of δ_{Rs} and net flux-predicted δ_{Rl} just one-third of the time over the four measurement nights (but note the inconsistency of assuming $\theta = 1$ with $C_a / C_c > 0$ and $\theta < 1$ with $C_a / C_c = 0$). Even when partitioning is possible, the net flux model both under- and over-estimates the proportional 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 CO_2 to partition the ecosystem isoflux, particularly close to sunset and sunrise. New online methods of isotopic analysis of CO_2 (e.g. online mass spectrometers: Schnyder *et al*. 2003; Klumpp *et al*. 2005; tunable diode laser absorption spectroscopy: Bowling *et al*. 2003*c*) will allow rigorous model testing at temporal resolutions orders of magnitude higher than previously attempted. We recommend applying Eqn 5 and the soil CO_2 invasion model (Tans 1998; Miller *et al*. 1999) to predict the $\delta^{18}O$ of CO_2 from the two main sources before attempting to partition the ecosystem respiratory flux.

Acknowledgments

Funding for this work by the Royal Society of New Zealand Marsden Fund (M.M.B., G.D.F. and D.W.; contract LCR201) is gratefully acknowledged. The original leaf respiration data were collected during work supported by funds from the Andrew W. Mellon Foundation (USA) and the Foundation for Research, Science and Technology (New Zealand).

References

Barbour MM, Roden JS, Farquhar GD, Ehleringer JR (2004) Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Péclet effect. *Oecologia* **138**, 426–435. doi: 10.1007/s00442-003-1449-3

Benyon RG (1999) Nighttime water use in an irrigated *Eucalyptus grandis* plantation. *Tree Physiology* **19**, 853–859.

Bottinga Y, Craig H (1969) Oxygen isotope fractionation between CO_2 and water, and the isotopic composition of marine atmospheric CO_2. *Earth and Planetary Science Letters* **5**, 285–295.

Bowling DR, McDowell NG, Welker JM, Bond BJ, Law BE, Ehleringer JR (2003*a*) Oxygen isotope content of CO_2 in nocturnal ecosystem respiration 1. Observations in forests along a precipitation transect in Oregon, USA. *Global Biogeochemical Cycles* **17**, 1120. doi: 10.1029/2003GB002081

Bowling DR, McDowell NG, Welker JM, Bond BJ, Law BE, Ehleringer JR (2003*b*) Oxygen isotope content of CO_2 in nocturnal ecosystem respiration. 2. Short-term dynamics of foliar and soil component fluxes in an old-growth ponderosa pine forest. *Global Biogeochemical Cycles* **17**, 1124. doi: 10.1029/2003GB002082

Bowling DR, Sargent SD, Tanner BD, Ehleringer JR (2003*c*) Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem–atmosphere CO_2 exchange. *Agricultural and Forest Meteorology* **118**, 1–19. doi: 10.1016/S0168-1923(03)00074-1

Boyer JS, Wong SC, Farquhar GD (1997) CO_2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiology* **114**, 185–191.

Brenninkmeijer C, Kraft P, Mook W (1983) Oxygen isotope fractionation between CO_2 and H_2O. *Isotope Geoscience* **1**, 181–190.

Bucci SJ, Scholz FB, Goldstein G, Meinzer FC, Hinojsa JA, Hoffmann WA, Franco AC (2004) Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. *Tree Physiology* **24**, 1119–1127.

Cappa CD, Hendricks MB, DePaulo DJ, Cohen RC (2003) Isotopic fractionation of water during evaporation. *Journal of Geophysical Research* **108**, 4525. doi: 10.1029/2003JD003597

Cernusak LA, Pate JS, Farquhar GD (2002) Diurnal variation in the stable isotope composition of water and dry matter in fruiting *Lupinus angustifolius* under field conditions. *Plant, Cell & Environment* **25**, 893–907. doi: 10.1046/j.1365-3040.2002.00875.x

Cernusak LA, Wong S-C, Farquhar GD (2003) Oxygen isotope composition of phloem sap in relation to leaf water in *Ricinus communis*. *Functional Plant Biology* **30**, 1059–1070. doi: 10.1071/FP03137

Cernusak LA, Farquhar GD, Wong SC, Stuart-Williams H (2004) Measurement and interpretation of the oxygen isotope composition of carbon dioxide respired by leaves in the dark. *Plant Physiology* **136**, 3350–3363. doi: 10.1104/pp.104.040758

Cernusak LA, Farquhar GD, Pate JS (2005) Environmental and physiological controls over carbon and oxygen isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. *Tree Physiology* **25**, 129–146.

Ciais P, Denning AS, Tans PP, Berry JA, Randall DA, *et al*. (1997) A three-dimensional synthesis study of $\delta^{18}O$ in atmospheric CO_2. 1. Surface fluxes. *Journal of Geophysical Research* **102**, 5857–5872. doi: 10.1029/96JD02360

Cowan IR, Farquhar GD (1977) Stomatal function in relation to leaf metabolism and environment. In 'Integration of activity in the higher plant'. (Ed. DH Jennings) pp. 471–505. (Cambridge University Press: New York)

Craig H, Gordon LI (1965) Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. In 'Proceedings of a conference on stable isotopes in oceanographic studies and palaeotemperatures'. (Ed. E Tongiorgi) pp. 9–130. (Lischi and Figli: Pisa)

Dongmann G, Nurnberg HW, Förstel H, Wagener K (1974) On the enrichment of $H_2{}^{18}O$ in the leaves of transpiring plants. *Radiation and Environmental Biophysics* **11**, 41–52. doi: 10.1007/BF01323099

Donovan LA, Linton MJ, Richards JH (2001) Predawn plant water potential does not necessarily equilibrate with soil water potential under well-watered conditions. *Oecologia* **129**, 328–335.

Ekblad A, Boström B, Holm A, Comstedt A (2005) Forest soil respiration rate and $\delta^{13}C$ is regulated by recent above ground weather conditions. *Oecologia* **143**, 136–142. doi: 10.1007/s00442-004-1776-z

Ethier GJ, Livingston NJ (2004) On the need to incorporate sensitivity to CO_2 transfer conductance into the Farquhar–von Caemmerer–Berry leaf photosynthesis model. *Plant, Cell & Environment* **27**, 137–153. doi: 10.1111/j.1365-3040.2004.01140.x

Farquhar GD, Lloyd J (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In 'Stable isotopes and plant carbon–water relations'. (Eds JR Ehleringer, AE Hall, GD Farquhar) pp. 47–70. (Academic Press: San Diego)

Farquhar GD, Cernusak LA (2005) On the isotopic composition of leaf water in the non-steady state. *Functional Plant Biology* **32**, 293–303.

Farquhar GD, Hubick KT, Condon AG, Richards RA (1989) Carbon isotope discrimination and water-use efficiency. In 'Stable isotopes in ecological research'. (Eds PW Rundel, JR Ehleringer, NA Nagy) pp. 21–46. (Springer-Verlag: New York)

Farquhar GD, Lloyd J, Taylor JA, Flanagan LB, Syvertsen JP, Hubick KT, Wong SC, Ehleringer JR (1993) Vegetation effects on the isotope composition of oxygen in atmospheric CO_2. *Nature* **363**, 439–443. doi: 10.1038/363439a0

Fisk MC, Fahey TJ, Groffman PM, Bohlen PJ (2004) Earthworm invasion, fine root distributions, and soil respiration in north temperate forests. *Ecosystems* **7**, 55–62.

Flanagan LB, Ehleringer JR (1991) Effects of mild water stress and diurnal changes in temperature and humidity on the stable oxygen and hydrogen isotopic composition of leaf water in *Cornus stolonifera* L. *Plant Physiology* **97**, 298–305.

Flanagan LB, Brooks JR, Varney GT, Ehleringer JR (1997) Discrimination against $C^{18}O^{16}O$ during photosynthesis and the oxygen isotope ratio of respired CO_2 in boreal forest ecosystems. *Global Biogeochemical Cycles* **11**, 83–98. doi: 10.1029/96GB03941

Flanagan LB, Kubien DS, Ehleringer JR (1999) Spatial and temporal variation in the carbon and oxygen stable isotope ratio of respired CO_2 in a boreal forest ecosystem. *Tellus* **51B**, 367–384.

Francey RJ, Tans PP (1987) Latitudinal variation in oxygen-18 of atmospheric CO_2. *Nature* **327**, 495–497. doi: 10.1038/327495a0

Gansert D (2003) Xylem sap flow as a major pathway for oxygen supply to the sapwood of birch (*Betula pubescens* Ehr.). *Plant, Cell & Environment* **26**, 1803–1814. doi: 10.1046/j.1365-3040.2003.01097.x

Gillon JS, Yakir D (2001) Influence of carbonic anhydrase activity in terrestrial vegetation on the ^{18}O content of atmospheric CO_2. *Science* **291**, 2584–2587. doi: 10.1126/science.1056374

Griffin KL, Anderson OR, Tissue DT, Turnbull MH, Whitehead D (2004) Variations in dark respiration and mitochondrial numbers within needles of *Pinus radiata* grown in ambient or elevated CO_2 partial pressure. *Tree Physiology* **24**, 347–353.

Grulke NE, Alonso R, Nguyen T, Cascio C, Dobrowolski W (2004) Stomata open at night in pole-sized and mature ponderosa pine: implications for O_3 exposure metrics. *Tree Physiology* **24**, 1001–1010.

Harwood KG, Gillon JS, Griffiths H, Broadmeadow MSJ (1998) Diurnal variation of $\Delta^{13}CO_2$, $\Delta C^{18}O^{16}O$, and evaporative site enrichment of $\delta H_2{}^{18}O$ in *Piper aduncum* under field condition in Trinidad. *Plant, Cell & Environment* **21**, 269–283. doi: 10.1046/j.1365-3040.1998.00276.x

Kato T, Nakazawa T, Aoki S, Sugawara S, Ishizawa M (2004) Seasonal variation of the oxygen isotopic ratio of atmospheric carbon dioxide in a temperate forest, Japan. *Global Biogeochemical Cycles* **18**, GB2020. doi: 10.1029/2003GB002173

Kays WM (1966) 'Convective heat and mass transfer.' (McGraw-Hill: New York)

Kelliher FM, Ross DJ, Law BE, Baldocchi DD, Rodda NJ (2004) Limitations to carbon mineralization in litter and mineral soil of young and old ponderosa pine forests. *Forest Ecology and Management* **191**, 201–213. doi: 10.1016/j.foreco.2003.12.005

Klumpp K, Schäufele R, Lötscher M, Lattanzi FA, Feneis W, Schnyder H (2005) C-isotope composition of CO_2 respired by shoots and roots: fractionation during dark respiration? *Plant, Cell & Environment* **28**, 241–250. doi: 10.1111/j.1365-3040.2004.01268.x

Li-Cor Biosciences (2002) 'Using the LI-6400 portable photosynthesis system.' (Li-Cor Biosciences Inc.: Lincoln, NE)

Litton CM, Ryan MG, Knight DH, Stahl PD (2003) Soil-surface carbon dioxide efflux and microbial biomass in relation to tree density 13 years after a stand replacing fire in a lodgepole pine ecosystem. *Global Change Biology* **9**, 680–696. doi: 10.1046/j.1365-2486.2003.00626.x

Masle J, Farquhar GD, Wong SC (1992) Transpiration ratio and plant mineral content are related among genotypes of a range of species. *Australian Journal of Plant Physiology* **19**, 709–721.

McDonald EP, Erickson JE, Kruger EL (2002) Can decreased transpiration limit plant nutrient acquisition in elevated CO_2? *Functional Plant Biology* **29**, 1115–1120. doi: 10.1071/FP02007

Miller JB, Yakir D, White JWC, Tans PP (1999) Measurement of $^{18}O/^{16}O$ in the soil–atmosphere CO_2 flux. *Global Biogeochemical Cycles* **13**, 761–774. doi: 10.1029/1999GB900028

Moroney JV, Bartlett SG, Samuelsson G (2001) Carbonic anhydrases in plants and algae. *Plant, Cell & Environment* **24**, 141–153.

Muchow RC, Ludlow MM, Fisher MJ, Myers RJK (1980) Stomatal behaviour of kenaf and sorghum in a semiarid tropical environment. I. During the night. *Australian Journal of Plant Physiology* **7**, 609–619.

Musselman RC, Minnick TJ (2000) Nocturnal stomatal conductance and ambient air quality standards for ozone. *Atmospheric Environment* **34**, 719–733. doi: 10.1016/S1352-2310(99)00355-6

Nobel PS (1983) 'Biophysical plant physiology and ecology.' (EH Freeman: New York)

Ogée J, Peylin P, Cuntz M, Bariac T, Brunet Y, Berbigier P, Richard P, Ciais P (2004) Partitioning net ecosystem carbon exchange into net assimilation and respiration with canopy-scale isotopic measurements: an error propagation analysis with $^{13}CO_2$ and $CO^{18}O$ data. *Global Biogeochemical Cycles* **18**, 2019.

Ometto JPH, Flanagan LB, Martinelli LA, Ehleringer JR (2005) Oxygen isotope ratios of waters and respired CO_2 in Amazonian forest and pasture ecosystems. *Ecological Applications* **15**, 58–70.

Parisi G, Perales M, Fornasaru MS, Colaneri A, Gonzalez-Schain N, *et al*. (2004) Gamma carbonic anhydrases in plant mitochondria. *Plant Molecular Biology* **55**, 193–207. doi: 10.1007/s11103-004-0149-7

Raschke K (1979) Movements of stomata. In 'Encyclopedia of plant physiology. Vol. 7'. (Eds W Haupt, ME Feinleib) pp. 383–441. (Springer: Berlin)

Šantrůček J, Šimáňová E, Karbulková J, Šimková M, Scheiber L (2004) A new technique for measurement of water permeability of stomatous cuticular membranes isolated from *Hedera helix* leaves. *Journal of Experimental Botany* **55**, 1411–1422. doi: 10.1093/jxb/erh150

Schnyder H, Schäufele R, Lötscher M, Gebbing T (2003) Disentangling CO_2 fluxes: direct measurements of mesocosm-scale natural abundance $^{13}CO_2$ / $^{12}CO_2$ gas exchange, ^{13}C discrimination, and labelling of CO_2 exchange flux components in controlled environments. *Plant, Cell & Environment* **26**, 1863–1874. doi: 10.1046/j.1365-3040.2003.01102.x

Seibt U, Wingate L, Berry JA, Lloyd J (2005) Diurnal discrimination against ^{13}C and ^{18}O during carbon uptake by *Picea sitchensis* branches: 3. Evidence for non-steady state effects in photosynthetic ^{18}O discrimination in the field. *Plant, Cell & Environment*, (In press).

Stern LA, Amundson R, Baisden WT (2001) Influence of soils on oxygen isotope ratio of atmospheric CO_2. *Global Biogeochemical Cycles* **15**, 753–760. doi: 10.1029/2000GB001373

Snyder KA, Richards JH, Donovan LA (2003) Night-time conductance in C_3 and C_4 species: do plants lose water at night? *Journal of Experimental Botany* **54**, 861–865. doi: 10.1093/jxb/erg082

Tans PP (1998) Oxygen isotopic equilibrium between carbon dioxide and water in soils. *Tellus* **50B**, 163–178.

Tissue DT, Griffin KL, Turnbull MH, Whitehead D (2005) Stomatal and non-stomatal limitations to photosynthesis in four tree species in a temperate rainforest dominated by *Dacrydium cupressinum* in New Zealand. *Tree Physiology* **25**, 447–456.

Turnbull MH, Whitehead D, Tissue DT, Schuster WSF, Brown KJ, Griffin KL (2001) Responses of leaf respiration to temperature and leaf characteristics in three deciduous tree species vary with site water availability. *Tree Physiology* **21**, 571–578.

Tyree MT, Wilmot TR (1990) Errors in the calculation of evaporation and leaf conductance in steady-state porometry: the importance of accurate measurement of leaf temperature. *Canadian Journal of Forestry Research* **20**, 1031–1035.

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387. doi: 10.1007/BF00384257

Wang X-F, Yakir D, Avishai M (1998) Non-climatic variations in the oxygen isotopic compositions of plants. *Global Change Biology* **4**, 835–849. doi: 10.1046/j.1365-2486.1998.00197.x

Whitehead D, Hall GMJ, Walcroft AS, Brown KJ, Landsberg JJ, *et al*. (2002) Analysis of the growth of rimu (*Dacrydium cupressinum*) in South Westland, New Zealand, using process-based simulation models. *International Journal of Biometeorology* **46**, 66–75. doi: 10.1007/s00484-001-0122-y

Whitehead D, Griffin KL, Turnbull MH, Tissue DT, Engel VC, Brown KJ, Schuster WSF, Walcroft AS (2004*a*) Response of total night-time respiration to differences in total daily photosynthesis for leaves in a *Quercus rubra* L. canopy: implications for modelling canopy CO_2 exchange. *Global Change Biology* **10**, 925–938. doi: 10.1111/j.1529-8817.2003.00739.x

Whitehead D, Walcroft AS, Griffin KL, Tissue DT, Turnbull MH, Engel V, Brown KJ, Schuster WSF (2004*b*) Scaling carbon uptake from leaves to canopies: insights from two forests with contrasting properties. In 'Forest at the land–atmosphere interface'. (Eds M Mencuccini, J Grace, J Moncrieff, KG McNaughton) pp. 231–254. (CAB International: Wallingford)

Yakir D, Wang X-F (1996) Fluxes of CO_2 and water between terrestrial vegetation and the atmosphere estimated from isotope measurements. *Nature* **380**, 515–517. doi: 10.1038/380515a0

Zeiger E, Field C, Mooney HA (1981) Stomatal opening at dawn: possible roles of the blue light response in nature. In 'Plants and the daylight spectrum'. (Ed. H Smith) pp. 391–407. (Academic Press: London)

Manuscript received 17 May 2005, accepted 12 August 2005