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Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Péclet effect

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Abstract There is an increasing ecological interest in understanding the gradients in $H_2{}^{18}O$ enrichment in leaf water (i.e. a Péclet effect), because an appreciation of the significance of the Péclet effect is important for improving our understanding of the mechanistic processes affecting the ^{18}O composition of leaf water and plant organic material. In data sets where both source water and leaf water ^{18}O data are available, we can evaluate the potential contribution of a Péclet effect. As an example, we recalculate data published earlier by Roden and Ehleringer (1999, Oecologia 121:467–477) as enrichments in leaf water (Δ_L) and cellulose (Δ_{cell}) above source water. Based on these recalculations, we present support for the relevance of a Péclet effect in leaves. Further, we demonstrate that the subtle variations in Δ_L and Δ_{cell} caused by a Péclet effect may be masked in experimental systems in which variation in the source water oxygen isotope ratio is considerable.

Keywords Oxygen-18 · Leaf water enrichment · Tree ring cellulose · Leaf gas exchange · Humidity

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Introduction

The oxygen isotope composition ($\delta^{18}O$) of plant cellulose is of considerable interest to a number of different disciplines, including paleoclimatology, agriculture and ecology. Paleoclimatological studies use tree ring isotope ratios as correlative parameters to estimate the local prevailing temperature at the time when the cellulose was formed by assuming that $\delta^{18}O$ of cellulose reflects $\delta^{18}O$ of water taken up by the tree (e.g. Gray and Thompson1977). The oxygen isotope composition of soil water is normally closely linked to that of rainfall, and because $\delta^{18}O$ of rainfall is known to vary with temperature, tree rings are viewed as "isotopic thermometers" (Libby et al.1976). Yet we also know that the isotopic composition of precipitation is a function of season (i.e. winter versus summer) instead of annual temperature, which restricts the potential of using ^{18}O as a simple thermometer (Ehleringer and Dawson1992; Lin et al.1996; Schwinning et al.2002). While variation in $\delta^{18}O$ of water taken up by a plant is the baseline for variation in $\delta^{18}O$ of cellulose, variation can also result from variation in evaporative enrichment of leaf water during transpiration. This evaporative effect has prompted plant breeders and physiological ecologists to suggest that $\delta^{18}O$ of cellulose may represent an integrated record of environmental and stomatal regulation of water loss (Farquhar et al.1994; Barbour and Farquhar2000; Barbour et al. 2000a).

Recent work (Helliker and Ehleringer2002) has demonstrated that when grass plants were grown in conditions of constant source water $\delta^{18}O$, variation in leaf water enrichment is recorded in $\delta^{18}O$ of leaf cellulose. However, in studies with variable $\delta^{18}O$ of source water, leaf enrichment effects might be less obvious. Barbour et al. (2003) suggested that whenever possible removing spatial and temporal variation in source water $\delta^{18}O$, by presenting cellulose $\delta^{18}O$ as enrichments above source water ($\Delta^{18}O$), would allow the more subtle effects of leaf evaporative enrichment to be studied. This paper aims to highlight an advantage to presenting isotope compositions relative to the source water by recalculating previously published

cellulose data from an experiment in which $\delta^{18}O$ of source water varied widely (by >10‰).

Materials and methods

Isotope theory

The absolute isotopic composition of a substance is difficult to measure directly, so isotope ratios are generally presented in relation to a standard (δ) or to the source material (Δ). In the case of $^{18}O/^{16}O$ the standard is commonly Vienna-Standard Mean Oceanic Water (V-SMOW), with an isotope ratio of 2.0052×10^{-3} (Gonfiantini et al.1965), and isotope compositions are expressed as deviations from this ratio:

$$\delta = \frac{R}{R_{st}} - 1 \tag{1}$$

where R and R_{st} are the isotope ratios of the substance of interest and the standard, respectively. Oxygen isotope ratios of plant water or organic material can also be expressed as deviations from the ratio of water taken up by the plant:

$$\Delta = \frac{R}{R_s} - 1 \tag{2}$$

where R_s is the $^{18}O/^{16}O$ of source water.

Recent work (e.g. Roden et al.2000; Barbour et al. 2000a, 2000b; Helliker and Ehleringer2002) has highlighted the main processes resulting in variation in oxygen isotope ratios in plant water and organic material. Variation in $\delta^{18}O$ of water in plants is a result of (1) variation in $\delta^{18}O$ of water taken up by the plant, (2) variation in the enrichment of leaf water during transpiration, (3) mixing of enriched leaf water and unenriched source water within the plant. Variation in $\delta^{18}O$ of organic material is a result of all of the above variation, and (4) variation in the extent of isotopic exchange between water and organic molecules during biosynthesis. These processes are formalized in the models described below. The models fall into two parallel forms: those that model variation in plant $^{16}O/^{16}O$ including variation in source water ($\delta^{18}O$) and those that model variation in $^{18}O/^{16}O$ of the plant independently of variation in source water ($\Delta^{18}O$).

Water at the sites of evaporation is enriched because the heavier $H_2{}^{18}O$ molecule diffuses more slowly and has a lower vapour pressure than $H_2{}^{16}O$. Craig and Gordon (1965) presented a model relating enrichment in an open water body to the isotopic fractionation during the phase change from liquid to vapour, and during diffusion. This model was extended to include leaf boundary layer effects and diffusion through stomata (Dongmann et al.1974; Flanagan et al. 1991b):

$$R_{lw} = \alpha \times \left[\alpha_k R_{wx}\left(\frac{e_i - e_s}{e_i}\right) + \alpha_{kb} R_{wx}\left(\frac{e_s - e_a}{e_i}\right) + R_a\left(\frac{e_a}{e_i}\right)\right] \tag{3a}$$

where R is the $^{18}O/^{16}O$ ratio of the substance, e is the water vapour pressure, and subscripts lw, a, i, s and wx refer to leaf water, bulk air, intercellular air spaces, leaf surface and xylem water, respectively. α^* is the liquid-vapour equilibrium fractionation factor, α_k is kinetic fractionation associated with diffusion through air, and α_{kb} is the kinetic fractionation associated with diffusion through the boundary layer. Equation 3a may also be expressed in $\delta^{18}O$ terms, and to a close approximation is given by (Farquhar et al.1989; Saurer et al.1997),

$$\delta_e = \delta_s + \varepsilon_k + \varepsilon* + (\delta_v - \delta_s - \varepsilon_k)\frac{e_a}{e_i} \tag{3b}$$

where δ_e, δ_s and δ_v are the oxygen isotopic compositions (relative to V-SMOW) of water at the evaporating sites, source water and atmospheric water vapour, respectively, ε_k is the kinetic fractionation as water vapour diffuses through the stomata and the boundary layer, and ε^* is the fractionation associated with the proportional depression of water vapour by $H_2{}^{18}O$ ($\varepsilon^*=1-\alpha^*$). ε^* (‰) is sensitive to temperature (T, in K) such that (Majoube1971):

$$\varepsilon* = \exp\left(\frac{1,137}{T^2} - \frac{0.4156}{T} - 0.0020667\right) - 1 \tag{4}$$

so that ε^* is 9.1‰ at 25°C and 9.5‰ at 20°C. $H_2{}^{18}O$ diffuses 1.032 times more slowly than $H_2{}^{16}O$ in air and through stomata (i.e. α_k=1.032; Cappa et al.2003), but 1.021 times more slowly through the laminar boundary layer (i.e. α_{kb}=1.021) from Polhausen analysis (Farquhar et al.1989). ε_k becomes:

$$\varepsilon_k = \frac{32r_s + 21r_b}{r_s + r_b} \tag{5}$$

where r_s and r_b are stomatal and boundary layer resistances to water flux, respectively. Note that values for α_k and α_{kb} have been revised from those published by Merlivat (1978; 1.028 and 1.019, respectively), reflecting the recent work of Cappa et al. (2003).

Equation 3a and b may also be derived in terms of enrichment above source water (Δ_e), so that Eq. 3a is exactly equivalent to (Farquhar and Lloyd1993; Farquhar and Gan2003):

$$\Delta_e = (1 + \varepsilon*)\left[1 + \varepsilon_k + (\Delta_v - \varepsilon_k)\frac{e_a}{e_i}\right] - 1 \tag{6a}$$

and Eq. 3b is approximated by,

$$\Delta_e = \varepsilon* + \varepsilon_k + (\Delta_v - \varepsilon_k)\frac{e_a}{e_i} \tag{6b}$$

where Δ_v is the oxygen isotope composition of atmospheric water vapour relative to plant source water (i.e. xylem water).

Roden and Ehleringer (1999a) confirmed the predictive power of Eq. 3a from measurements of leaf water (δ_{lw}) made under wide-ranging environmental conditions. A good fit of modelled δ_e on measured δ_{lw} was made with the inclusion of a factor (p_v) describing the proportion of leaf water unenriched by evaporation:

$$\delta_{lw} = (1 - p_v)\delta_e + p_v\delta_s \tag{7}$$

or, in terms of enrichment above source water:

$$\Delta_{lw} = \Delta_e(1 - p_v) \tag{8}$$

Roden and Ehleringer (1999a, 2000) found that when the mid-vein was removed from the sample prior to the extraction of water, a value of 0.1 for p_v best fitted measured δ_{lw}.

Other studies also found that the modified Craig-Gordon model overestimated leaf water enrichment (e.g. Allison et al.1985; Yakir et al.1990; Flanagan et al. 1991a, 1991b; Wang et al.1998), and some found that the discrepancy between measured and modelled leaf water enrichments increased with increasing transpiration (Walker et al.1989; Flanagan et al.1994). Farquhar and Lloyd (1993) suggested that this response may be partly explained by the transpirational convection of unenriched water to the evaporating sites opposed by the backward diffusion of $H_2{}^{18}O$ into the leaf: a Péclet effect. The Péclet effect is characterized by a dimensionless number, $\wp$, which describes the ratio of convection to diffusion by:

$$\wp = \frac{LE}{CD} \tag{9}$$

where L is the effective length (= actual distance in meters from the evaporating surface multiplied by a scaling factor that describes the tortuous path of water through the leaf), E is the evaporation rate (mol $m^{-2}s^{-1}$), C is the molar density of water (55.5×10^3 mol m^{-3}) and D the diffusivity of the $H_2{}^{18}O$ in water (2.66×10^{-9} m^2 s^{-1}). Average leaf water enrichment above source water (Δ_L) is then given by (Farquhar and Lloyd1993):

$$\Delta_L = \frac{\Delta_e(1 - e^{-\wp})}{\wp} \tag{10}$$

where Δ_e is derived from Eq. 6b. The effective length is expected to be much larger than the actual distance between the vein and the evaporating surface because of the tortuous pathway for water movement. As such, L is expected to increase with increasing leaf thickness and to vary if the arrangement of cells within the leaf changes the pathway for water movement. For example, Flanagan et al. (1993) calculated (from Eqs. 9 and 10) an effective length for the mistletoe *Phoradendron juniperinum* and its host *Juniperus osteosperma* of 22 and 27 mm, respectively, while Flanagan et al. (1994) estimated L to be 6.2 and 8.5 mm for *Phaseolus vulgaris* grown in different conditions. Wang et al. (1998) calculated a range in L from 4 to 166 mm for a wide range of species grown in a common garden.

Strong, although indirect, evidence of a Péclet effect in leaf water was found from $\Delta^{18}O$ measurements of sucrose bled from castor bean leaves (Barbour et al. 2000b). Further, other work has shown that anatomical dimensions and velocities of water movement within leaves could allow significant gradients in enrichment to develop (Barbour and Farquhar 2003). Gan et al. (2002) recently examined the progressive enrichment of water in the veins of cotton, and concluded that there needed to be treatments of the Péclet effect over different scales within the leaf. These scales include the distance between the minor veins and the stomata, and along the leaf. Farquhar and Gan (2003) attempted such a model, and found that Eq. 10 still holds as a model of average enrichment of lamina mesophyll water and so is applicable to data in this paper.

Equations 9 and 10 imply that the fractional difference between the isotopic enrichment of laminar mesophyll water and that at the sites of evaporation should increase with increasing E in a curvilinear fashion. Formally expressed; $1–\Delta_L/\Delta_e$ should be positively related to E. Such a relationship has been found for both common bean (Flanagan et al.1994) and, indirectly, for castor bean (Barbour et al. 2000b). No relationship between the two is expected if Eqs. 6a and 8 adequately model leaf water enrichment.

Oxygen in organic molecules often reflects the isotopic ratio of water in which the molecules formed, due to isotopic exchange between carbonyl oxygen and water (Sternberg et al.1986). At equilibrium the carbonyl oxygen in acetone is 27‰ more enriched than the water with which it exchanged (Sternberg and DeNiro1983). Many intermediates in the biochemical pathways leading to cellulose synthesis contain carbonyl oxygen, and evidence of the importance of this exchange was found in cellulose from aquatic plants, which was 27±4‰ more enriched than the water in which the plants grew (Yakir and DeNiro1990).

While oxygen atoms in sucrose are not able to exchange with water during export from a source leaf, exchange between oxygen in triose phosphates and chloroplastic water may occur during the Calvin cycle, and further exchange with cytosolic water may occur during sucrose formation (Farquhar et al.1998). Chloroplastic and cytosolic water may be some distance from water at the evaporating sites, suggesting that sucrose may reflect laminar mesophyll $\Delta^{18}O$ rather than evaporation site $\Delta^{18}O$. Or in other words, $\Delta^{18}O$ of the water with which sucrose has exchanged may be closer to the Péclet Δ_L than the Craig-Gordon Δ_e. Sucrose bled from castor bean leaves had $\Delta^{18}O$ values differing significantly from Craig-Gordon modelled values, and accurately predicted by Eq. 10 (modified to describe sucrose rather than leaf water $\Delta^{18}O$), with an effective length of 13.5 mm.

When sucrose reaches the sink tissue, it is cleaved to form hexose phosphates, allowing one of the five oxygen atoms in a cellulose unit to exchange with local water (Farquhar et al.1998). A proportion (y) of hexose phosphates also passes through a futile triose phosphate cycle (Hill et al.1995), allowing a further three of the five oxygen atoms in a cellulose unit to exchange with local water, before becoming non-exchangeable in cellulose. The proportion of oxygen atoms in cellulose that have exchanged with local water in the cell synthesising cellulose (p_{ex}) is given by (Barbour and Farquhar2000):

$$p_{ex} = 0.2 + y\left(0.6 + \frac{0.2}{2 - y}\right) \tag{11}$$

The term $(1–y)$ is the proportion of hexose phosphates that immediately form cellulose without recycling through triose phosphates. Hence, when all hexose phosphates are recycled through triose phosphates, i.e. y=1, then p_{ex} will also be 1, and $\delta^{18}O$ of cellulose will reflect only variation in source water $\delta^{18}O$.

However, cellulose has been shown to record leaf evaporative conditions (e.g. Roden and Ehleringer 1999b; Barbour and Farquhar2000; Helliker and Ehleringer2002). As such, y (and p_{ex}) must be numerically less than unity, and cellulose from transpiring plants must reflect both exchange with enriched leaf water during sucrose synthesis, and local stem water during cellulose synthesis. Roden and Ehleringer (1999b) formalized this understanding by suggesting that the $\delta^{18}O$ of cellulose (δ_{cell}) is related to the fraction of oxygen atoms in cellulose that exchange during cellulose synthesis (f_0), and the equilibrium fractionation factor between carbonyl oxygen and water (ε_{wc}) by:

$$\delta_{cell} = f_0(\delta_s + \varepsilon_{wc}) + (1 - f_0)(\delta_e + \varepsilon_{wc}) \tag{12a}$$

For a number of riparian tree species f_0 was found to be 0.42 (Roden et al.2000). Evidence presented by Barbour et al. (2000b) suggests that oxygen atoms in sucrose have exchanged with laminar mesophyll water, rather than water at the evaporation sites, so that Eq. 12a may be rewritten:

$$\delta_{cell} = f_0(\delta_s + \varepsilon_{wc}) + (1 - f_0)(\delta_{lw} + \varepsilon_{wc}) \tag{12b}$$

As with Eq. 3b, Eqs. 12a and 12b may also be re-written in terms of enrichment of cellulose above source water (Δ_{cell}) as (Barbour and Farquhar2000):

$$\Delta_{cell} = \Delta_e(1 - p_{ex}) + \varepsilon_{wc} \tag{13a}$$

and:

$$\Delta_{cell} = \Delta_L(1 - p_{ex}p_x) + \varepsilon_{wc} \tag{13b}$$

where p_{ex} is the proportion of exchangeable oxygen in cellulose formed from sucrose (p_{ex}=f_0) and p_x is the proportion of unenriched (xylem) water in the cell forming the cellulose. Both Eqs. 12b and 13b assume that sucrose exported to the stem to form cellulose is in equilibrium with leaf water, as demonstrated by Barbour et al. (2000b). However, Eq. 13b differs from Eqs. 12a, 12b and 13a in the inclusion of the p_x term, which allows for the possibility of enriched leaf water being unloaded into the developing cell with sucrose (Barbour and Farquhar2000).

Comparing leaf water and meristem water $\delta^{18}O$ in a number of grass species, Helliker and Ehleringer (2002) calculated p_x between 0.50 and 0.62. Barbour et al. (2002) argue that water in the stems of large trees may be expected to be unaffected by leaf water enrichment (i.e. p_x=1) because the distance between source leaf and sink tissue would allow significant exchange of water between the phloem and the xylem. We make no assumptions as to the value

of either p_{ex} or p_x in the analysis presented here. Rather, the combined factor $p_{ex}p_x$ is varied to produce the best fit of measured on modelled Δ_{cell}.

The Péclet effect means that the fractional difference (F, when calculated from leaf water isotope ratios, or F' when calculated from organic matter isotope ratios) between leaf water and evaporation site enrichment (F=1–Δ_L/Δ_e) should increase with increasing transpiration rate. Rearranging Eq. 13b we find that:

$$\Delta_L = \frac{\Delta_{cell} - \varepsilon_{wc}}{1 - p_{ex}p_x} \quad (14)$$

so that the function:

$$F' = 1 - \frac{\left(\frac{\Delta_{cell} - \varepsilon_{wc}}{1 - p_{ex}p_x}\right)}{\Delta_e} \quad (15)$$

should increase with increasing E. ε_{wc} is thought to be constant, at 27‰, and if $p_{ex}p_x$ is also assumed to be rather constant, then:

$$F' = 1 - \frac{\Delta_{cell} - \varepsilon_{wc}}{\Delta_e} \quad (16)$$

will increase with increasing E if the Péclet effect is relevant to leaf water enrichment.

Methods

Data in this paper were taken from previously published work (Roden and Ehleringer 1999b) in which alder (*Alnus incana* L. Moench), water birch (*Betula occidentalis* Hook) and cottonwood (*Populus fremontii* Wats) were grown hydroponically in glasshouses at low and high humidity (average relative humidity of 40 and 73%, respectively) and with six different $\delta^{18}O$ of tank water between −15.5 and +9.8‰. The average air temperature was 25°C and 27°C for high and low humidity glasshouses, respectively. The experimental design and setup are described in more detail in Roden and Ehleringer (1999b).

As pointed out by Roden et al. (2000), errors in relative humidity (RH) measurement have the largest effect on modelled values of Δ_L when water vapour $\delta^{18}O$ widely departs from equilibrium with source water $\delta^{18}O$ minus ε_k. The slope of the relationship between Δ_e and RH with changing Δ_v is shown in Fig.1. When Δ_v is −10‰, a 10% error in RH measurement will result in a 3.2‰ error in Δ_e calculation, while if Δ_v is –30‰ the same error in RH measurement will result in a 4.9‰ error in the modelled value. Given that RH varied between 55 and 95% and between 23 and 55% for the high and low humidity glasshouses, respectively, we exclude data collected from treatments in which Δ_v is lower than –16‰. Only trees grown with source water $\delta^{18}O$ of –15.5, –10.3 and –5.5‰ are included in this analysis.

At the end of the growing season wood from the outer growth ring was sampled, and α-cellulose extracted using the soxhlet solvent extraction technique (e.g. Leavitt and Danzer1992). Tank water and atmospheric water vapour were also sampled for $\delta^{18}O$ analysis throughout the growth season as described in Roden and Ehleringer (1999b). Stomatal conductance (g_s) and leaf temperature (T_l) were measured using a steady-state diffusion porometer (Li Cor 1600, Lincoln, Neb., USA) on three occasions during the growth period. As gas exchange measurements did not coincide with leaf water sampling, an average leaf temperature over the growth period is more appropriate. Average T_l (°C) was estimated from regression analysis of measured T_l on air temperature (T_a; °C) and RH (%) from porometer measurements. For alder:

$$T_l = 1.453T_a + 0.0086\text{RH} - 10.74,\ r^2 = 0.97 \quad (17)$$

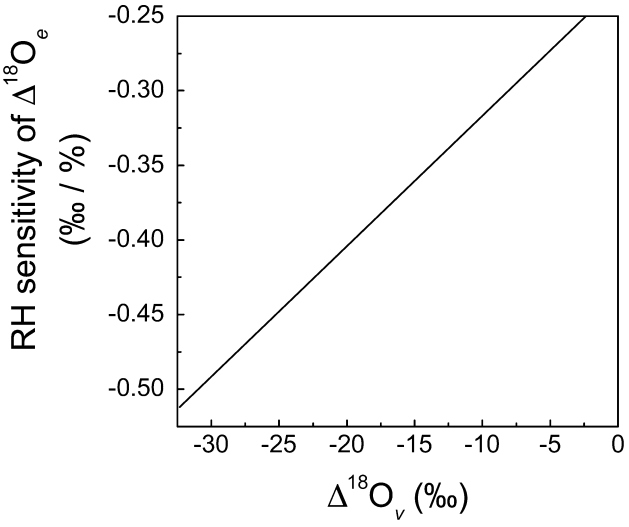

Fig. 1 The sensitivity of Craig-Gordon modelled $H_2{}^{18}O$ enrichment at the evaporating sites (Δ_e) to errors in relative humidity measurement (a ‰ decrease in Δ_e with a 1% increase in relative humidity) when the difference between atmospheric and source water oxygen isotope ratios (Δ_v) varies

for birch:

$$T_l = 1.307T_a + 0.0059\text{RH} - 7.13,\ r^2 = 0.90 \quad (18)$$

and for cottonwood:

$$T_l = 1.267T_a + 0.0120\text{RH} - 6.24,\ r^2 = 0.82 \quad (19)$$

Using estimated T_l, measured RH and T_a, and average g_s, w_i and w_a were calculated and E was estimated from:

$$E = (w_i - w_a)\left(\frac{1}{r_s + r_b}\right) \quad (20)$$

where w_i and w_a are mol fractions of water vapour in the leaf intercellular spaces and ambient air, respectively, and r_b is assumed to be 1 m^2 s mol^{-1}. Average parameter values over the growth period were used to model cellulose enrichments, and daily values were used to model leaf water enrichment.

Leaf water samples from the plants grown at –15.5‰ source water were collected four times during the growth period, as described in Roden and Ehleringer (1999a). Briefly, leaf material with the midvein removed was frozen in a sealed glass vial, and the water extracted cryogenically. This unpublished leaf water data set, from the same experiment for which cellulose samples were taken (Roden and Ehleringer 1999b), is presented in Fig. 2. Also presented are measured T_a, RH and δ_v, and estimated T_l and E for each day of gas exchange measurement.

Results

Stomatal conductance and transpiration rate

Average stomatal conductance (g_s), and estimated transpiration rate and leaf temperature for each species and humidity treatment are presented in Table 1. g_s was not significantly different between humidity treatments, but the estimated transpiration rate in the high humidity

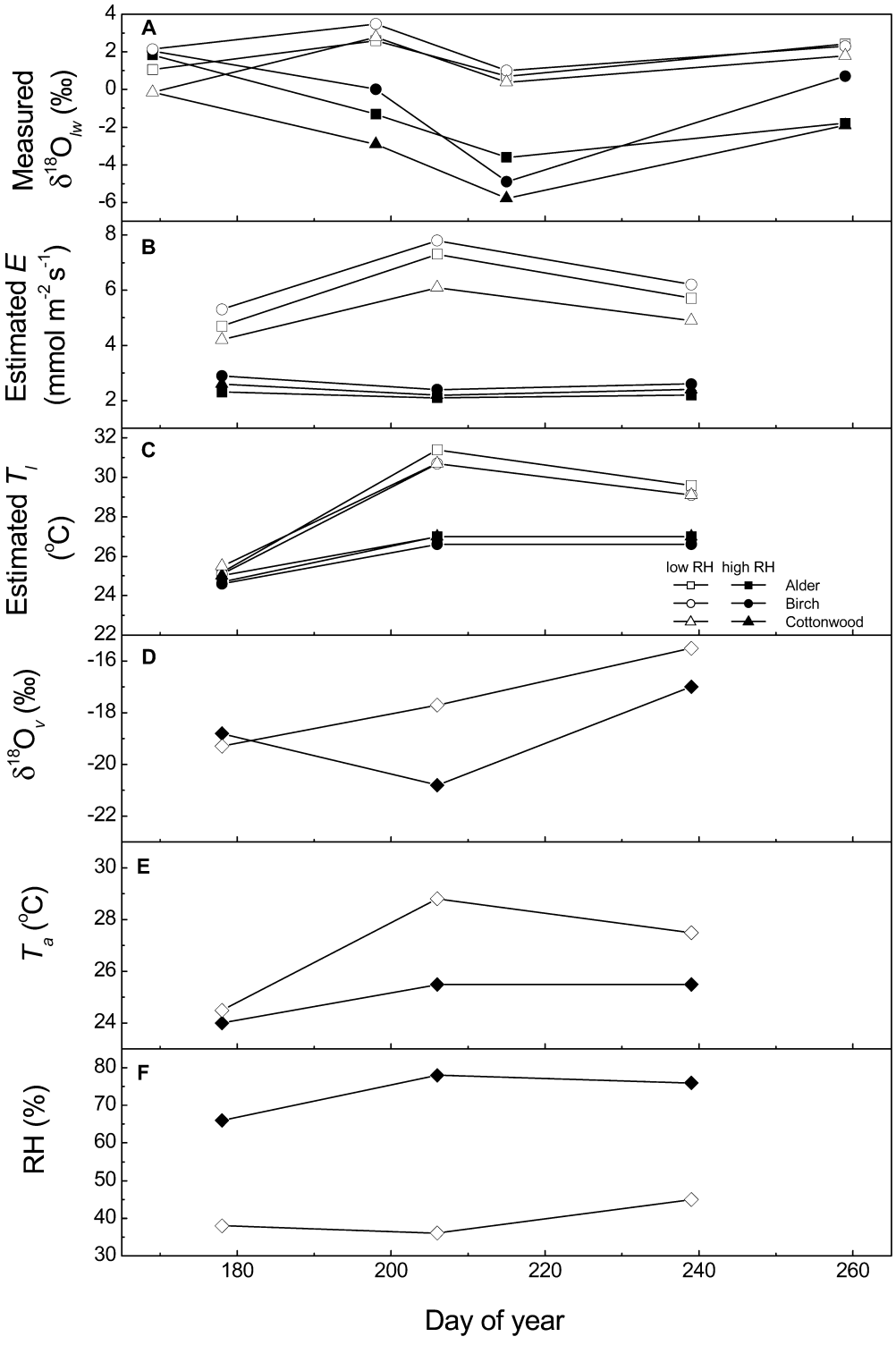

Fig. 2 Environmental conditions (**D**, $\delta^{18}O$ of glasshouse water vapour; **E**, air temperature; **F**, relative humidity), estimated leaf evaporative conditions (**B**, transpiration rate; **C**, leaf temperature) and measurements of leaf water $\delta^{18}O$ (**A**) during the growth period. For **A–F** *filled symbols* represent data from the high humidity glasshouse and *open symbols* data from the low humidity glasshouse. In **A–C** *square*, *circular* and *triangular symbols* represent alder, birch and cottonwood data, respectively

glasshouse was about 50% of that in the low because of a reduced (w_i–w_a) gradient. Birch leaves had the highest g_s and E, on average, and cottonwood the lowest (Fig. 2 and Table 1).

Leaf water enrichment

In a previous experiment, Roden and Ehleringer (1999a) found very good agreement between measured and modelled leaf water $\delta^{18}O$ using the Craig-Gordon model of leaf water enrichment. However, for leaf water samples taken during the cellulose experiment (Roden and Ehleringer 1999b) Eqs. 6a and 8 tend to overestimate

Table 1 Average gas exchange measurements and oxygen isotope composition of leaf water for three riparian tree species grown at two relative humidities (*RH*) and used for modelling of cellulose ^{18}O enrichment. Leaf temperatures (T_l) and evaporation rates (E) are estimates over the growth period, and are calculated as described in the Methods section. Stomatal conductance (g_s) values are averages from measurements made over 3 days throughout the growing season

Species	RH (%)	T_a (°C)	Measured g_s (mol m^{-2} s^{-1})	Estimated T_l (°C)	Estimated E (mol m^{-2} s^{-1})
Alder	40	27	0.24±0.02	28.7	5.9
Alder	73	25	0.21±0.02	26.2	2.2
Birch	40	27	0.29±0.02	28.3	6.4
Birch	73	25	0.28±0.03	26.0	2.6
Cottonwood	40	27	0.21±0.02	28.4	5.1
Cottonwood	73	25	0.23±0.03	26.3	2.4

leaf water enrichment above source water at low humidity (by about 2‰) when 10% of the leaf water is assumed to be unaffected by evaporation (i.e. p_v=0.1). If p_v is allowed to vary, to optimise the fit of measured on modelled Δ_L values, data from high and low humidity treatments never fall on the 1:1 line at the same time (see Fig. 3A). This suggests that the Craig-Gordon model does not include an important process (or processes) affecting Δ_L. Using the Péclet extension of the Craig-Gordon model, data from both high and low humidity treatments fall closer to the 1:1 line when the effective length for the Péclet effect is 23 mm (Fig. 3B).

A better test of the relevance of a Péclet effect to leaf water $H_2{}^{18}O$ enrichment may be made by plotting the fractional difference between measured and modelled leaf water $\Delta^{18}O$ versus transpiration rate (E). The Péclet effect proposes a positive relationship between E and F, while no relationship should exist if the Craig-Gordon model adequately describes Δ_L. Fig. 4 shows that as E increases, F increases in all species, in support of the Péclet model. The species seem to differ in the effective length (L) over which the Péclet effect occurs. Cottonwood seems to have the longest effective length, of about 32 mm, while birch seems to be rather smaller, and about 19 mm. When values of L for each individual species taken from Fig. 4 are used in the Péclet model of leaf water enrichment, measured Δ_L is very well predicted. Fig. 3C shows that the model including estimated L for each species explains 90% of variation in measured Δ_L, with a slope of 1.12 (not significantly different from unity; P<0.05) and an intercept of –1.79‰ (not significantly different from 0; P<0.05).

^{18}O enrichment in cellulose

The Craig-Gordon model and Eq. 12a predict 97% of variation in δ_{cell} when f_0 = 0.60 (Fig. 5A). The model accurately predicts δ_{cell} at δ_s=–10.3‰, but tends to underestimate δ_{cell} at δ_s=–15.5‰, resulting in a slope of less than one. The model including a Péclet effect also predicts 97% of variation in δ_{cell} when L = 20 mm and

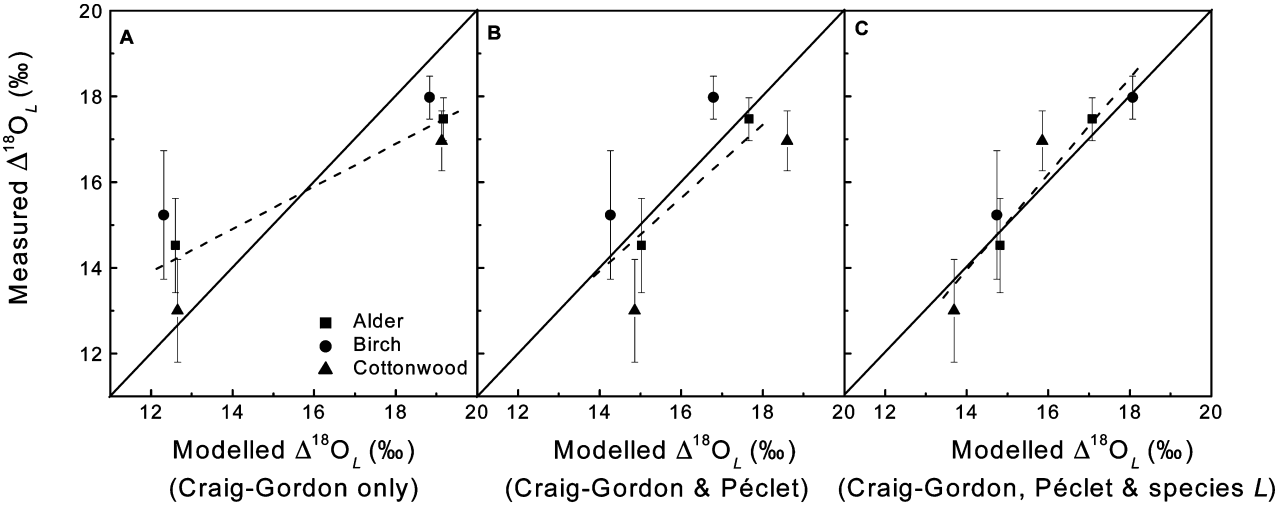

Fig. 3 The relationship between measured and modelled laminar mesophyll water enrichment (Δ_L) for tree species grown at high and low humidity: **A** the Craig-Gordon model, **B** the Péclet effect model using a single fitted effective length, and **C** the Péclet effect model using effective lengths for each species taken from Fig. 4. *Solid lines* represent the 1:1 relationship and *dashed lines* a least squares regression. In **A** p_v=0.28 and Δ_L measured =8.22+0.48 Δ_L modelled, r^2=0.80, in **B** L=23 mm and Δ_L measured =1.08+0.98 Δ_L modelled, r^2=0.56, and in **C** L=25, 19 and 34 mm for alder, birch and cottonwood, respectively, and Δ_L measured =–1.79+1.12 Δ_L modelled, r^2=0.90. *Error bars* represent standard errors of the mean values

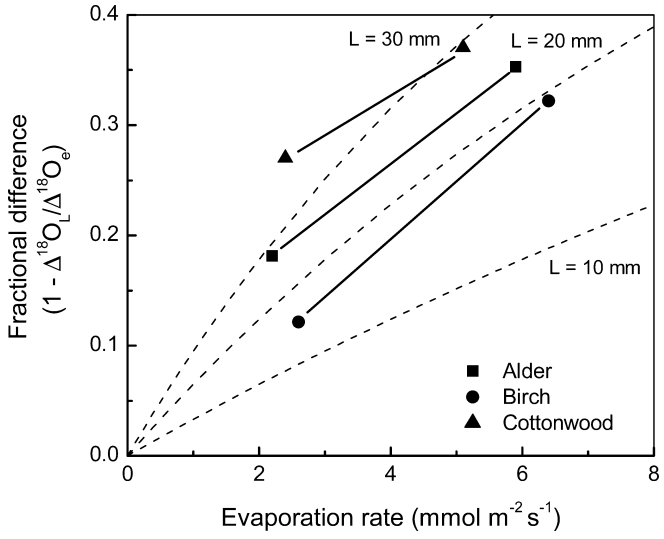

Fig. 4 The relationship between evaporation rate and the fractional difference between measured bulk leaf water enrichment (Δ_L) and Craig-Gordon modelled enrichment (Δ_e), $1-\Delta_L/\Delta_e$, for three riparian tree species grown at high and low humidity. The predicted relationships at different effective lengths for the Péclet effect are plotted as *dashed lines*

$p_{ex}p_x$=0.40, but the slope of the relationship is closer to one (0.80) (see Fig. 5B).

When cellulose isotope ratios are presented relative to the source water, the inaccuracy of the Craig-Gordon model in predicting Δ_{cell} becomes more apparent, with just 75% of measured variation in Δ_{cell} explained by the model when p_{ex}=0.60 (Fig. 6A). In contrast, if a Péclet effect is included in the model of leaf water enrichment, the model explains 89% of variation in measured Δ_{cell} (Fig. 6B). The best fit of measured on modelled data emerges when the effective length is 20 mm and $p_{ex}p_x$ is 0.33.

As for leaf water enrichment, the best test of the relevance of a Péclet effect to Δ_{cell} is to compare the fractional difference between the water with which oxygen atoms in cellulose have exchanged and evaporation rate. As described above, if ε_{wc} and $p_{ex}p_x$ are assumed to be constant, $1-\Delta_{cell}/\Delta_e$ will be positively related to E. The modelled response of F' is shown in Fig. 7 when ε_{wc} is assumed to be 27‰, $p_{ex}p_x$ is either 0.3 or 0.4, and L is either 10 or 30 mm. The value of $p_{ex}p_x$ gives the minimum value for $1-(\Delta_{cell}-\varepsilon_{wc})/\Delta_e$ when E is zero, and F' becomes more sensitive to increasing E as the effective length increases.

The fractional differences between measured $\Delta_{cell}-\varepsilon_{wc}$ and the Craig-Gordon-modelled Δ_e (assuming ε_{wc}=27‰) for each species are shown in Fig. 8. In support of theoretical predictions, F' increased with increasing E for all three species, with data fitting between the modelled lines when $p_{ex}p_x$=0.40 and L is between 20 and 36 mm.

Discussion

In the most detailed series of experiments in this area to date, Roden and Ehleringer investigated the processes affecting the oxygen isotope composition of leaf water ($\delta^{18}O_l$) and tree ring cellulose ($\delta^{18}O_{cell}$), concluding that the modified Craig-Gordon model adequately describes leaf water enrichment for wide-ranging environmental conditions (Roden and Ehleringer 1999a). By varying $\delta^{18}O$ of source water from –15 to +10‰, they were able to demonstrate that the dependence of δ_L (Roden and Ehleringer 1999a) and δ_{cell} (Roden and Ehleringer 1999b, 2000) on relative humidity varied according to the difference between source water and water vapour $\delta^{18}O$. A further observation from their work was the absence of a clear relationship between evaporation rate (E) and the difference between measured and Craig-Gordon modelled leaf water enrichment. This led the authors to conclude that the effects of gradients in $H_2{}^{18}O$ enrichment within leaves caused by the ratio of convection to diffusion (a Péclet effect) were lost within other causes of variation in $\delta^{18}O$ of leaf water, such as nonsteady state conditions, compartmentation of water within the leaf, patchy stomatal conductance and leaf morphological effects.

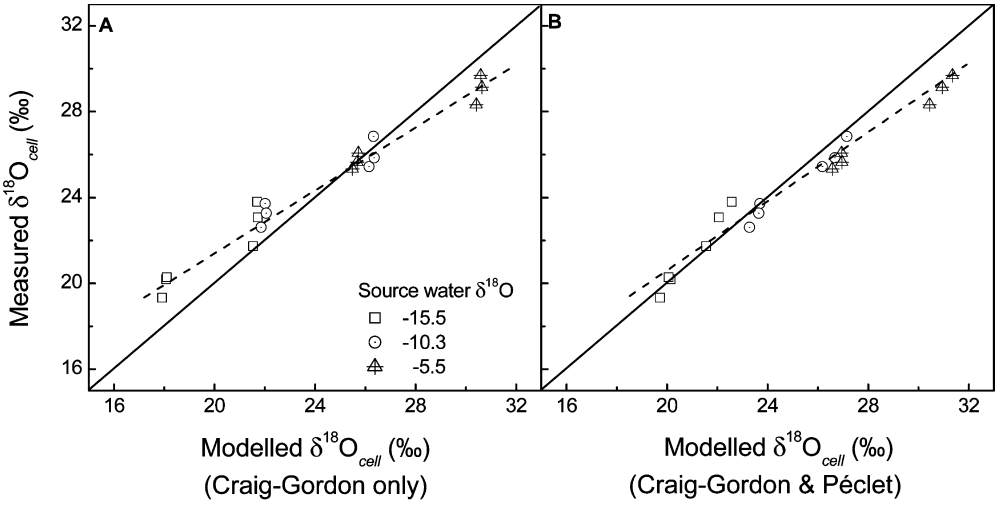

Fig. 5 The relationship between measured and **A** Craig-Gordon modelled and **B** Péclet effect modelled oxygen isotope composition of cellulose relative to the V-SMOW standard for trees grown at three different source water $\delta^{18}O$ and two humidities. Fitted parameters are: **A** f_0 =0.60, and **B** L =20 mm and $p_{ex}p_x$=0.40. The solid lines represent 1:1 relationships, and the dashed lines least squares regressions: **A** δ_{cell} measured =7.13+0.72 δ_{cell} modelled, r^2=0.97, **B** δ_{cell} measured =4.38+0.80 δ_{cell} modelled, r^2=0.97

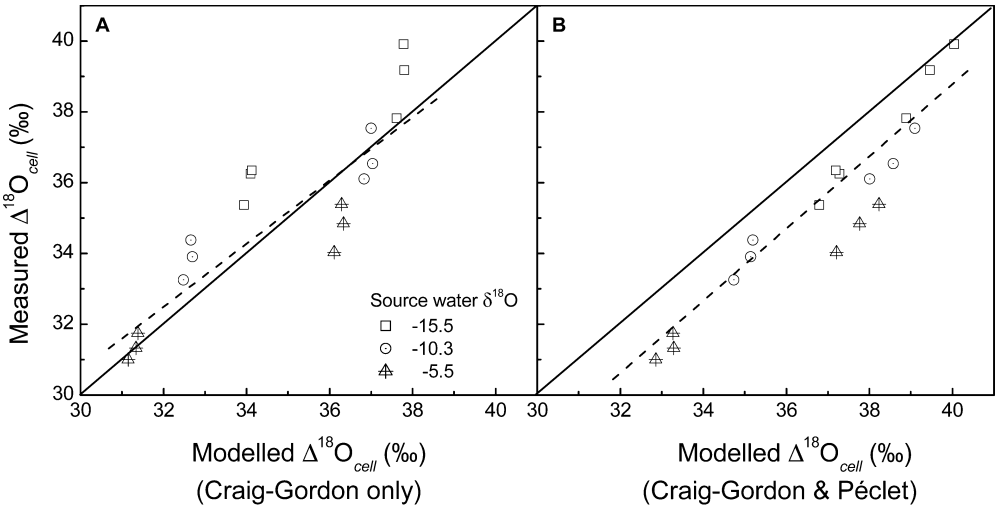

Fig. 6 The relationship between measured and **A** Craig-Gordon modelled and **B** Péclet modelled oxygen isotope enrichment of cellulose above source water for trees grown in three different source water $\delta^{18}O$ and two humidities. The *solid line* in both **A** and **B** represents a 1:1 relationship, and the dashed line the least squares regression. In **A** fitted parameters are $p_{ex}p_x$=0.60, and the fitted line Δ_{cell} measured =3.84+0.90 Δ_{cell} modelled, r^2=0.75. In **B** L and $p_{ex}p_x$ were fitted to be 20 mm and 0.33, respectively, and the fitted line Δ_{cell} measured =−3.81+1.06 Δ_{cell} modelled, r^2=0.89

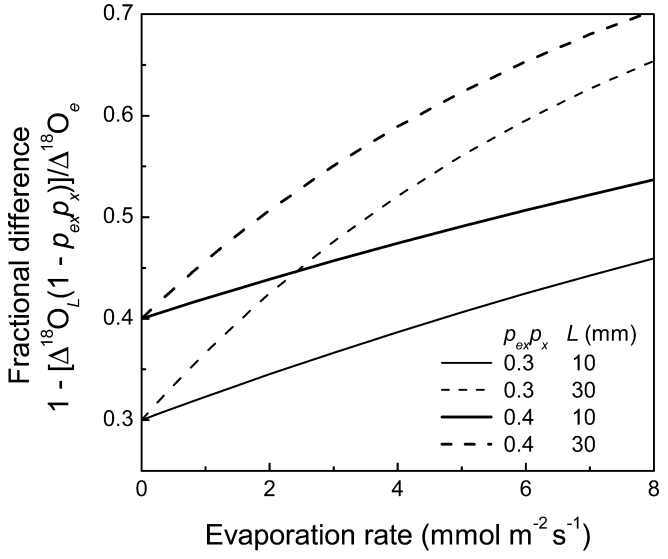

Fig. 7 The modelled relationships between evaporation rate and the fractional difference between laminar mesophyll water and that at the sites of evaporation (Δ_e), where laminar mesophyll water is estimated from the enrichment in cellulose above source water (Δ_{cell}) minus the equilibrium fractionation factor between carbonyl oxygen and water (ε_{wc}). Two values for $p_{ex}p_x$ are shown, and at each $p_{ex}p_x$, relationships for an effective length of either 10 mm (*solid*) or 30 mm (*dashed*)

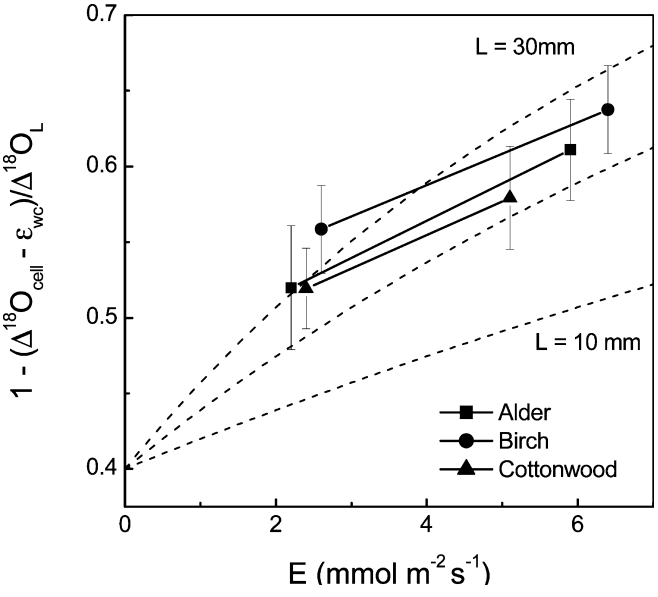

Fig. 8 The relationship between evaporation rate and the average fractional difference between $\Delta_{cell}-\varepsilon_{wc}$ [which is the effective water isotopic enrichment in equilibrium with cellulose, and equals $\Delta_L(1-p_{ex}p_x)$] and Craig-Gordon modelled enrichment at the sites of evaporation (Δ_e) for three riparian tree species grown at high and low humidity. The predicted relationships at $p_{ex}p_x$=0.40 and effective lengths for the Péclet effect of 10, 20 and 30 mm are plotted as *dashed lines*. *Error bars* represent standard errors of the mean values

Recalculation of leaf water and cellulose data collected in the Roden and Ehleringer experiments supports the suggestion of Barbour et al. (2003) that presentation of oxygen isotope composition relative to the V-SMOW standard rather than as enrichment above source water compositions, particularly when source water $\delta^{18}O$ varies considerably, will not allow full interpretation of the data. By recalculating leaf water and cellulose $\delta^{18}O$ values as enrichments above source water we are able to demonstrate positive relationships between E and the fractional difference between measured and modelled leaf water isotope ratios ($1-\Delta_L/\Delta_e$), supporting the relevance of a Péclet effect to whole leaf water (Figs. 4, 8). Evidence of a Péclet effect was not clear when data were presented relative to the standard because variation in leaf water and cellulose due to gradients in $H_2{}^{18}O$ within the leaf were masked by the huge variation in leaf water due to variation in source water $\delta^{18}O$.

Inclusion of a Péclet effect into models of leaf water and cellulose oxygen isotope composition improved the accuracy of model predictions, particularly when data were recalculated as enrichments above source water. For example, Roden and Ehleringer (1999b) found that 93% of variation in $\delta^{18}O_{cell}$ could be explained by the Craig-Gordon model and Eq. 12a (when f_0=0.42). However, when data were recalculated relative to source water, these models explained only 75% of measured variation. In

contrast, the model including a Péclet effect (Eq. 10) explained 89% of the measured variation, and the measured: modelled relationship had a slope of 1.06 when the effective length was 20 mm and $p_{ex}p_x$=0.33.

Figures 4 and 8 show that effective lengths of between 15 and 36 mm, and 22 and 36 mm are estimated for leaf water and cellulose data, respectively. When L is varied to produce the best fit of measured on modelled Δ_L and Δ_{cell}, the fitted values for L lie within the range in estimated L. L was fitted to be 23 mm from leaf water data and 20 mm from cellulose data. These values compare well with previously published effective lengths in a wide range of species (4–166 mm; Wang et al.1998). The similarity between fitted values of L for leaf water and cellulose found for these data suggest that the water with which sucrose has exchanged in the leaf has an isotopic composition rather similar to lamina mesophyll water, as noted by Barbour et al. (2000b).

When cellulose is formed from sucrose in the stem, a proportion of oxygen atoms (p_{ex}) exchange with local water, which may be a mixture of enriched leaf water and unenriched stem xylem water, resulting in the inclusion of the $p_{ex}p_x$ term in Eq. 13b (where p_x is the proportion of local water that is from the xylem). The $p_{ex}p_x$ term was fitted to be 0.33 for these data, a value that compares well with previous estimates. Barbour and Farquhar (2000) found that a $p_{ex}p_x$ of 0.38 produced the best fit of measured on modelled $\Delta^{18}O$ in whole-leaf tissue from cotton plants, while Helliker and Ehleringer (2002) found that $p_{ex}p_x$ of 0.25 fitted cellulose $\delta^{18}O$ values from ten C_3 and C_4 grasses grown at varying humidity. Differences in $p_{ex}p_x$ between species are expected as both turnover times of biochemical intermediates, (determining p_{ex}) and exchange of enriched leaf water with unenriched xylem water (determining p_x) are expected to vary.

We suggest that the Péclet effect is important when variation in Δ_{cell} is interpreted in terms of evaporative effects on leaf water enrichment, such as in studies investigating control of water loss in plant breeding or physiological ecology settings. If variation in the oxygen isotope composition is to be interpreted in terms of the temperature of source water at the time of cellulose synthesis (i.e. in paleoclimatic reconstructions), and so presented as $\delta^{18}O$ relative to the V-SMOW standard, the Péclet effect may be less important. As demonstrated by Roden and Ehleringer (1999a, 1999b), the modified Craig-Gordon model (Eqs. 3a, 7, 12a) predicts variation in δ_{cell} adequately over a wide range in environmental conditions. Fine-tuning of the Craig-Gordon model by including a Péclet effect should improve prediction of temperature from δ_{cell} in paleoclimatic studies (particularly when $\delta^{18}O$ of source water varies over a relatively small range), but will require estimates of $p_{ex}p_x$ and L from modern relatives to parameterize the model.

An EXCEL version of the leaf water enrichment model including the Péclet effect is available to interested investigators at: ftp://ecophys.biology.utah.edu/tree_ring/.

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