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On the isotopic composition of leaf water in the non-steady state

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Abstract. An expression is derived for the isotopic composition of water in leaves under conditions where the composition of water entering the leaf is not necessarily the same as that of water being transpired. The treatment is simplified and considers the average composition of the lamina and of the sites of evaporation. The concept of 'isostorage' is introduced as the product of leaf water content and the isotopic enrichment of leaf water above source water. It is shown that the rate of increase of isostorage is minus the 'isoflux' through the stomata, with the latter expressed as the product of the transpiration flux and the enrichment of the transpired water beyond source water. The approach of the isostorage to the steady state depends on the deviation of the isotopic enrichment of water at the evaporating sites from the steady value, and on the gross (one way) diffusive flux out of the leaf. To achieve model closure, it is assumed that the relationship between leaf water enrichment and that at the sites of evaporation depends on the radial Péclet number in the same manner as in the steady state. The equations have an analytical solution, and we also show how to calculate the results simply using a commonly available computer tool. The form of the equations emphasises that the one-way fluxes of water into and out of the stomata must sometimes be considered separately, rather than as a net outward flux. In this narrow sense we come to the interesting conclusion that more water usually enters the leaf from the air than from the roots.

Keywords: isotopic signal, leaf conductance, leaf water content, leaf water enrichment, non-steady state, transpiration.

Introduction

The isotopic composition of water in leaves is of interest for several reasons: it is a key determinant of the isotopic composition of organic matter made by the plant, and the latter is of interest for palaeoclimatic reconstruction (Gray and Thompson 1976; Libby *et al*. 1976; Epstein *et al*. 1977), for assessment of physiological and genetic changes in stomatal conductance and crop yield (Barbour and Farquhar 2000; Barbour *et al*. 2000) and as a tool for interpreting, e.g. effects of pollution (Saurer *et al*. 2001), and resource utilisation by mistletoes (Cernusak *et al*. 2004*b*). Further, the oxygen isotope composition of leaf water contributes to the Dole effect (Dole *et al*. 1954), the enrichment of $^{18}O/^{16}O$ in O_2 in the atmosphere compared with that ratio in sea water, and the Dole effect has been used to assess changes in the ratio of terrestrial and marine productivity (Bender *et al*. 1994). Leaf water isotopic composition also affects the $^{18}O/^{16}O$ composition of atmospheric CO_2 via both photosynthesis and respiration. Measurements of the latter composition are used to distinguish, for example, an increase in gross primary production from a decrease in heterotrophic (also roots) respiration (Farquhar *et al*. 1993; Ciais *et al*. 1997; Cuntz *et al*. 2003). The $^{17}O/^{16}O$ signal has other possibilities, for example, in determining the ratio of gross photosynthetic oxygen evolution to stratospheric photochemical processes (Luz *et al*. 1999).

The isotopic composition of water in a leaf is heterogeneous (Yakir *et al*. 1989; Bariac *et al*. 1994*b*; Helliker and Ehleringer 2000; Gan *et al*. 2002). The water in the veins usually has an isotopic composition near that of source (soil) water. The maximum value is normally at the sites of evaporation, and is usually calculated using equations developed by Craig and Gordon (1965) for the surface of water bodies, and adapted by Dongmann *et al*. (1974) for leaves. The fractionation during gaseous diffusion

Abbreviations used: E, flux out of leaf lamina by evaporation; J, flux in to leaf lamina from the source / soil via roots, stem and veins; R_E, isotopic ratio of evaporating water (heavy to light); R_L, isotopic ratio of lamina water (heavy to light); R_S, isotopic ratio of source (heavy to light); W, water content in the leaf lamina.

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was related to conventional plant gas-exchange equations by Farquhar *et al*. (1989). The isotopic variation in water between the veins and the sites of evaporation is thought to depend on the ratio of convection of unenriched water to back diffusion of enriched water, the Péclet effect (Farquhar and Lloyd 1993).

This paper is concerned with what happens in the non-steady state and follows the suggestion by Wang and Yakir (1995) that in a normal diurnal humidity regime the leaf is rarely in a steady state. For an early example of such observations see Lesaint *et al*. (1974). Such also appeared to be the case in the field study conducted by Harwood *et al*. (1998), and also, especially at night, in the studies by Cernusak *et al*. (2002, 2005). It is probable that non-steady state treatments will be useful in a range of models from leaf to global scales, including, for example, attempts to check or validate the representation of the hydrological cycle using stable water isotopes. [see IPILPS program at http://ipilps.ansto.gov.au (validated 18 March 2005)]. At an intermediate canopy scale we show, for example, that consideration of the non-steady state is necessary before using Keeling plots to partition between transpiration and evaporation.

We derive an equation for leaf water enrichment that is simple to apply. The derivation is similar to that by Dongmann *et al*. (1974) and subsequently applied by Farris and Strain (1978), Bariac *et al.* (1994*a*) and Cuntz *et al.* (2003). However, the equation of Dongmann *et al*. (1974) takes into account neither the Péclet effect, nor effects of changes in leaf water content, as noted by White (1989) and Yakir (1998). Therefore, White (1989) extended the Dongmann *et al*. (1974) equations, but again assumed that the average enrichment in the leaf is the same as at the sites of evaporation. Yakir (1998) wrote the mass balance eqns (1) and (2) below in the context of a 2-dimensional numerical model of leaf water isotopic heterogeneity, and included the effects of changing water content in the numerical results. The following treatment provides a compact form for a zero-dimensional model, including changing water content and Péclet effects, and which is easy to apply, and to apply consistently in daytime and nighttime.

Theory

We consider the water content (strictly of ^1H_2O if we are considering the leaf enrichment in deuterium, or of $H_2{}^{16}O$ if we are interested in ^{18}O or ^{17}O) in the leaf lamina ($W\,\mathrm{mol\,m^{-2}}$) and the flux in to it from the source / soil via roots, stem and veins ($J\,\mathrm{mol\,m^{-2}\,s^{-1}}$) and that from it by evaporation ($E\,\mathrm{mol\,m^{-2}\,s^{-1}}$). Both J and E are also fluxes of the major (light) molecules:

$$\frac{dW}{dt} = J - E. \qquad (1)$$

We denote as R_L the isotopic ratio [heavy to light] ($^{18}O / ^{16}O$, $^{17}O / ^{16}O$, $^2H / ^1H$) of the lamina water, that of source as R_S and that of evaporating water as R_E so that the fluxes of the heavy isotope ($R_S J$ and $R_E E$), and the rate of change of the amount of the heavy isotope $\frac{d(R_L W)}{dt}$, are correspondingly linked by:

$$\frac{d(R_L W)}{dt} = R_S J - R_E E. \qquad (2)$$

We note that eqn (2) ignores complications arising from consideration of, say, isotopic interconversion between leaf water and carbon dioxide and subsequent photosynthetic metabolism (Farquhar *et al.* 1998) or of the export of water in phloem (Cernusak *et al.* 2003) and, therefore, limits our accuracy to ~0.1‰ for ^{18}O.

We now express the isotopic ratios as enrichments beyond source water, as was done by Lesaint *et al.* (1974) for their experimental data. Thus, dividing eqn (2) by R_S and using:

$$\Delta_L = R_L / R_S - 1, \qquad (3)$$

$$\Delta_E = R_E / R_S - 1, \qquad (4)$$

so that eqn (2) becomes:

$$\frac{d[(1+\Delta_L)W]}{dt} = J - (1+\Delta_E)E.$$

Expanding, and using eqn (1) to eliminate J:

$$(1+\Delta_L)\frac{dW}{dt} + W\frac{d\Delta_L}{dt} = E + \frac{dW}{dt} - (1+\Delta_E)E,$$

so that

$$\frac{d(W \cdot \Delta_L)}{dt} = -E\Delta_E. \qquad (5)$$

Thus, by expressing eqn (5) with all isotopic compositions as enrichments above source water, the flux into the leaf from the soil carries no isoflux, and eqn (5) says that the rate of change of 'isostorage', which we define here as the product $W\Delta_L$, is minus the 'net isoflux' through the stomata, here defined as the product $E\Delta_E$, of net flux of transpiration, E, and, Δ_E the enrichment of the transpired water (and not of the sites of evaporation).

To express eqn (5) in terms of environmental variables we need to expand the right hand side. To that end we re-derive the equation for enrichment (Craig and Gordon 1965; Dongmann *et al*. 1974; Farquhar *et al*. 1989; Flanagan *et al*. 1991) and follow the approach and notation of Farquhar and Gan (2003). Thus the rate of transpiration of isotopically light water, E, is the product of leaf [stomata and boundary layer in series (Farquhar *et al*. 1989)] conductance (g, $\mathrm{mol\,m^{-2}\,s^{-1}}$) to diffusion of the light molecules of water (in air) and the difference ($w_i - w_a$, mol / mol) between the mole fraction of (light) water vapour in air in the intercellular spaces, w_i (mol / mol) and that in the air outside the leaf, w_a (mol / mol):

$$E = g(w_i - w_a), \tag{6}$$

and the flux of the heavy water molecules is given by:

$$R_E E = \frac{g}{\alpha_k}\left(R_e \frac{w_i}{\alpha^+} - R_v w_a\right), \tag{7}$$

where α_k is the fractionation factor (> 1) for diffusion, α^+ is that (> 1) for the saturated vapour pressure in equilibrium with the liquid water. Thus, the diffusivity of $H_2{}^{16}O$ is greater than that of $H_2{}^{18}O$, as is the saturation vapour pressure. R_e is the isotopic ratio of liquid water at the sites of evaporation and R_v is the isotope ratio of water vapour outside the leaf.

Equation (7) becomes:

$$R_E E = \frac{g w_i}{\alpha_k \alpha^+}(R_e - \alpha^+ R_v h), \tag{8}$$

where h is the relative humidity (light isotopes) of the ambient air compared with that of water vapour saturated at the temperature and chemical potential of the water at the sites of evaporation, and given by:

$$h = \frac{w_a}{w_i}. \tag{9}$$

In the steady state $R_E = R_S$ and we denote as R_{es} the steady value to which R_e would relax were w_a, w_i, R_v, R_S, α_k, and α^+ to remain constant. Thus,

$$R_S E = \frac{g w_i}{\alpha_k \alpha^+}(R_{es} - \alpha^+ R_v h). \tag{10}$$

We rewrite the right hand side of eqn (5) as:

$$-E \cdot \Delta_E = -E \cdot \frac{R_E - R_S}{R_S} = -\frac{R_E E - R_S E}{R_S},$$

and using eqns (10) and (8) to replace the terms in the numerator:

$$-E\Delta_E = -\frac{g w_i}{\alpha_k \alpha^+} \cdot \frac{(R_e - R_{es})}{R_S} = -\frac{g w_i}{\alpha_k \alpha^+}(\Delta_e - \Delta_{es}). \tag{11}$$

We also note that eqns (11), (6) and (9) yield a simple formula for Δ_E:

$$\Delta_E = \frac{\Delta_e - \Delta_{es}}{\alpha_k \alpha^+ (1-h)}, \tag{12}$$

where:

$$\frac{R_{es}}{R_S} = \alpha^+ \left[\alpha_k (1-h) + h \frac{R_v}{R_S}\right], \tag{13}$$

$$\Delta_e = R_e / R_S - 1, \tag{14}$$

$$\Delta_{es} = R_{es} / R_S - 1, \tag{15}$$

and R_{es} could be equally denoted as R_C and Δ_{es} as Δ_C, where the subscript 'C' denotes the modified Craig–Gordon value. So finally returning to eqn (5) and inserting eqn (11):

$$\frac{d(W\Delta_L)}{dt} = -\frac{g w_i}{\alpha_k \alpha^+}(\Delta_e - \Delta_{es})$$

or

$$\Delta_e = \Delta_{es} - \frac{\alpha_k \alpha^+}{g w_i}\frac{d(W\Delta_L)}{dt}. \tag{16}$$

Equation (16) in its first form relates the change in isostorage (the product of lamina water content and its enrichment) to leaf conductance, intercellular humidity and the difference between the transient enrichment at the site of evaporation and its steady (modified Craig–Gordon) value.

To illustrate the use of the above equations, we examine data of Cernusak *et al.* (2002) on the oxygen isotope enrichment of lamina water of lupins. Because we know the lamina water content as well as its enrichment, we can use eqn (5) to calculate the time-course of the isoflux of transpired water, $E\Delta_E$ (see 'calculated' values in Fig. 1). Notice that the isoflux is strongly negative from about dawn to midday on both days, corresponding to the times of strong progressive leaf water enrichment. Similarly the isoflux is positive from 1700 to 0700 h during the night, as the leaf loses its afternoon enrichment. We use the isoflux, rather than the enrichment of the transpired water, Δ_E, because Δ_E becomes very large and erratic as transpiration approaches zero at night. Similarly, we can back-calculate the enrichment at Δ_e, using eqn (16). See 'calculated' values of Δ_e in Fig. 2,

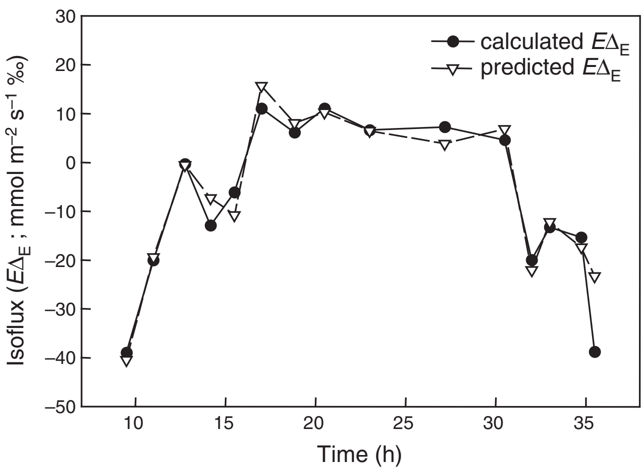

Fig. 1. Time-course of variation in the calculated and predicted leaf water isoflux, $E\Delta_E$, for *Lupinus angustifolius* leaves sampled (Cernusak *et al.* 2002) in Western Australia on 1 and 2 November 2000. Calculated $E\Delta_E$ was obtained with eqn (5), using measured values of Δ_L and W. Predicted $E\Delta_E$ was obtained using the same equation, but replacing measured values of Δ_L with predicted values, obtained using eqn (21).

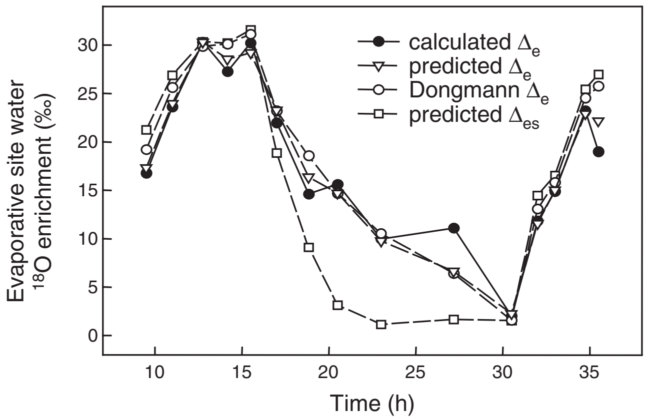

Fig. 2. A comparison of the time-courses of variation in calculated and predicted evaporative site water enrichment, Δ_e, for *Lupinus angustifolius*. Also shown is the predicted steady-state evaporative site enrichment, Δ_{es}, equal to the modified Craig–Gordon enrichment, Δ_C. Calculated Δ_e was obtained using eqn (16) and measured values for Δ_L and W (Cernusak *et al.* 2002). Predicted Δ_e was obtained using eqn (22), also employing the measured values for W. The Dongmann *et al.* (1974) prediction of Δ_e was made using eqn D29.

and note how much greater the observed night-time values are compared with the Craig–Gordon steady-state predictions, Δ_{es}.

Brave assumption to obtain a closed form of the equations for use in a forward mode

For many applications there are no data on lamina enrichment to analyse. Rather, there is the need to predict such values in a 'forward model', and for that purpose we need a link between the average enrichment of the lamina and that at the sites of evaporation. In the steady state the relation between Δ_L and Δ_e is thought to depend on the ratio of advection to diffusion of enrichment within the leaf characterised by the Péclet number, P, via Farquhar and Lloyd 1993:

$$\Delta_{Ls} = \frac{\Delta_{es}(1 - e^{-P})}{P}$$

i.e., (17)

$$\Delta_{es} = \frac{P\Delta_{Ls}}{(1 - e^{-P})},$$

where Δ_{Ls} and Δ_{es} are the steady-state values of Δ_L and Δ_e.

This steady relation is thought to hold even in conditions of enrichment along a leaf provided the transpiration rate is uniform (Farquhar and Gan 2003). In the present treatment we do not consider progressive enrichment along a leaf and so do not consider the longitudinal Péclet number. The relevant Péclet number is then the radial one, which in turn depends on transpiration rate via:

$$P = \frac{EL}{CD}, \tag{18}$$

where C (mol m^{-3}) is the density of water, D is the diffusivity in water of the heavier water species in question, and L is the scaled effective 'radial' length over which liquid phase diffusion occurs. Thus, L is a scaled value of the distance from, for example, a xylem element to the site of evaporation in the sub-stomatal cavity (Barbour and Farquhar 2004).

While eqn (17) may describe the relationship between Δ_L and Δ_E in the steady state, the relationship during dynamic changes will be complex in detail. If the change in conditions involves a factor that affects water relations (such as a change in humidity, temperature or stomatal conductance), the propagation of changes in water potential within the leaf would need to be considered, and this involves the elasticity of cell walls and the permeability of cell membranes to water movement (Passioura and Boyer 2003). If the change were something (artificial) that involved a change only in isotopic composition, with no change in water relations, such as a change in the isotopic composition of vapour, at constant absolute humidity, consideration would be needed of the time course of isotopic exchange between water flowing from veins to the sites of evaporation with any pools of water, such as those in the vacuole, perhaps, that may have little involvement in the flow path under steady-state conditions.

To avoid these complications, we bravely assume that eqn (17) holds in the non-steady state and can be combined usefully on the time scale of interest (roughly hourly), with eqn (16) to obtain the following closed forms:

$$\frac{d(W \cdot \Delta_L)}{dt} = -\frac{gw_i}{\alpha_k\alpha^+}\frac{P}{1 - e^{-P}}(\Delta_L - \Delta_{Ls}), \tag{19}$$

and

$$\frac{d\left(W \cdot \frac{1-e^{-P}}{P} \cdot \Delta_e\right)}{dt} = -\frac{gw_i}{\alpha_k\alpha^+}(\Delta_e - \Delta_{es}), \tag{20}$$

or, equivalently,

$$\Delta_L = \frac{1 - e^{-P}}{P}\left(\Delta_{es} - \frac{\alpha_k\,\alpha^+}{gw_i}\frac{d(W\Delta_L)}{dt}\right)$$
$$= \Delta_{Ls} - \frac{\alpha_k\alpha^+}{gw_i} \cdot \frac{1 - e^{-P}}{P} \cdot \frac{d(W \cdot \Delta_L)}{dt} \tag{21}$$

and

$$\Delta_e = \Delta_{es} - \frac{\alpha_k\alpha^+}{gw_i}\frac{d\left(W \cdot \frac{1-e^{-P}}{P} \cdot \Delta_e\right)}{dt}. \tag{22}$$

We emphasise again that this treatment is an approximation, in that when a change in Δ_{es} occurs, its effect will take some time to propagate spatially through the leaf.

Solution

The form in eqns (19) and (20), with the minus sign on the right hand side, was to emphasise the negative feedback between the rate of approach to the steady state, and the offset or 'error' between the transient and steady state that drives it. That is, the enrichment changes more quickly towards the steady value when it is far from it than it does when close. The form in eqns (21) and (22) shows that the enrichment lags the value that would have been calculated if steady state occurred instantaneously. Thus, when the value is decreasing, making the time derivatives negative, the enrichment is greater than the steady value. So as the night progresses and leaf enrichment decreases, enrichment is greater than one would calculate using the steady-state-modified Craig–Gordon values (see Fig. 3). Note that the open circles in Figs 2, 3 have identical values, because the treatment by Dongmann *et al*. (1974) assumes Δ_e and Δ_L are identical.

To obtain an analytical solution to the general case where W and P are functions of time, we rewrite eqn (19) as:

$$\frac{d(W \cdot \Delta_L)}{dt} = -E_1(p\Delta_L - \Delta_{es}), \tag{23}$$

where $E_1 = \frac{gw_i}{\alpha_k \alpha^+}$ and is so denoted since gw_i is the one-way flux of vapour out of the stomata, and where $p(>1) = \frac{P}{1-e^{-P}}$.

As an aside we note that White (1989) suggested that an equation for R_L in which W is varying can be expressed in terms of a solution for R_L in which W is assumed to be constant. This is deceptively simple to do in the present treatment including Péclet effects [eqn (23)] by expanding the left hand side and rearranging and yields:

$$\Delta_L = \frac{\Delta_L^0}{1 + \frac{dW/dt}{pE_1}}, \tag{23a}$$

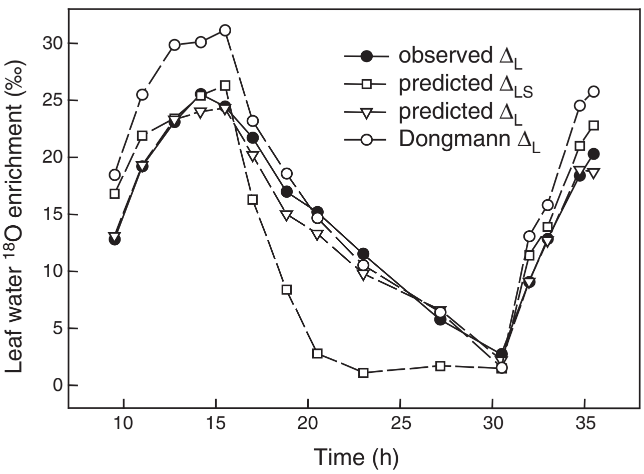

Fig. 3. Time-course of variation in predicted steady state (Δ_{Ls}) and non-steady state (Δ_L) leaf water enrichment for *Lupinus angustifolius*. Also shown is the observed leaf water enrichment (Cernusak *et al*. 2002). Predicted Δ_L was calculated using eqn (21). Dongmann *et al*. (1974) Δ_L was calculated using eqn D29, and employing the assumption $\Delta_L = \Delta_e$. Thus, the open circles have the same values here as in Fig. 2, aiding comparison. Data used to make the predictions are detailed in Table 1.

where Δ_L^0 is the value of Δ_L that would be calculated if dW/dt were ignored. However, eqn (23*a*) seems unlikely to aid computation as any solution for Δ_L^0 actually involves W, and the issue arises immediately as to what value of W one would use when it is actually changing over time.

We need then to be able to predict the time course of Δ_L for given time courses of environmental variables and leaf water content. To that end we expand the left hand side of eqn (23) and rearrange:

$$\frac{d\Delta_L}{dt} + u\Delta_L = \frac{E_1}{W}\Delta_{es}, \tag{24}$$

where:

$$u = \frac{E_1 p}{W} + \frac{1}{W}\frac{dW}{dt}. \tag{25}$$

Multiplying both sides of eqn (24) by $\exp \int u \cdot dt$ it may then be rewritten as:

$$\frac{d}{dt}\left(e^{\int u \cdot dt}\Delta_L\right) = e^{\int u \cdot dt}\frac{E_1}{W}\Delta_{es}, \tag{26}$$

and so integrating with respect to time:

$$e^{\int u \cdot dt}\Delta_L = \int e^{\int u \cdot dt}\frac{E_1}{W}\Delta_{es}dt + \text{Constant},$$

and at time 0, $\Delta_L(0) = \text{Constant}$, yielding:

$$\Delta_L(t) = e^{-\int_0^t u \cdot dt'}\left[\int_0^t e^{\int_0^{t''} u \cdot dt'}\frac{E_1}{W}\Delta_{es}dt'' + \Delta_L(0)\right]. \tag{27a}$$

$\Delta_e(t)$ is then calculated as:

$$\Delta_e = p \cdot \Delta_L. \tag{27b}$$

The analytical solution [eqn (27)] can be calculated when the time courses of u, E_1, W and Δ_{es} are available, and so can be used for forward modelling of Δ_L and Δ_e. Because the start time, $t(0)$ is arbitrary, the formula can also be used in an iterative manner, setting the result as the new $\Delta_L(0)$ and adjusting the time accordingly. Nevertheless, for comparing experimental data with predictions, eqn (27) is not convenient to calculate, and we present a simpler method below. But first we consider an earlier, approximate solution that involved keeping the parameters (other than Δ_e and Δ_L) constant, the exponential approach of Dongmann *et al*. (1974). The latter authors assumed that $\Delta_L = \Delta_e$ and that the water content, W, is constant. In that case eqn (16) becomes:

$$\frac{d\Delta_L}{dt} = -\frac{gw_i}{W\alpha_k\alpha^+}(\Delta_L - \Delta_{Ls}), \tag{D28}$$

where the prefix in the equation number indicates that this approximation is an older result of Dongmann *et al*. (1974) for which the solution is (after a step change at time zero to a new condition, of humidity for example, that initially sets Δ_L to Δ_{L0}, a value different from what would be its steady value, Δ_{Ls}):

$$\Delta_L = \Delta_{Ls} + (\Delta_{Lo} - \Delta_{Ls})e^{-t/\tau}, \tag{D29}$$

where the time constant, τ, is given by:

$$\tau = \frac{W \alpha_k \alpha^+}{g w_i} = \frac{W}{E_1}, \qquad \text{(D30)}$$

and where:

$$\alpha_k \alpha^+ \approx 1. \qquad \text{(D31)}$$

Of the three assumptions made by Dongmann *et al.* (1974), that involved in setting the product of the fractionation factors to unity involves an error in the rate of approach to Δ_{Ls} (in the case of $^{18}O/^{16}O$ only ~4%, but for D/H the error is ~11%). The error in ignoring time changes in W can be estimated from eqn (23*a*). For a particular example, White (1989) estimated $(dW/dt)/E$ as ~0.052 in the data of Farris and Strain (1978). This means that the denominator of eqn (23*a*) would be approximately 1.03, implying an error of only ~3%. Indeed Farris and Strain (1978) cite an unpublished report by Wagener that variation in W has little effect on calculated values of enrichment. The error in assuming $\Delta_L = \Delta_e$ depends on the Péclet number. From eqn (17) the proportional error in using Δ_e and Δ_L interchangeably is roughly $P/2$. This can be seen by comparing Figs 2 and 3, where the open circles have the same values in both figures. It is clear that at night, when P is small, Δ_e and Δ_L have similar values. However, during daylight hours, Δ_L is typically ~20% less than Δ_e (with P being ~0.4).

While the value of P varies with species and conditions, its value in a range of species has been estimated by Wang and Yakir (1998) to vary between 0.1 and 1.6 in leaves they examined in the Jerusalem Botanical Garden during the day. Our own experience is that at night, E, and hence P, are small. Thus, accepting the approximation of eqn (D29), and taking P as negligibly small at night, we obtain the form used by Cernusak *et al.* (2002):

$$\Delta_e = \Delta_{es} - \frac{1}{g w_i} \cdot \frac{d(W\Delta_e)}{dt}, \qquad \text{(C32)}$$

where the prefix 'C' refers to the approximations made by Cernusak *et al.* (2002) and:

$$\Delta_L = \Delta_{Ls} - \frac{1}{g w_i} \cdot \frac{d(W\Delta_L)}{dt}. \qquad \text{(C33)}$$

In the experimental context of Cernusak *et al.* (2002), we found the numerical solution of eqns (C32) and (C33) to be simple using 'Solver' in the Microsoft Excel package. It is apparent that the most important difference between the steady-state and dynamic calculations occurs at night (Flanagan and Ehleringer 1991; Cernusak *et al.* 2002). In these conditions P is small. In the daytime, P is not negligible, and one needs to apply eqns (21) or (22), or (27). In practice, because $g w_i$ is comparatively large during the day in stress-free conditions when stomata are wide open, the errors involved in ignoring time variation under such conditions are not as large as at night. Indeed the steady-state equations should often be a reasonably good approximation in daylight hours under unstressed conditions, but should break down under stress conditions when stomata are predominantly closed.

Nevertheless, eqns (21) or (22), or (27) improve the predictions considerably compared with the steady state [see Fig. 3 applying the equations to the data of Cernusak *et al.* (2002)]. Rather than using the analytical solution, eqn (27), we find it simpler to solve eqns (21) and (22) by the 'Solver' method.

We describe the method in detail, to ensure that it is easily understood. It is necessary to first define the initial rates of change of Δ_e or Δ_L and W. This is effectively a best guess of what Δ_e or Δ_L and W would have been at time $t = 0$, if their first prediction is to take place at time $t = 1$. The initial guesses can then be used along with the observations at time $t = 1$ to calculate the term on the right hand side of eqn (21) or (22), and the Solver function can be used to set the difference between Δ_e or Δ_L on the left and right hand sides of eqn (21) or (22) to zero by changing that term on the right side for time $t = 1$. The Δ_e or Δ_L at time $t = 2$ is then calculated based on the values for time $t = 1$, and so on. Table 1 provides an example dataset demonstrating these calculations. Using this procedure, we have found that the affect of varying the boundary conditions for Δ_e or Δ_L and W rapidly diminishes after only a few time steps (Fig. 4). However, this would not be the case if the first guess occurred in the middle of the night.

Discussion

Figure 1 shows that the inferred isoflux in the lupin data of Cernusak *et al.* (2002) was rarely zero for long, so that the leaves were rarely at steady state. This was also true for the evaporative site enrichment (Fig. 2). Here the predictions from the model of Dongmann *et al.* (1974) (○) match the values calculated from observations (●) reasonably well, although not quite as well in the daytime as the new non-steady-state model (▽). The steady-state (modified Craig–Gordon) model (□) predicts poorly at night. In Fig. 3, we see that leaf water enrichment (●) is well predicted by both the Dongmann *et al.* (1974) model (○) and the new one (▽) at night, but that the Dongmann *et al.* (1974) model overestimates enrichment during daylight hours. The steady-state model including the Péclet effect (□) is better in the daylight than the Dongmann *et al.* (1974) version, but not as good as the new non-steady-state model. The steady-state model does poorly at night. Taking into account the ability to model the enrichment of transpired water, the enrichment at the sites of evaporation as well as leaf water enrichment, both day and night, the new non-steady-state model is an improvement on what was previously available.

Table 1. A dataset to demonstrate the prediction of Δ_L

Measurements are for *Lupinus angustifolius*, and were described previously by Cernusak *et al*. (2002). Symbols are as defined in the text. The effective length, L, for calculation of P in column 10, was assumed to be 15 mm. The format of the table mimics the way in which one might set up a spreadsheet for predicting Δ_L. Values for W and Δ_L at time 8.50 h comprise the boundary condition. It is necessary to define a boundary condition so that $dW\Delta_L / dt$ can be calculated at 9.50 h, the time at which the first observations took place. The term Δ_L represents the guess for the predicted non-steady state leaf water enrichment and Δ_L* is calculated from it using the right hand side of eqn (21). The values shown below are the successful guesses where the two match. They were obtained using the Solver function in Microsoft Excel. Solver is used to set the difference between Δ_L* and Δ_L, the final column in the table, to zero by changing the corresponding value of Δ_L, which in turn changes the value of Δ_L* until a match is found

Time (h)	dt (s)	W (mol m^{-2})	Δ_L (‰)	$W\Delta_L$ (mol m^{-2} ‰)	d$W\Delta_L$ (mol m^{-2} ‰)	d$W\Delta_L$/dt (mol m^{-2} ‰ s^{-1})	g_t (mol m^{-2} s^{-1})	w_i (mol mol^{-1})	$\frac{(1-e^{-P})}{P}$	$\alpha^+\alpha_k$	Δ_{Ls} (‰)	Δ_L* (‰)	Δ_L* − Δ_L (‰)
8.50	–	17.0	6.0	102.0	–	–	–	–	–	–	–	–	–
9.50	3600	18.9	13.1	247.7	145.7	0.0405	0.375	0.0241	0.79	1.040	16.8	13.1	0.0
11.00	5400	18.2	19.3	352.6	104.9	0.0194	0.218	0.0292	0.81	1.041	21.9	19.3	0.0
12.75	6300	15.3	23.3	356.1	3.5	0.0006	0.185	0.0373	0.77	1.041	23.4	23.3	0.0
14.17	5100	16.4	24.0	393.6	37.4	0.0073	0.127	0.0358	0.84	1.041	25.4	24.0	0.0
15.50	4800	18.3	24.3	445.3	51.7	0.0108	0.134	0.0348	0.83	1.041	26.3	24.3	0.0
17.00	5400	17.9	20.2	360.7	−84.6	−0.0157	0.155	0.0238	0.87	1.041	16.3	20.2	0.0
18.83	6600	20.5	15.0	307.6	−53.1	−0.0080	0.073	0.0158	0.92	1.042	8.4	15.0	0.0
20.50	6000	18.5	13.3	246.1	−61.5	−0.0103	0.073	0.0127	0.90	1.042	2.8	13.3	0.0
23.00	9000	19.3	9.8	188.0	−58.1	−0.0065	0.073	0.0107	1.00	1.043	1.1	9.8	0.0
27.17	15000	19.7	6.6	130.7	−57.3	−0.0038	0.073	0.0109	1.00	1.043	1.7	6.6	0.0
30.50	12000	21.5	2.2	48.3	−82.3	−0.0069	0.740	0.0138	1.00	1.040	1.5	2.2	0.0
32.00	5400	18.4	9.1	167.4	119.1	0.0221	0.426	0.0185	0.79	1.041	11.4	9.1	0.0
33.00	3600	16.7	12.7	211.5	44.1	0.0122	0.426	0.0197	0.84	1.040	13.9	12.7	0.0
34.75	6300	16.9	18.9	321.0	109.5	0.0174	0.268	0.0266	0.83	1.041	21.0	18.9	0.0
35.50	2700	20.5	18.7	383.9	62.8	0.0233	0.171	0.0296	0.84	1.041	22.8	18.7	0.0

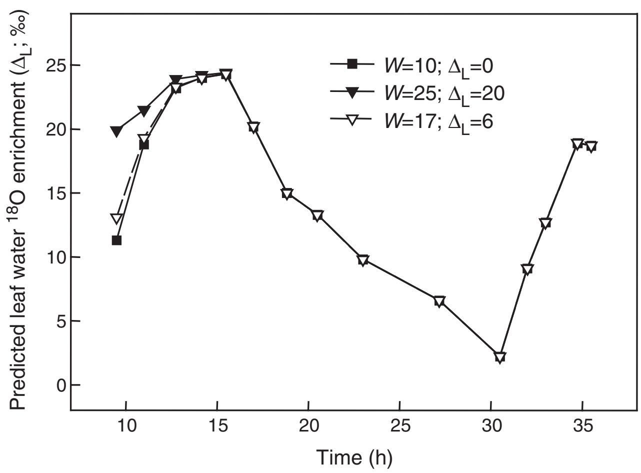

Fig. 4. A demonstration of the effect of changing the boundary condition on subsequent predictions of Δ_L. In this particular dataset, the effect rapidly diminishes after the first few time steps, even when the changes to the boundary condition are rather extreme.

It may be that some features of the model will be largely unnecessary, for example, dealing with changes in leaf water content. Our experience has been limited to testing equations similar to (21) and (22) and the variability between leaves made it difficult to see trends in W. For many purposes it may be simpler to regard W as constant, particularly as W can be variable between individual leaves. However, White (1989) and Yakir (1998) emphasised the importance of variation in leaf water content and Yakir suggested that failure to include its effects may have contributed to reports where measured Δ_L exceeded calculated Δ_C. He investigated a two-dimensional simulation of ^{18}O composition in leaf water and noted that 'leaves' which decreased in volume during the day had significantly greater Δ_L compared with constant volume leaves. In our own experience to date the effects on Δ_L of changing W have been small.

We note that the time constant that applies in the absence of changes in water content is given by eqn (D30). Dongmann *et al.* (1974) did not present the result in that format but rather as the equivalent $\tau = \frac{W\alpha_k\alpha^+(1-h)}{E}$, where $h = w_a / w_i$. Farris and Strain (1978) made measurements of the time constant involved following a change in environmental conditions. However, they discussed the results in terms of the leaf water turnover time, W/E, which is different from W/E_1. We suggest that (D30) is a better measure of the leaf water isotopic turnover time. That is, $E_1 = E / [\alpha_k\alpha^+ (1 - h)] \approx gw_i$ is the appropriate flux, and not E. Farris and Strain (1978) found that the observed isotopic equilibration was faster than the 'theoretical prediction'. But the latter was based incorrectly on the use of E/W for the rate constant, as opposed to $E_1 / W \approx gw_i / W$, and it seems likely that the disparities the authors record in their table 2 (factors of 1.3 to 2) are caused by this mistake, roughly a factor of $w_i / (w_i - w_a)$.

To the extent to which the enrichment, Δ_e, at the site of evaporation is the parameter of interest, eqns (21) and (22) also show that gw_i is the flux of interest. The net uptake from the soil can be thought of as matching the net loss from the canopy $g(w_i - w_a)$ where g is the total conductance, w_i is the mole fraction of water vapour in air inside the leaves and w_a is that in the atmosphere. The one-way rate of loss from the leaves is gw_i and the one-way rate of gain from the atmosphere is gw_a. So the total 'gross' flux into the leaves is gw_i, of which gw_a is from the atmosphere. That is w_a / w_i is the proportion of water coming to the leaves that originates from the aerial environment. It is commonly thought that the source of plant water is solely the roots. In fact, we see from an isotopic point of view that a lot of water comes from the atmosphere to the leaf, as well as *vice versa*. Since w_a / w_i is typically approximately two-thirds, it follows that the rate of water entry into the leaf from the atmosphere is approximately two-thirds of the rate at which it leaves through the stomata, but twice the rate at which water enters the leaf from the roots. Parenthetically, the rate of water entry into roots may exceed the rate of delivery from roots via the stem to the leaves. This would be the case when there is diffusion of water vapour from roots to the soil. Our quantitative argument strictly compares water entry into leaves from roots with that entering from the atmosphere. The clear qualitative argument is that it is the one-way fluxes that must be taken into account when assessing isotope effects. Such is the case when assessing the isoflux of ^{18}O in CO_2 during photosynthesis (Farquhar and Lloyd 1993; Farquhar *et al.* 1993) and dark respiration (Cernusak *et al.* 2004*a*).

Equations 5, 12 and 16 appear to be reasonably robust forms (at the whole-leaf level) for describing the rate of change of isostorage ($W\Delta_L$) in terms of isoflux ($E\Delta_E$) (see Fig. 1), to relate transient changes in Δ_e and Δ_E, and to relate changes in isostorage to the difference between Δ_e and Δ_{es}, respectively. The process of leaf enrichment in the morning as relative humidity diminishes requires that the isoflux be negative, just as the leaf depletion in the evening requires the isoflux to be positive (see Fig. 1). Harwood *et al.* (1998) showed results for *Piper aduncum* in the field that are consistent with this; in the morning the transpired water was depleted compared with the stem water, and more enriched in the afternoon. From both Fig. 1 and the data of Harwood *et al.* (their Fig. 6) one sees that isotopic steady state holds only briefly during the day (typically the early afternoon). Harwood *et al.* (1999) demonstrated a negative relationship between Δ_E and leaf-to-air vapour pressure deficit (VPD) within a *Quercus petraea* canopy. However, we see from the theory that this relationship should not be a direct one; Δ_E should relate to time variation in Δ_L, and factors driving that variation, rather than to static VPD. Indeed from Fig. 1 we infer that the opposite occurred in the mornings to the lupin canopy. These factors need to be taken into account when using Keeling plots of water vapour enrichment *v.* the reciprocal of ambient water vapour pressure to partition soil evaporation from plant transpiration (e.g. Yepez *et al.* 2003). The assumption in such an approach has been that the vapour from transpiration has the same enrichment as stem water. Interpretation of the Keeling plots needs to take into account that the transpired water vapour will be systematically depleted in the morning and enriched in the late afternoon. Measurements of the time course of leaf water enrichment in such studies would allow the estimation of changing values of Δ_E and hence, perhaps, appropriate correction of the plots.

Such issues are important on a diurnal time scale, but not over longer time scales where integrated $E{\cdot}\Delta_E$ must approach zero, i.e. the isotopic composition of water leaving the leaf must average out close to that entering the leaf (Harwood *et al.* 1999). Similarly, the rate of change of isostorage must be nearly zero averaged over long periods. The isostorage itself, however, is normally positive over all time scales.

Equation 16 should be particularly useful in field studies of the isoflux of O^{18} in CO_2 at night, since the latter relates to Δ_e, which can differ substantially from Δ_{es} (see Fig. 2), and during the day under conditions when stomatal conductance is low because of stress. The Dongmann *et al.* (1974) predictions work reasonably well for Δ_e, although not as well as the present formulation (see Fig. 5).

The subsequent equations [after (16)] follow our assumption that the Péclet number holds in the non-steady state. They are approximations but seem to give reasonable fit to the limited data available. One test of the assumption that the Péclet number holds in the non-steady state is to compare calculations of Δ_e from eqn 16, based on measured values of Δ_L and *W*, with predictions of Δ_e from eqn 22, based on modelled values of Δ_L. This comparison is shown in Fig. 5 and an equivalent plot for Δ_L is shown in Fig. 6. This initial result appears to suggest that the steady-state Péclet treatment approximates reasonably well with observations in the non-steady state. More experiments are needed to further test the validity of this assumption. One would expect in practice that a change in atmospheric conditions would affect Δ_e before Δ_L as the effect propagates into the leaf. There is a hint of this after 16 h in Fig. 2, but more, and less noisy, data would be needed to test such a weakness of the present approach. Figure 6 shows that the present treatment gives much better fit to the data on Δ_L than does the Dongmann *et al.* (1974) prediction, mainly because of the improved fit during the day.

The present equations have the advantage of including the Péclet effect, which was missing from the treatment by Dongmann *et al.* (1974) Like the latter treatment, the present one emphasises that nocturnal leaf enrichment is

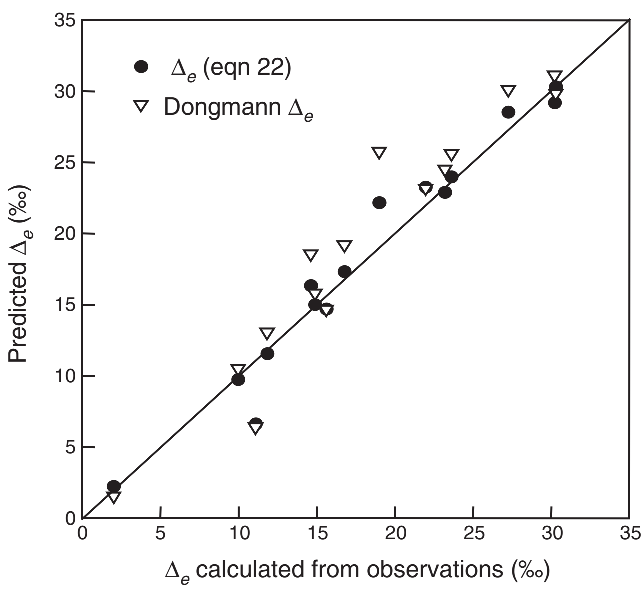

Fig. 5. A comparison of Δ_e calculated from observations (Cernusak *et al.* 2002) and predicted values for Δ_e. Calculated Δ_e was obtained using eqn (16) and measured values for Δ_L and *W*. The comparison is shown for predictions made both by eqn 22, and for the Dongmann *et al.* (1974) approach using eqn D29. The solid line is the one-to-one line. Linear regression of Δ_e predicted by eqn 22 against calculated Δ_e yields the equation $y = 1.04x - 0.58$, with R^2=0.96 and $n = 15$. Linear regression of Dongmann *et al.* (1974) predicted Δ_e against calculated Δ_e yields the equation $y = 1.08x - 0.23$, with R^2=0.92 and $n = 15$.

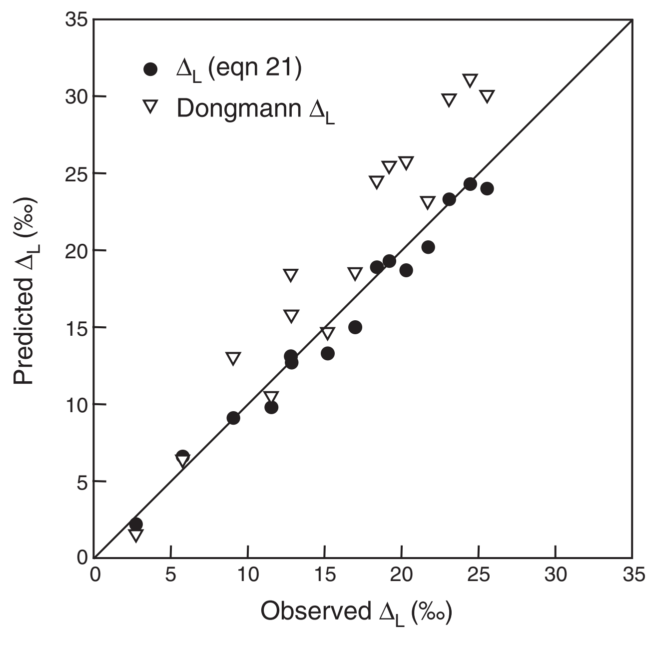

Fig. 6. A comparison of observed (Cernusak *et al.* 2002) and predicted Δ_L. The comparison is shown for calculations made both by eqn 21, and for the Dongmann *et al.* (1974) approach using eqn D29, which assumes that $\Delta_L = \Delta_e$. The solid line is the one-to-one line. Linear regression of Δ_L predicted by eqn 21 against observed Δ_L yields the equation $y = 0.96x - 0.02$, with R^2=0.98 and $n = 15$. Linear regression of Dongmann *et al.* (1974) predicted Δ_L against observed Δ_L yields the equation $y = 1.27x - 1.02$, with R^2=0.93 and $n = 15$.

considerably greater than would be predicted by a quasi steady-state model, and this difference is significant in examining the isoflux of ^{18}O or ^{17}O in respired CO_2 from leaves at night. The non-steady-state treatment is less important during the day (and hence for determining enrichment in organic matter, or in O_2, or in the exchange affecting CO_2) for the data examined here (Cernusak *et al*. 2002) where the stomata were more open, but would be required, particularly in thick leaves with large W, if closure occurred with stress for example.

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