The effects on isotopic composition of leaf water and transpiration of adding a gas-exchange cuvette

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ii. Abstract:

An expression was earlier derived for the non-steady state isotopic composition of a leaf when the composition of the water entering the leaf was not necessarily the same as that of the water being transpired (Farquhar and Cernusak 2005). This was relevant to natural conditions because the associated time constant is typically sufficiently long to ensure that the leaf water composition and fluxes of the isotopologues are rarely steady. With the advent of laser-based measurements of isotopologues, leaves have been enclosed in cuvettes and time courses of fluxes recorded. The enclosure modifies the time constant by effectively increasing the resistance to the one-way gross flux out of the stomata because transpiration increases the vapour concentration within the chamber. The resistance is increased from stomatal and boundary layer in series, to stomata, boundary layer and chamber resistance, where the latter is given by the ratio of leaf area to the flow rate out of the chamber. An apparent change in concept from one-way to net flux, introduced by Song, Simonin, Loucos and Barbour (2015) is resolved, and shown to be unnecessary, but the value of their data is reinforced.

iii. Keywords:

Oxygen; deuterium; isotopologues; laser spectrometry; non steady state; oxygen isotope; transpiration; leaf water

iv. Main text:

Introduction

Isotopes of oxygen and hydrogen remain important tools to a wide range of disciplines. The application of water isotopes in plant physiological studies include (1) interpretation of the oxygen isotope composition of atmospheric carbon dioxide (Farquhar et al. 1993, Cuntz et al. 2003; Welp et al. 2011) and of atmospheric oxygen (Dole et al. 1954; Hoffmann et al. 2004; Luz & Barkan 2011), (2) estimates of stomatal conductance from measurements of the isotopic composition of organic matter, in turn strongly influenced by the leaf water composition (Barbour &Farquhar 2000, Werner et al. 2012), (3) estimates of the 'mesophyll conductance' to carbon dioxide diffusion from the intercellular spaces to the sites of oxygen exchange between water and carbon dioxide (Barbour et al. 2016), (4) of the humidity inside leaves, which may not always be 100% (Cernusak et al. 2018; Holloway-Phillips et al., 2019; Wong et al., unpublished), and (5) partitioning of ecosystem vapour fluxes into transpiration and soil evaporation (Yepez et al. 2007).

Theoretical consideration and interpretation of experimental data are common for leaf water at isotopic steady state, despite widespread recognition that leaf water is unlikely to be at isotopic steady state (e.g. Wang and Yakir 1995; Harwood et al. 1998; Simonin et al. 2013; Dubbert et al. 2014). An incorrect assumption of isotopic steady state can cause significant errors in interpretation of the influence of leaf water isotope composition on all the applications described above. For example, Yepez et al. (2007) reported errors of up to 25% in the proportion of evapotranspiration from transpiration when comparing assumptions of leaf water isotopic steady state with non-steady state for a riparian woodland dominated by mesquite.

The formal theory underlying our understanding of leaf water isotope composition originates from Dongmann et al.(1974). They derived expressions for the changes in time of leaf water isotopic composition. They expressed the changes in terms of a relaxation time, equivalent in a first order linear system to a time constant, the time taken for the difference between composition and final composition to fall to e^{-1} of the initial difference. Their expression for the time constant, τ, of such changes was

$$\tau = \frac{W(1-h)}{E} \tag{1}$$

where W mol water m^{-2} is the leaf water content, E mol water $m^{-2}s^{-1}$ is the transpiration rate, h (dimensionless) is the relative humidity at leaf temperature. i.e.

$$h = w_a/w_i$$

with w_a and w_i being the ambient and intercellular humidities (mol water vapour/mol moist air). Dongmann and colleagues recognised, without writing it out explicitly, that a more precise expression was

$$\tau = \frac{W\alpha_k\alpha^{+}(1-h)}{E} \quad , \tag{2}$$

where α_k (>1) is the kinetic fractionation during diffusion from the leaf, and α^+ (>1) is the fractionation at the liquid/air interface. They also noted "Since E is proportional to (1-h), τ is independent of the relative humidity h." It is unclear why they did not cancel that term of proportionality, but it was possibly because there was an intuitive feeling that the 'turnover time', W/E, had to be involved.

Farris and Strain (1978) rederived the equations and this time they emphasised the 'turnover rate', E/W, 'the fraction of total leaf water volume transpired per unit time'. They carried out experiments and were puzzled that the observed time constant was less than W/E.

Farquhar and Cernusak (2005) divided the numerator and denominator of Eq (2) by (1-h) and obtained

$$\tau = \frac{W\alpha_k\alpha^+}{gw_i} = \frac{W\alpha_k\alpha^+}{E_1} \quad , \tag{3}$$

where g is the leaf conductance (stomata and boundary layer in series), and E_1 denotes the one-way flux out of the leaf. Because E_1 is greater than the net transpiration rate, the predicted time constant was reduced in magnitude compared to W/E and fitted well the observations of changes in leaf water isotopic composition in the field. They further modified the equation to take into account the observation that lamina water is often less enriched than expected for the sites of evaporation, which they associated with a Péclet effect (Farquhar & Lloyd, 1993; Farquhar & Gan, 2003), where enriched water at the sites of evaporation diffuses back along the path of liquid water movement, but is opposed by the advection of unenriched water. Thus Farquhar & Cernusak assumed that the enrichment of the sites of evaporation beyond source water was p (>1) times that of the enrichment of lamina water, and Eq (3) became

$$\tau = \frac{W\alpha_k\alpha^+}{pgw_i} = \frac{W\alpha_k\alpha^+}{pE_1} \quad . \tag{4}$$

With the advent of laser-based measurements of the various isotopologues (Wang et al., 2012; Simonin et al., 2013; Dubbert et al., 2014; Song et al., 2015a,b; Dubbert et al., 2017) it has become possible to follow the isotopic composition of transpired water in cuvettes and thereby infer the changes in leaf composition, in a manner analogous to the introduction of gas exchange measurements of carbon dioxide exchange to study carbon assimilation. The cuvette introduces an extra element when predicting lamina enrichment and the time constant for response. In 2015 Song et al. (2015b) derived expressions for these phenomena that they described as suggesting a net-flux-based leaf water turnover. We discuss those ideas later, but first derive appropriate equations to describe the response to a step change of the isotopic composition of the water vapour in the air entering the chamber. The results apply to both $^{18}O/^{16}O$ and $^{2}H/^{1}H$ in water.

It may seem somewhat counter-intuitive, and perhaps artificial, to explore leaf water isotopic steady state and turnover time through a label introduced through vapour rather than a label introduced to the transpiration stream entering the leaf through xylem, given that the net flux of water is from the xylem to the atmosphere. However, it should be kept in mind that under some conditions the one-way flux of vapour into the leaf can be up to twice the one-way flux of water entering the leaf through the petiole (Farquhar and Cernusak 2005). Further, field studies have confirmed that rapid changes in the stable isotope composition of atmospheric vapour are common (Huang & Wen, 2014; Lai & Ehleringer 2011; Lee, Smith, & Williams, 2006; Tremoy et al., 2012; Yu, Tian, Ma, Xu, & Qu, 2015), and strongly influence the isotope composition of both leaf water (Goldsmith et al. 2017) and organic matter (Lehmann et al. 2018, 2020). Further studies on the influence of vapour isotopes of leaf water enrichment will improve interpretation of observed variation in leaf water and organic oxygen isotope compositions. Finally, label introduction through vapour has a very clear experimental advantage over a liquid-phase label in that a clear and rapid step change is able to be applied to the whole leaf (assuming there is no patchy stomatal closure). In contrast, a step change in xylem water has a variable lag time because the label will not move at uniform rates through the petiole, leaf vasculature and mesophyll, obscuring the effects of the step change and making application of mathematical models extremely challenging.

Theory

To simplify matters we consider the case where gas exchange rates are steady, and the only change imposed is the isotopic composition of the water vapour entering the cuvette. We ignore ternary effects (von Caemmerer and Farquhar, 1981; Farquhar & Cernusak, 2012). A schematic of the model system considered is illustrated in Figure 1.

The rate of transpiration of the major isotopologue of water is given by

$$E = \frac{w_i - w_a}{r_s + r_b} \quad , \tag{5}$$

where r_s and r_b are the stomatal and boundary layer resistances [$m^2 s$ (mol air)$^{-1}$] to the diffusion of water vapour.

The rate of transpiration of the minor isotopologue (eg $H_2{}^{18}O$) is then

$$R_E E = \frac{\frac{R_e w_i}{\alpha^+} - R_a w_a}{\alpha_s r_s + \alpha_b r_b} \quad , \tag{6}$$

where *R* denotes ‘isotope ratio’ (eg ^{18}O /^{16}O), R_E is that of transpired water and R_e is that of water at the evaporating sites, and α_s and α_b are the fractionation factors associated with stomatal and boundary layer resistances. We now need an expression for $R_a w_a$ and so we use the conservation of the minor isotopologue:

$$u_a R_a w_a = u_{in} R_{in} w_{in} + a R_E E \ , \tag{7}$$

where u_{in} and u_a are the flow rates of moist air into and out of the chamber, with a humidity w_{in} and an isotope ratio of R_{in}, and a m^2 is the leaf area. We consider R_{in} to be a function of time, involving a step change at time *t*=0. It is sometimes convenient to work with the flow rate of dry air, u_d , so that $u_a = \frac{u_d}{1-w_a}$ and $u_{in} = \frac{u_d}{1-w_{in}}$. Equation 7 becomes

$$\frac{u_d}{1-w_a} R_a w_a = \frac{u_d}{1-w_{in}} R_{in} w_{in} + a R_E E \quad ,\text{(7a)}$$

and dividing both sides by $u_a = \frac{u_d}{1-w_a}$ we obtain

$$R_a w_a = \frac{1-w_a}{1-w_{in}} R_{in} w_{in} + \frac{a R_E E}{u_a} \quad . \tag{8}$$

Substituting Equation 8 into Equation 6 and rearranging

$$R_E E = \frac{\frac{R_e w_i}{\alpha^+} - \frac{1-w_a}{1-w_{in}} R_{in} w_{in}}{\alpha_s r_s + \alpha_b r_b + a/u_a} = \frac{\frac{R_e w_i}{\alpha^+} - \frac{1-w_a}{1-w_{in}} R_{in} w_{in}}{\Sigma \alpha r} , \tag{9}$$

where $\Sigma\alpha r = \alpha_s r_s + \alpha_b r_b + a/u_a$. (10)

In other words, addition of the chamber adds a non-fractionating resistance, a/u_a, as shown in Figure 1, due to the transpiration-driven increase in humidity surrounding the leaf.

A step change in R_{in}, δR_{in}causes a change $\frac{-\frac{1-w_a}{1-w_{in}} w_{in}\ \delta R_{in}}{E.\Sigma\alpha r}$ in R_E. In the steady state (a long time after the step change in R_{in}), the isotope ratio of the transpiration, R_E, will equal that of the source water, R_S, and R_e will be at a steady value of R_{es}, while R_{in}will be unchanged at its value after the step change (*t*=0+), and so in the steady state Equation (9) becomes

$$R_S E = \frac{\frac{R_{es} w_i}{\alpha^+} - \frac{1-w_a}{1-w_{in}} R_{in} w_{in}}{\Sigma \alpha r} \quad . \tag{11}$$

Subtracting the steady-state equation (11) from the transient (in the sense that R_E and R_e will be changing, although not R_{in} after *t*=0+) Equation (9) becomes

$$(R_E - R_S)E = \frac{\frac{(R_e - R_{es})w_i}{\alpha^+}}{\Sigma\alpha r} \quad . \tag{12}$$

Dividing through by R_S we obtain the enrichment, $\in_E = (R_E - R_S)/R_S$, of transpired water in the transient with respect to the isotope ratio of the source water, and similarly the enrichment at the sites of evaporation in the transient, $\in_e = (R_e - R_S)/R_S$, and in the steady state, $\in_{es} = (R_{es} - R_S)/R_S$. This is a change in notation: enrichment was previously denoted Δ, but some confusion arose because that symbol is more widely used for discrimination (see discussion by Tcherkez (2010), who wrote “La convention internationale est malheureusement d’utiliser la notation Δ pour ces enrichissements, ce qui introduit quelque peu la confusion avec les fractionnements”). Thus

$$\in_E E = \frac{(\in_e - \in_{es})w_i}{\alpha^+ . \Sigma\alpha r} \quad . \tag{13}$$

Following Farquhar & Cernusak (2005) we make the assumption that

$$\frac{\in_e}{\in_L} = \frac{\in_{es}}{\in_{Ls}} = p, \tag{14}$$

where $\in_L$ is the enrichment of the whole leaf water compared to source water, so that

$$\in_E E = \frac{p(\in_L - \in_{Ls})w_i}{\alpha^+ . \Sigma\alpha r} \quad . \tag{15}$$

By expressing all isotopic compositions as enrichments above source water, the flux into the leaf from the soil carries no isoflux. This means that the rate of change of ‘isostorage’, the product $W\frac{d\in_L}{dt}$, is minus the ‘net isoflux’ through the stomata, the product $E\in_E$, of net flux of transpiration, E, and $\in_E$, the enrichment of the transpired water. Thus (Farquhar & Cernusak, 2005)

$$W\frac{d\in_L}{dt} = -\in_E E \quad , \tag{16}$$

and using equation (15) this yields

$$W\frac{d\in_L}{dt} = -\frac{p(\in_L - \in_{Ls})w_i}{\alpha^+ \Sigma\alpha r} \tag{17}$$

which may be integrated

$$\int \frac{d(\in_L - \in_{Ls})}{(\in_L - \in_{Ls})} = \int -\frac{dt}{W\alpha^+ \Sigma\alpha r/(pw_i)}$$

That is

$$\int d ln\,(\in_L - \in_{Ls}) = \int \frac{dt}{\tau}$$

$$ln\frac{(\in_L - \in_{Ls})}{(\in_{L0} - \in_{Ls})} = -\frac{t}{\tau}$$

or

$$\in_L\ (t) = \in_{Ls} + (\in_{L0} - \in_{Ls})e^{-t/\tau}\ , \tag{18}$$

with $\in_{L0}$ being the value of the enrichment of the lamina at time zero, immediately after the step change in ambient enrichment (but in this case identical to the value immediately before the step change in $\in_{in}$); τ is the time constant for response and τ is given by

$$\tau = \frac{W\alpha^+ \Sigma\alpha r}{p.w_i} = \frac{W\alpha^+(\alpha_b.r_b + \alpha_s.r_s + \frac{a}{u_a})}{p.w_i} = \frac{W.\overline{\alpha}_k.\alpha^+}{p.E_1}\ , \tag{19}$$

where

$$E_1 = g_t.w_i \tag{20}$$

and represents the one-way flux out through the stomata (note the slight change in notation compared to Farquhar & Cernusak (2005) who absorbed the factor $\overline{\alpha}_k\alpha^+$ into the definition of E_1), and

$$g_t = 1/(r_b + r_s + \frac{a}{u_a}) \tag{21}$$

is the associated conductance, taking boundary layer and chamber resistances into account, and the resistance- weighted kinetic fractionation is

$$\overline{\alpha}_k = \frac{(\alpha_b.r_b+\alpha_s.r_s+\frac{a}{u_a})}{(r_b+r_s+\frac{a}{u_a})} \;. \tag{22}$$

Given that $\in_L - \in_{Ls}$ has an initial value $\in_{L0} - \in_{Ls}$ and an exponential response with time constant τ, approaching zero at large *t* (Equation 18), then so too from Equations (14) does $\in_e - \in_{es}$ and from Equations (17) $\frac{d\in_L}{dt}$ does also, and from Equation (16) transpiration enrichment $\in_E$, and from Equation (9) it follows that the enrichment, $\in_{E1} - \in_{E1s}$, of the one-way flux out of the leaf does also. From Equation (8), the enrichment of the ambient air minus the steady enrichment, $\in_a - \in_{as}$, also follows. Thus, the shape of the curve represented by Equation (18) becomes generic with

$$\frac{(\in_L - \in_{Ls})}{(\in_{L0} - \in_{Ls})} = e^{-t/\tau} = \frac{(\in_e - \in_{es})}{(\in_{e0} - \in_{es})} = \frac{-\tau.\frac{d\in_L}{dt}}{\in_{L0} - \in_{Ls}} = \frac{\in_E}{\in_{E0}} = \frac{\in_{E1} - \in_{E1s}}{\in_{E10} - \in_{E1s}} = \frac{\in_a - \in_{as}}{\in_{a0} - \in_{as}} \tag{23}$$

From the foregoing, and noting that the enrichment of transpiration rate over source water is zero in the steady state, we can describe the isofluxes at time zero, when the disturbance is greatest, by

$$E.\in_{E0} = -E_1.(\in_{E1s} - \in_{E10}) = -\frac{E_1.(\in_{es} - \in_{e0})}{\overline{\alpha}_k.\alpha^+} = -\frac{E_1.p(\in_{Ls} - \in_{L0})}{\overline{\alpha}_k.\alpha^+} = \frac{-\frac{1-w_a}{1-w_{in}} w_{in}(\in_{in} - \in_{in}(t=0^-))}{\Sigma\alpha r} \;, \tag{24}$$

So a step change (assume an increase for argument's sake) in $\in_{in}$ from its previous value $\in_{in}$ $(t = 0^{-})$ causes an initial positive step change in $\in_a$ and that causes a negative step change in $\in_E$ leading to the negative initial value of $\in_{E0}$, followed by an exponential recovery to zero. See Figure 2 for an illustration. The negative $\in_E$ causes $\in_L$ and $\in_e$ and hence $\in_{E1}$ to commence to increase. An important point is that for the previous equations to make sense, time zero starts just after the step change in $\in_{in}$, $\in_a$, and $\in_E$ and thus $\in_{in}$ for our purposes is taken as a constant for t=0+. Again, the steady state refers to the settled condition after the responses to the step change, and not to any steady state before the step change. For an example system characterised by the parameters in Table 1, the theoretical enrichment responses to a step increase in $\in_{in}$ are presented in Figure 2 and Table 2.

Referring again to Equation (23), after a positive step change in $\in_{in}$ there is a transient period characterised by $\in_{a0}<\in_{as}$, $\in_{E0}<\in_{Es}$, $\in_{e0}<\in_{es}$, $\in_{L0}<\in_{Ls}$, $\frac{d\in_L}{dt}(0) > \frac{d\in_L}{dt}(s)$ [see Table 2 for examples], and *vice versa* for all if the initial change in $\in_{in}$ was negative.

Verification

It is a property of the first order linear differential equations like (18) that the initial speed of action in elimination of the transient enrichment $(\in_{L0}-\in_{Ls})$, if maintained, would have the task completed in time τ. This may be seen by differentiating Equation (18) with respect to time

$$\frac{d\in_L}{dt} = \frac{-1}{\tau}(\in_{L0}-\in_{Ls})e^{-t/\tau} \tag{25}$$

and examining the value at time t=0:

$$\frac{d\in_L}{dt} = \frac{-1}{\tau}(\in_{L0}-\in_{Ls}) \tag{26}$$

showing that the initial enrichment would disappear in time τ, if the initial rate were maintained. Of course the rate declines and at time τ the enrichment has only reduced to $(\in_{L0} - \in_{Ls})e^{-1}$, i.e. to 0.37 of its initial value. See Figure 3.

The expression for τ (Equation 19) may be examined in this light, by multiplying above and below by $(\in_{e0} - \in_{es})$,

$$\tau = \frac{W.\overline{\alpha}_k.\alpha^+}{p.E_1} = \frac{W.(\in_{e0} - \in_{es})}{p} . \frac{\overline{\alpha}_k.\alpha^+}{(\in_{e0} - \in_{es}).E_1} \quad . \qquad (27)$$

But from Equation (14)

$$\frac{W.(\in_{e0} - \in_{es})}{p} = W.(\in_{L0} - \in_{Ls})$$

and this is divided by

$$\frac{(\in_{e0} - \in_{es}).E_1}{\overline{\alpha}_k.\alpha^+} = (\in_{E10} - \in_{E1s}).E_1$$

so that Equation (27) becomes

$$\tau = \frac{W.(\in_{L0} - \in_{Ls})}{E_1(\in_{E10} - \in_{E1s})} . \qquad (28)$$

which can be interpreted as $W.(\in_{L0} - \in_{Ls})$ representing the enrichment ('isostorage') to be 'removed', and $E_1(\in_{E10} - \in_{E1s})$ being the isoflux doing the removal.

Of course, from Equation (23) the denominator of Equation (27) is identical to the isoflux carried by net transpiration, $E.\in_{E0}$, so that τ can also be written as

$$\tau = \frac{W.(\in_{L0} - \in_{Ls})}{E.\in_{E0}} \quad , \qquad (29)$$

which is a more intuitive formula. The equivalence of Equations (28) and (29) may at first sight be confusing. The numerators or isostorage are of course identical. The denominators or isofluxes are also equal, but the one-way flux E_1 is greater than the net flux, E, meaning that the enrichment of the transpiration flux $\in_{E0}$ must be greater than ($\in_{L0} - \in_{Ls}$) by an equal proportion, as was indeed shown in the derivation of Equation (27). We also see that the isostorage would be eliminated in time τ if the isoflux remained steady at the initial rate of $E.\in_{E0}$.

Why is τ given to a good approximation by W/E_1, where E_1 is the one-way flux out of the leaf, and not by W/E, where E is the net transpiration rate? Consider the total flux coming into a leaf. A flux $g_t w'_{in}$ (where $w'_{in} = \frac{1-w_a}{1-w_{in}} w_{in}$) enters in the gas phase through the stomata, and E enters through the petiole. So the total flux entering the leaf is $E + g_t w'_{in}$. But $E = g_t.w_i - g_t w_{in}$, so $E + g_t w_{in} = g_t.w_i$ and that is why it is the flux $g_t.w_i$ that must turn over W mol.m^{-2}, which would take (at the initial rate) $W/(g_t.w_i)$ seconds. Hence $\tau \approx \frac{W}{g_t w_i}$

Summarising the theory thus far, Equation (4) (Farquhar & Cernusak 2005) has been modified to Equation (19) to include the additional chamber resistance a/u (m^{2}s.mol^{-1}) [which does not fractionate], but the overall form is unchanged, with the time constant of relaxation remaining as the leaf water content divided by the one-way efflux, with the corrections for fractionation and the Péclet effect equivalent to those as found in Equation (4). The Péclet term, p, arises as the correction made to the volume of the leaf to account for water that is unenriched, or inaccessible. We recognise that p is at this stage a largely empirical correction. Hence W is replaced by W/p and so $\tau \approx \frac{W}{p \cdot g_t w_i}$.

Discussion

Comparison with Song et al. (2015)

Song et al. (2015) derived equations (here with numbers denoted S) to deal with the effect of including a chamber and came to a different conclusion. They noted on page 2626 that *"emphasizing a 'gross flux' was previously discussed by Farquhar & Cernusak (2005), who* *pointed out that alternate use of 'net flux'-based leaf water turnover time (i.e. W/E) is incorrect and can potentially lead to biased interpretation of NSS ΔL data. However, it is interesting that such a 'gross flux' viewpoint is no longer valid in our cuvette experimental setting, as Eqn S21* clearly indicates that it is W/E but not W/g.*w_i *that exerts control on τ. Our observation that the new model predicted a larger time constant than did the F&C model is therefore a reflection of the fact that 'net-flux'-based leaf water turnover is slower than its 'gross-flux' based counterpart (i.e.W/E >W/g.*w_i*)."*

The present treatment (Equation 19), in contrast, suggests that the reason for the larger time constant when a chamber is present is because the one-way flux, E_1, is reduced by the presence of the additional (chamber) resistance, a/u. It is easy to see that as the flow rate, u, increases, the solution degenerates to that derived by Farquhar & Cernusak for the case where there is no chamber.

Song et al. (2015b) showed that their Equation matched observations of changes with time in the isotopic composition of transpired water. How can this be resolved?

Equation S21 to which Song et al. refer (above) was written as follows:

$$\tau \approx (1-f)W/E$$

where (1-f) is equivalent to 1/p above. It may be seen from Equation 19 that this relationship only works when $E=E_1$, and this is only the case when the incoming air is dry ($w_{in} = 0$). Indeed their experiments were carried out with dry incoming air. However, the experimental comparison was not with Equation S21, but with their more detailed Equation S20:

$$\tau = (1-f).\frac{W}{E}.\alpha^{+}[\alpha_k(1-\frac{w_a}{w_i}) + (\frac{w_a}{w_i})]$$

Equation S20 was actually a simplification of what was written in their Equation (S14) as

$$\tau = (1-f).\frac{W}{E}.\alpha^{+}.K$$

$$= (1-f).\frac{W}{E}.\alpha^{+}[\alpha_k(1\text{-}\frac{w_a}{w_i}) + (\frac{w_a - w_{in}}{w_i})(\frac{1}{1-w_{in}})] \qquad \text{(S14)}$$

However the square-bracketed term in Equation S14, which they denoted as *K,* may be rewritten as

$$K = \left[\alpha_k\left(1-\frac{w_a}{w_i}\right) + \left(\frac{w_a - w_{in}}{w_i}\right)\left(\frac{1}{1-w_{in}}\right)\right] = [\alpha_k E(\frac{r_b + r_s}{w_i}) + (\frac{w_a - w_{in}}{w_i})(\frac{1}{1-w_{in}})]$$

and because their experiments were with dry air entering the chamber (w_{in}=0), *K* was approximately 1, so that the time constant would appear to relate to *W/E*. However use of dry air is not generally the case. It is shown in Appendix 1 that

$(\frac{w_a - w_{in}}{1-w_{in}}) = \frac{aE}{u_a}$.

So that

$$K = [\alpha_k E(\frac{r_b + r_s}{w_i}) + \frac{aE}{u_a w_i}]$$

And recognising that in their notation $\alpha_k = \frac{\alpha_b.r_b + \alpha_s.r_s}{r_b + r_s}$ this means that Equation S14 may be rewritten as

$$\tau = \frac{(1-f)W\alpha^{+}(\alpha_b.r_b + \alpha_s.r_s + \frac{a}{u_a})}{w_i} = \frac{(1-f)W.\overline{\alpha}_k.\alpha^{+}}{E_1}$$

which is numerically and algebraically equivalent to Equation (19) above, with *p* replaced by 1/(1-*f*). It confirms that even when a chamber is used it is still the one-way flux that is determining the time constant. Put simply, it is the one-way flux E_1 that determines the time constant, and if the incoming air happens to be perfectly dry then E_1 equals *E*, the transpiration rate.

All the equations discussed above are derived assuming that the leaf water content W is unchanging. If W is not steady the analytical solution becomes that given in Equations (27) of Farquhar and Cernusak (2005), with E_1 adjusted for the extra chamber resistance.

On the ratio of evaporative site enrichment to lamina enrichment

The ratio, p (>1), of evaporative site enrichment to lamina enrichment, $\in_e/\in_L$, derives from Péclet theory (Farquhar & Lloyd, 1993) and the Péclet number, P, as

$$\in_L/\in_e = 1/p = \frac{1-e^{-P}}{P} \approx 1 - P/2$$

and since Song et al. (2015) defined (1-f) as $\in_L/\in_e$ it means that $f \approx P/2$. From the results of their experiments, a typical value of f was 0.15 for both ^{2}H/^{1}H and ^{18}O/^{16}O, implying that P would be about 0.3 if the effect was a Péclet one. Holloway-Phillips at al. (2016), also working with cotton, obtained values of f of about 0.3, implying an even greater value of P, if the Péclet effect is the source of non-zero f. Of course, f may well represent the proportion of leaf water that is not isotopically accessible on the time scale of the experiment.

Our recent experimental study (Barbour et al. 2020) indicates a possible mechanistic link between the hydraulic design of leaves and the presence of Péclet-like responses of leaf water ^{18}O enrichment to transpiration rate. Leaves with hydraulically well-connected vasculature and epidermes, but hydraulically isolated mesophyll (hydraulic design two, as described by Zwieniecki, Brodribb and Holbrook 2007), were found to have leaf water isotope compositions best modelled by a Péclet effect. In contrast, leaves with either hydraulically isolated mesophyll and epidermes (hydraulic design one), or hydraulically connected mesophyll (hydraulic design three) had leaf water isotope compositions best modelled using a simple two-pool approach without a strong Péclet effect. More species, and more diversity in leaf anatomy and hydraulic architecture, are required to determine the generality of these observations. However, the suggestion above that *f* may represent the proportion of leaf water that is not isotopically accessible, implies hydraulically- and isotopically-distinct pools of water in the leaf consistent with hydraulic design one which is common in coniferous needles for example. As Simonin et al. (2013) point out, multiple pools of isotopically-distinct water in leaves may each have different turn-over times which would complicate mathematical modelling and experimental interpretation. We are currently assessing experimental data to determine if this additional complication is warranted; note that Simonin et al. (2013) found no evidence of more than one pool in experimental data using citrus and tobacco leaves.

Effect of adding a cuvette

The effect on the transpiration rate of adding a leaf of a m^2 to a cuvette with air flow after the chamber of u_a mol s^{-1} is essentially that of adding a resistance of a/u_a. An analogous effect occurs for the description of CO_2 assimilation rate, *A* (Farquhar, 1973). Consider a leaf where a simplified description is

$$A = \frac{C_a - \Gamma}{r_b^c + r_s^c + r_m^c + r_{chem}^c}$$

where C_a is the ambient [CO_2] and Γ is the CO_2 compensation point, and the resistances are those of the boundary layer, the stomata, the mesophyll, and the underlying biochemistry. This expression works if C_a is an independent variable. If the concentration entering the chamber, C_{in} , is the independent variable, then the above equation is modified to

$$A = \frac{\frac{1-w_a}{1-w_{in}}C_{in} - \Gamma}{r_b^c + r_s^c + r_m^c + r_{chem}^c + a/u_a} \ .$$

There is another effect of adding a cuvette. The volume of the chamber and associated tubing and apparatus upstream of the leaf (V mol) introduces a delay of $\tau_a = V/u_a$. If the cuvette is sufficiently small and the flow rate sufficiently large, the delay would be less than a minute, and insignificant compared to τ in most leaves. However, for some experiments where one wants to assess the whole leaf, or perhaps a whole plant, larger cuvettes are necessary, and so in Appendix 2 we solve for the inclusion of cuvette volume, assumed to be well mixed. The resulting expressions for ambient and leaf water enrichment respectively, are:

$$\epsilon_a\ (t) = \epsilon_{as} - \left\{\beta e^{-\frac{t}{\tau_1}} + \gamma e^{-\frac{t}{\tau_2}}\right\} / (\tau_2 - \tau_1) \qquad \text{(A43)}$$

and

$$\epsilon_L\ (t) = \epsilon_{Ls} - \left\{\omega e^{-\frac{t}{\tau_1}} + \theta e^{-\frac{t}{\tau_2}}\right\} / (\tau_2 - \tau_1) \qquad \text{(A44)}$$

where,

$$\beta = \epsilon_{as}\ (\tau - \tau_1) - \epsilon_{a0}\ (\tau_2 - \tau_a) - (\epsilon_{Ls} - \epsilon_{L0}) b\tau_a$$

$$\gamma = \epsilon_{as}\ (\tau - \tau_2) - \epsilon_{a0}\ (\tau_1 - \tau_a) - (\epsilon_{Ls} - \epsilon_{L0}) b\tau_a$$

$$\omega = \epsilon_{Ls}\ (\tau_a - \tau_1) - \epsilon_{L0}\ (\tau - \tau_2) - (\epsilon_{as} - \epsilon_{a0}) \upsilon\tau$$

$$\theta = \epsilon_{Ls}\ (\tau_2 - \tau_a) - \epsilon_{L0}\ (\tau_1 - \tau) - (\epsilon_{a0} - \epsilon_{as}) \upsilon\tau$$

$$\upsilon = \frac{V}{u_a} \frac{w_a}{\Sigma \alpha r.W}$$

$b = \frac{aW}{Vw_a}$ (the ratio of the amount of liquid water in the leaf to the amount of water vapour in the cuvette system.)

and τ_2 is the modified τ, and τ_1 the modified τ_a.

Figure 4 illustrates the difference accounting for a range of delays ($\tau_a = V/u_a$) associated with the cuvette and upstream apparatus makes to the response of $\in_a$ and $\in_L$ over time.

The analysis given here will, we hope, be useful for studies of water relations, including water movement in leaves, and in isotope hydrology more generally. It may be of interest, also, as an analog of large-scale gas exchange, such as that discussed by Helliker et al. (2004).

v. Acknowledgements

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vi. Conflict of interest

The authors declare no conflict of interest.

vii. Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

viii. Figure Legends

Figure 1: Schematic of model system comprising a leaf enclosed within a cuvette.

Top: moist air enters the cuvette with flow rate u_{in} [mol moist air s^{-1}] , humidity w_{in} [mol water vapour/mol moist air], and isotope ratio R_{in}; and exits with flow rate u_a, humidity w_a, and isotope ratio R_a. The leaf of area a [m^2] contains water (W [mol water m^{-2}]) at R_L . The flux into the leaf from the stem J [mol water m^{-2}s^{-1}] carries source water with isotopic composition R_s and the net flux out from it by transpiration, E [mol water m^{-2} s^{-1}], carries water vapour at R_E.

Inset: The components of the net transpiration flux, namely the one way gross flux out ($E1$) and in ($E1$-E) through the stomata are represented by red dashed arrows. The effect of the cuvette is to increase the resistance by adding the chamber resistance a/u_a to the stomatal and boundary layer resistance (r_s and r_b) in series. The gross fluxes can be thought of as a flux out from the leaf where humidity is w_i and a flux in from the air where humidity is $w'_{in} = \frac{1-w_a}{1-w_{in}} w_{in}$.

The isotopic composition of water at the evaporating sites is R_e and that of the intercellular vapour leaving the leaf (light blue) is R_e/α^+, whilst the vapour entering is R_{in}.

Figure 2: Enrichment responses to a step increase in the enrichment of water vapour entering a cuvette ϵ_{in}, ignoring any time delay introduced by the cuvette and associated volume.

Figure 3: All enrichment responses to either a step increase (as in figure 2) or step decrease in the enrichment of water vapour entering a cuvette, ϵ_{in}, can be represented by a single generic exponential function when scaled according to Eq. 23 (ignoring any time delay introduced by the cuvette and associated volume, i.e. V=0).

Figure 4: The effect of accounting for the time delay introduced by the cuvette and associated volume on the response of ϵ_a and ϵ_L for the example illustrated in figure 2 (a step increase in ϵ_{in} at t=0).

ϵ_a (black dash) and ϵ_L(green dash) are reproduced from figure 2 and ignore the time delay introduced by the cuvette and associated volume (i.e V=0 and $\tau_a = 0$). The series of curves ϵ_a (red) and ϵ_L(blue) are produced using equation A43 and A44, respectively, account for a range of characteristic times, τ_a related to a non-zero volume, V.

ix. Table Legends

Table 1: Parameters characterising the example theoretical system that is subjected to a step increase in the enrichment of water vapour entering the cuvette, ϵ_{in} in figure 2.

Table 2: Calculated enrichment values just prior to and immediately after a 82.1 ‰ step increase in ϵ_{in} for the example described in Table 1 and illustrated in figure 2. Steady state is assumed just prior to the step increase (at $t = 0^-$) to determine the enrichment values just prior to the increase. The time constant, τ predicted by the theory (Eqn 19) is used to model the transient response of the enrichments following the step increase as they approach steady state in figure 2.

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xi. Tables:

Table 1: Parameters characterising the example theoretical system that is subjected to a step increase in the enrichment of water vapour entering the cuvette, ϵ_{in} in figure 2.

R_{in} ($t= 0^-$)	1.985×10^{-3}
R_{in} ($t \geq 0$)	2.153×10^{-3}
R_S	1.997×10^{-3}
ϵ_{in}($t= 0^-$)	-5.91 ‰
ϵ_{in}($t \geq 0$)	77.8 ‰
A	1.2×10^{-3} m^2
u_{in}	600×10^{-6} mol s^{-1}
w_{in}	9.20×10^{-3} mol mol^{-1}
u_a	606×10^{-6} mol s^{-1}
w_a	19.8×10^{-3} mol mol^{-1}
w_i	34×10^{-3} mol mol^{-1}
E	5.4 mmol m^{-2} s^{-1}
E_1	7.4 mmol m^{-2} s^{-1}
T_{leaf}	25 °C
r_s	2.30 m^2 s mol^{-1}
r_b	0.33 m^2 s mol^{-1}
W	18.7 mol m^{-2}
P	1.44

Table 2: Calculated enrichment values just prior to and immediately after a 82.1 ‰ step increase in ϵ_{in} for the example described in Table 1 and illustrated in figure 2. Steady state is assumed just prior to the step increase (at $t = 0^{-}$) to determine the enrichment values just prior to the increase. The time constant, τ predicted by the theory (Eqn 19) is used to model the transient response of the enrichments following the step increase as they approach steady state in figure 2.

ϵ_{as}(t= 0^{-})	$\in_{as}\ (t = 0^{-}) = \frac{w'_{in}}{w_a}(1 +\in_{in}\ (t = 0^{-}))+\frac{aE}{u_a w_a}$ -1	−2.7 ‰
ϵ_{a}(t= 0^{+})	$\in_{a0}= \frac{w'_{in}}{w_a}(1 +\in_{in})+\frac{aE}{u_a w_a}(1 + w'_{in}(\frac{\in_{in}(t=0^{-})-\in_{in}}{E\sum \alpha r}))$-1 where $w'_{in} = \frac{1-w_a}{1-w_{in}} w_{in}$	19.5‰
ϵ_{as}	$\in_{as}= \frac{w'_{in}}{w_a}(1 +\in_{in})+\frac{aE}{u_a w_a}$ -1	35.8‰
ϵ_{es}(t= 0^{-})	$\in_{es}\ (t = 0^{-}) = \frac{E\square\sum \alpha r + w'_{in}\left(1 +\in_{in}\ (t = 0^{-})\right)}{w_i/\alpha^{+}} - 1$	19.1‰
ϵ_{e}(t= 0^{+})	$\in_{e0} = \ \in_{es}\ (t = 0^{-})$	19.1‰
ϵ_{es}	$\in_{es}= \frac{E\square\sum \alpha r + w'_{in}(1 +\in_{in})}{w_i/\alpha^{+}} - 1$	41.7‰
ϵ_{Es}(t= 0^{-})	$\in_{Es}\ (t = 0^{-}) = \ 0$	0 ‰
ϵ_{E}(t= 0^{+})	$\in_{E0}= \frac{-w'_{in}\left(\in_{in}-\in_{in}\ (t = 0^{-})\right)}{E\sum \alpha r}$	−30.1‰
ϵ_{Es}	$\in_{Es}= 0$	0 ‰
ϵ_{Ls}(t= 0^{-})	$\in_{Ls}\ (t = 0^{-}) = \left\{\frac{E\cdot\Sigma\alpha r + w'_{in}(1 +\in_{in}\ (t = 0^{-}))}{w_i/\alpha^{+}} - 1\right\}/p$	13.3 ‰

ϵ_L(t= 0^+)	$\in_{L0} = \in_{Ls}\ (t = 0^-)$	13.3‰
ϵ_{Ls}	$\in_{Ls} = \left\{\frac{E \cdot \Sigma\alpha r + w'_{in}(1 + \in_{in})}{w_i/\alpha^+} - 1\right\}/p$	29.0‰
τ	$\tau = \frac{W.\overline{\alpha}_k.\alpha^+}{p.E_1}$	1.805×10^3 s

xii. Figure legends:

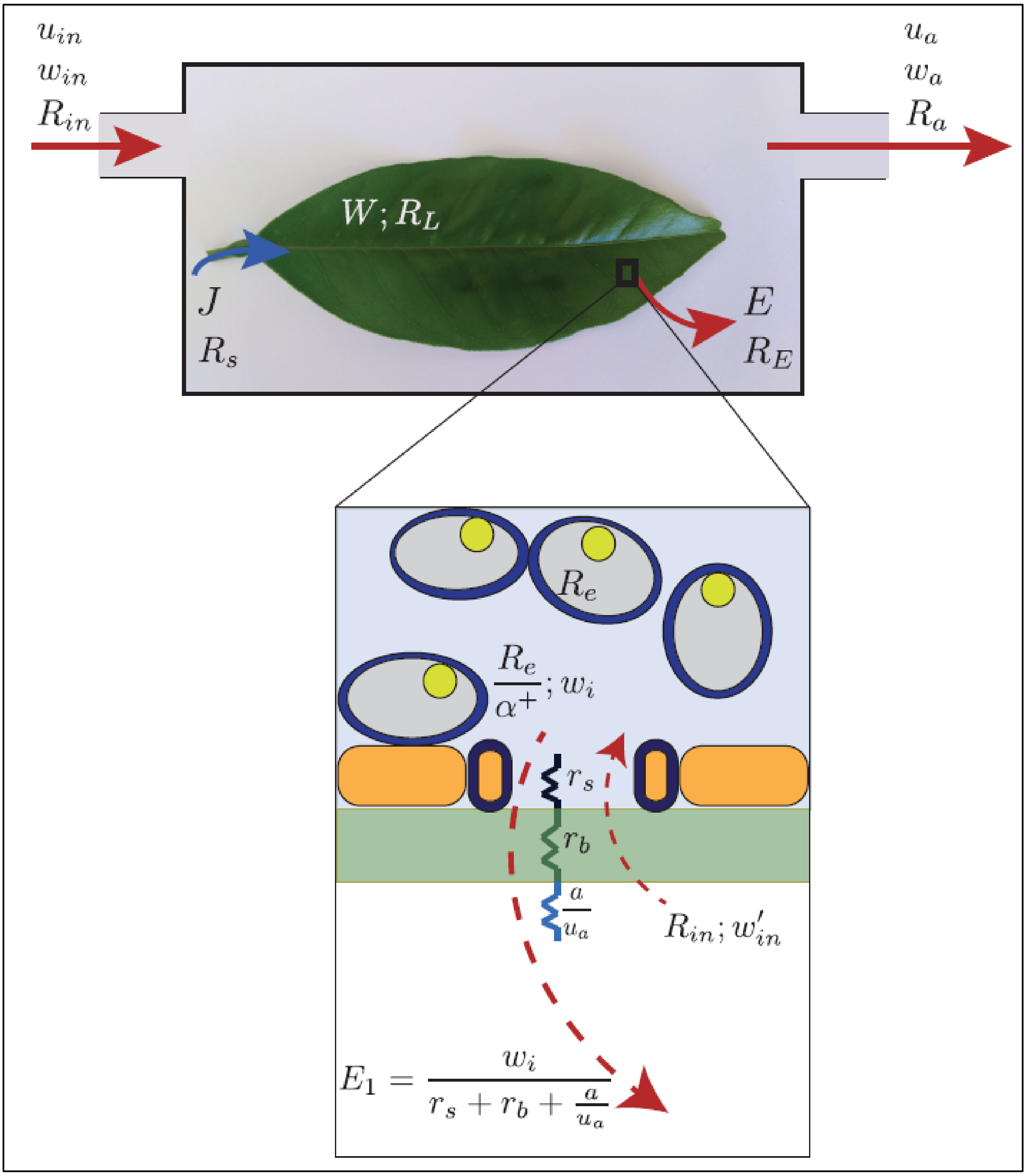


Figure 1: Schematic of model system comprising a leaf enclosed within a cuvette.

Top: moist air enters the cuvette with flow rate u_{in} [mol moist air s^{-1}] , humidity w_{in} [mol water vapour/mol moist air], and isotope ratio R_{in}; and exits with flow rate u_a, humidity w_a, and isotope ratio R_a. The leaf of area a [m^2] contains water (W [mol water m^{-2}]) at R_L . The flux into the leaf from the stem J [mol water m^{-2}s^{-1}] carries source water with isotopic composition R_s and the net flux out from it by transpiration, E [mol water m^{-2} s^{-1}], carries water vapour at R_E.

Inset: The components of the net transpiration flux, namely the one way gross flux out ($E1$) and in ($E1$-E) through the stomata are represented by red dashed arrows. The effect of the cuvette is to increase the resistance by adding the chamber resistance a/u_a to the stomatal and boundary layer resistance (r_s and r_b) in series. The gross fluxes can be thought of as a flux out from the leaf where humidity is w_i and a flux in from the air where humidity is $w'_{in} = \frac{1-w_a}{1-w_{in}} w_{in}$.

The isotopic composition of water at the evaporating sites is R_e and that of the intercellular vapour leaving the leaf (light blue) is R_e/α^+, whilst the vapour entering is R_{in}.

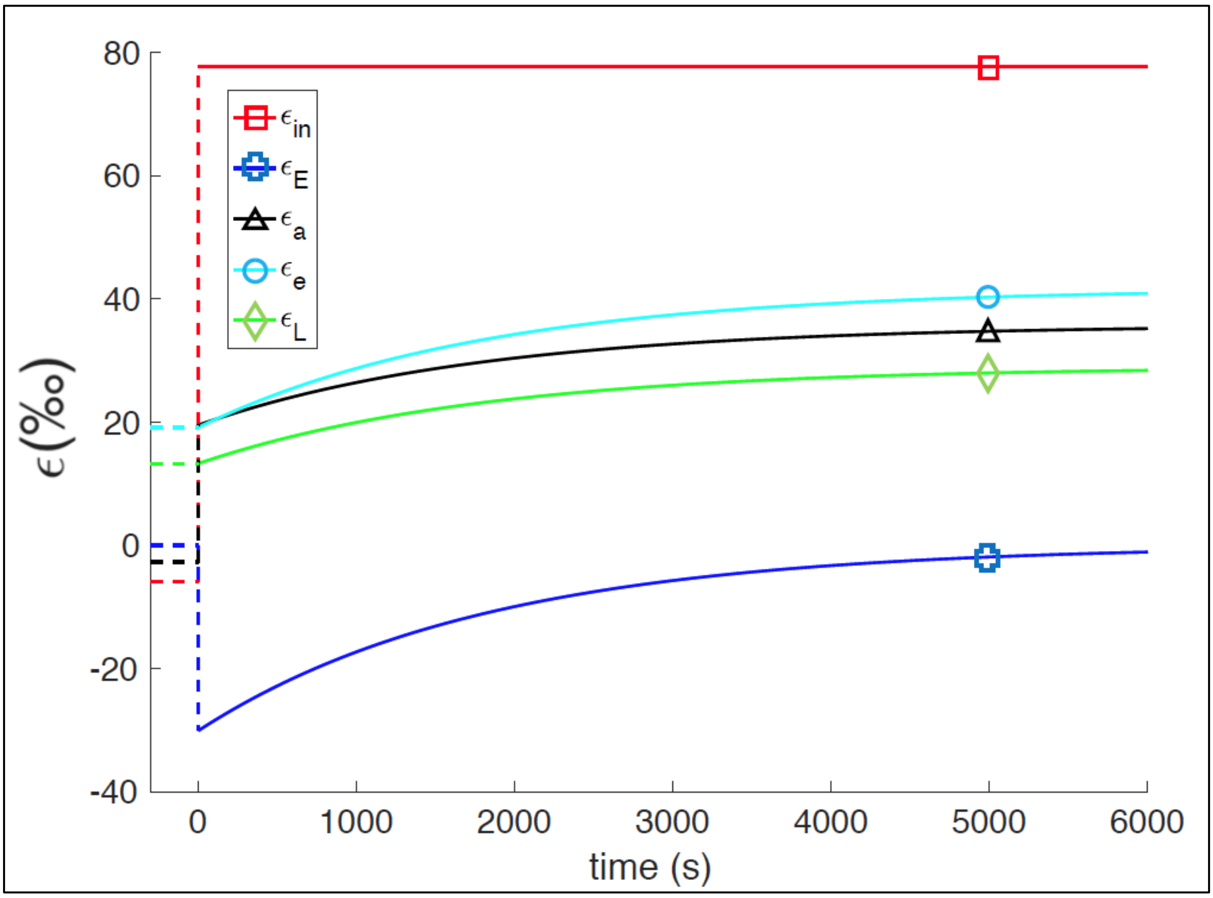


Figure 2: Enrichment responses to a step increase in the enrichment of water vapour entering a cuvette ϵ_{in}, ignoring any time delay introduced by the cuvette and associated volume.

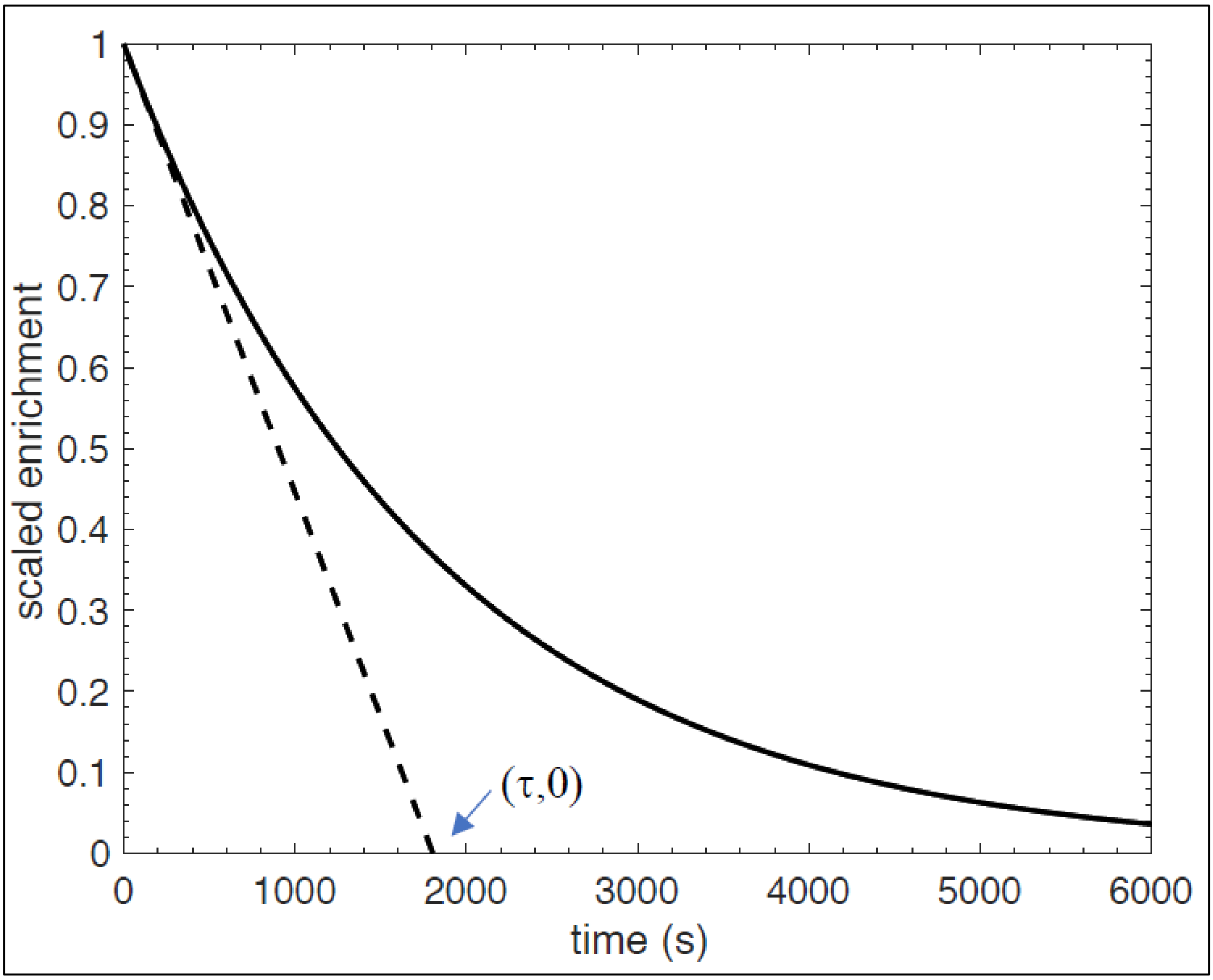
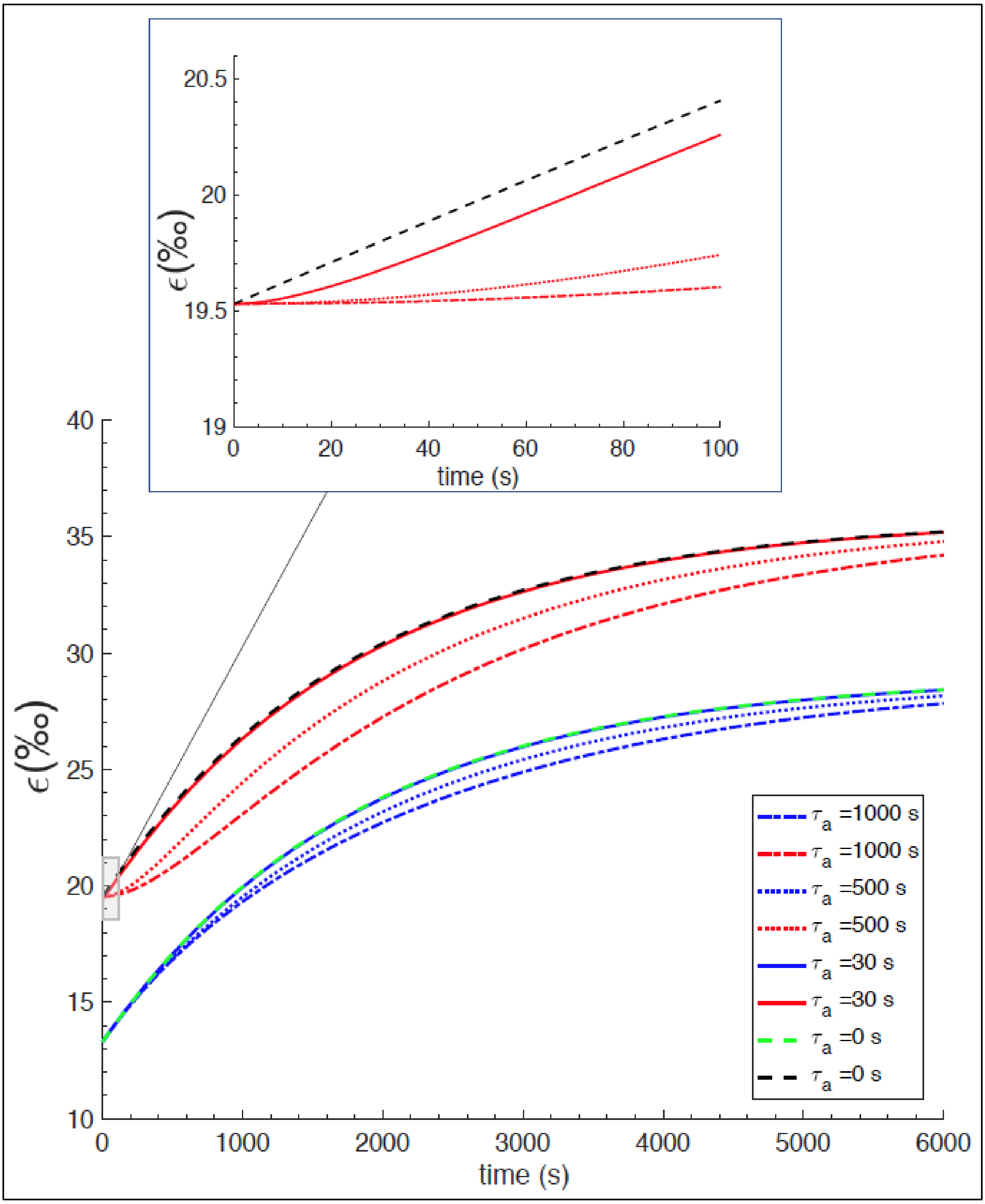


Figure 3: All enrichment responses to either a step increase (as in figure 2) or step decrease in the enrichment of water vapour entering a cuvette, ϵ_{in}, can be represented by a single generic exponential function when scaled according to Eq. 23 (ignoring any time delay introduced by the cuvette and associated volume, i.e. V=0).



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ϵ_a (black dash) and ϵ_L(green dash) are reproduced from figure 2 and ignore the time delay introduced by the cuvette and associated volume (i.e V=0 and $\tau_a = 0$). The series of curves ϵ_a (red) and ϵ_L(blue) are produced using equation A43 and A44, respectively, account for a range of characteristic times, τ_a related to a non-zero volume, V.

Appendix 1: **Proof that**

$$\boldsymbol{\frac{aE}{u_a} = \frac{w_a - w_{in}}{(1 - w_{in})}}$$

Conservation of water gives

$u_{in}.w_{in} + aE = u_a.w_a$ (A1)

where u_{in} and u_a are the flow rates (mol moist air s^{-1}) into and out of the chamber, and w_{in} and w_a (mol water vapour/mol moist air) are the incoming and ambient (outgoing in a well-mixed chamber) humidities. It is convenient to replace u_{in} and u_a by the flow rates of dry air, u_d, using $u_d/(1 - w_{in})$ and $u_d/(1 - w_a)$, respectively. Eq (A1) becomes

$$aE = \frac{u_d.w_a}{1 - w_a} - \frac{u_d.w_{in}}{1 - w_{in}}$$

Dividing through by u_d and bringing the RHS to a common denominator, we obtain

$$\frac{aE}{u_d} = \frac{w_a - w_{in}}{(1 - w_a)(1 - w_{in})}$$

And converting u_d back to u_a we obtain

$$\frac{aE}{u_a} = \frac{w_a - w_{in}}{(1 - w_{in})} \qquad \text{(A5)}$$

Appendix 2: **Including time delay introduced by cuvette and associated volume**

Following the theory in the main text, Equation (6) is unchanged.

$$R_E E = \frac{\frac{R_e w_i}{\alpha^+} - R_a w_a}{\alpha_s r_s + \alpha_b r_b} \quad . \qquad (6)$$

Equation (7) is replaced by

$$V\frac{d(R_a w_a)}{dt} + u_a R_a w_a = u_{in} R_{in} w_{in} + aR_E E \quad , \tag{A7}$$

where V mol is the volume of the chamber, and (8) by

$$R_a w_a = \frac{1-w_a}{1-w_{in}} R_{in} w_{in} + \frac{aR_E E}{u_a} - \frac{V}{u_a}\frac{d(R_a w_a)}{dt} \quad . \tag{A8}$$

Dividing through by R_S

$$(1+\in_a)w_a = \frac{1-w_a}{1-w_{in}}\left(1+\in_{in}\right)w_{in} + \frac{a(1+\in_E)E}{u_a} - \frac{V}{u_a}\frac{d((1+\in_a)w_a)}{dt}$$

Substituting (A8) into (6) and rearranging,

$$R_E E = \frac{\frac{R_e w_i}{\alpha^+} - \frac{1-w_a}{1-w_{in}} R_{in} w_{in} + \frac{V}{u_a}\frac{d(R_a w_a)}{dt}}{\alpha_s r_s + \alpha_b r_b + a/u_a} = \frac{\frac{R_e w_i}{\alpha^+} - \frac{1-w_a}{1-w_{in}} R_{in} w_{in} + \frac{V}{u_a}\frac{d(R_a w_a)}{dt}}{\Sigma\alpha r} \, . \tag{A9, A10}$$

In the steady state, the transpiration has the isotopic composition of the source water, and so (11) is unchanged

$$R_S E = \frac{\frac{R_{es} w_i}{\alpha^+} - \frac{1-w_a}{1-w_{in}} R_{in} w_{in}}{\Sigma\alpha r} \, . \tag{11}$$

Subtracting the steady-state equation (11) from the transient equation (A9)

$$(R_E - R_S)E = \frac{\frac{(Re - R_{es}) w_i}{\alpha^+} + \frac{V}{u_a}\frac{d(R_a w_a)}{dt}}{\Sigma\alpha r} \quad . \tag{A12}$$

Dividing through by R_S

$$\in_E E = \frac{(\in_e - \in_{es}) w_i/\alpha^+ + \frac{V}{u_a}\frac{d((1+\in_a)w_a)}{dt}}{\Sigma\alpha r} \ ,$$

and again assuming w_a is constant

$$\in_E E = \frac{(\in_e - \in_{es}) w_i/\alpha^+ + \frac{V}{u_a}\frac{w_a . d\in_a}{dt}}{\Sigma\alpha r} \quad . \tag{A13}$$

Again we assume

$$\frac{\in_e}{\in_L} = \frac{\in_{es}}{\in_{Ls}} = p, \tag{14}$$

so that

$$\in_E\ E = \frac{p(\in_L - \in_{Ls})w_i/\alpha^+ + \frac{V}{u_a}\frac{w_a . d\in_a}{dt}}{\Sigma\alpha r} \quad . \tag{A15}$$

The rate of change of ‘isostorage’ is

$$W\frac{d\in_L}{dt} = -\in_E\ E \tag{16}$$

$$= -\frac{p(\in_L - \in_{Ls})w_i/\alpha^+ + \frac{V}{u_a}\frac{w_a . d\in_a}{dt}}{\Sigma\alpha r} \qquad \text{A17}$$

or

$$\frac{d\in_L}{dt} + \frac{V}{u_a}\frac{w_a}{\Sigma\alpha r . W}\frac{d\in_a}{dt} = -\frac{pw_i}{\alpha^+\Sigma\alpha r . W}(\in_L - \in_{Ls})\ , \qquad \frac{d\in_L}{dt} + v\frac{d\in_a}{dt} = = -\frac{1}{\tau}(\in_L - \in_{Ls}) \tag{A18a}$$

where, as before,

$$\tau = \frac{W\alpha^+\Sigma\alpha r}{p.w_i} = \frac{W(\alpha_b.r_b + \alpha_s.r_s + \frac{a}{u_a})}{\frac{p.w_i}{\alpha^+}} = \frac{W.\overline{\alpha}_k.\alpha^+}{p.E_1} \tag{19}$$

and the complicating factor is

$$v = \frac{V}{u_a}\frac{w_a}{\Sigma\alpha r . W} \tag{A18b}$$

which has a characteristic time, τ_a, given by

$$\tau_a = V/u_a \quad . \tag{A18c}$$

This can be seen more clearly by solving for $\in_a$ rather than $\in_L$, so rewriting (A8)

$$\frac{aR_E E}{u_a} = R_a w_a - \frac{1-w_a}{1-w_{in}} R_{in} w_{in} + \frac{V}{u_a}\frac{d(R_a w_a)}{dt} \quad .$$

$$\frac{a(1+\in_E)E}{u_a} = (1+\in_a)w_a - \frac{1-w_a}{1-w_{in}}(1+\in_{in})w_{in} + \frac{V}{u_a}\frac{d((1+\in_a)w_a)}{dt}$$

Subtracting Eq (A5)

$$\frac{a\in_E E}{u_a} = (1+\in_a)w_a - \frac{w_a-w_{in}}{(1-w_{in})} - \frac{1-w_a}{1-w_{in}}(1+\in_{in})w_{in} + \frac{V}{u_a}\frac{w_a\, d\in_a}{dt}$$

$$=\in_a w_a + \frac{w_a - w_a w_{in} - w_a + w_{in}}{(1-w_{in})} - \frac{1-w_a}{1-w_{in}}(1+\in_{in})w_{in} + \frac{V}{u_a}\frac{w_a\, d\in_a}{dt}$$

$$=\in_a w_a + \frac{w_{in}(1-w_a)}{(1-w_{in})} - \frac{1-w_a}{1-w_{in}}(1+\in_{in})w_{in} + \frac{V}{u_a}\frac{w_a\, d\in_a}{dt}$$

$$=\in_a w_a - \frac{1-w_a}{1-w_{in}}\in_{in} w_{in} + \frac{V}{u_a}\frac{w_a\, d\in_a}{dt}$$

$$\in_E E = \frac{u_a}{a}\in_a w_a - \frac{u_a}{a}\frac{1-w_a}{1-w_{in}}\in_{in} w_{in} + \frac{V}{a}\frac{w_a\, d\in_a}{dt} \qquad \text{(A20)}$$

In the steady state

$$\in_{Es} E = \frac{u_a}{a}\in_{as} w_a - \frac{u_a}{a}\frac{1-w_a}{1-w_{in}}\in_{in} w_{in} \;.$$

Subtracting the steady state

$$\in_E E = \frac{u_a}{a}(\in_a - \in_{as})w_a + \frac{V}{a}\frac{w_a\, d\in_a}{dt} \quad . \qquad \text{(A21)}$$

Inserting in Eqn (16)

$$W\frac{d\in_L}{dt} = -\frac{u_a}{a}(\in_a - \in_{as})w_a - \frac{V}{a}\frac{w_a\, d\in_a}{dt}$$

and rearranging

$$\frac{d\in_a}{dt} + b\frac{d\in_L}{dt} = -\frac{1}{\tau_a}(\in_a - \in_{as}), \qquad \text{(A22)}$$

where

$$b = \frac{aW}{Vw_a} \qquad \text{(A23)}$$

is the ratio of the amount of liquid water in the leaf to the amount of water vapour in the cuvette system.

<u>So for a very large volume chamber, with little leaf area in it</u>, the equation would become

$$\frac{d\in_a}{dt} = -\frac{1}{\tau_a}(\in_a - \in_{as})$$

for which the solution would be

$$\in_a = \in_{as} - (\in_{a0} - \in_{as})e^{-\frac{t}{\tau_a}} \qquad \text{(A24)}$$

<u>**The general solution**</u> to (A18a) and (A22) may be found by taking Laplace transforms. Thus

$$\frac{d\in_L}{dt} + v\frac{d\in_a}{dt} = -\frac{1}{\tau}(\in_L - \in_{Ls}) \qquad \text{(A25)}$$

transforms to

$$s.\overline{\in_L} - \in_{L0} + v.s\,\overline{\in_a} - v.\in_{ao} = \frac{-\overline{\in_L}}{\tau} + \frac{\in_{Ls}}{\tau.s} \qquad \text{(A26)}$$

where *s* is the Laplace operator and the overbar denotes a transformed variable.

Similarly (A22) transforms to

$$\mathrm{s}.\,\overline{\in_a} - \in_{a0} + \; b.s\,\overline{\in_L} - b.\in_{L0} = \frac{-\,\overline{\in_a}}{\tau_a} + \frac{\in_{as}}{\tau_a.s} \qquad \text{(A27)}$$

Rearranging (A27)

$$\overline{\in_a} = \frac{\in_{a0} - b.s.\overline{\in_L} + b.\in_{L0} + \frac{\in_{as}}{\tau_a.s}}{\left(\mathrm{s} + \frac{1}{\tau_a}\right)} \qquad \text{(A28)}$$

Substituting (A28) for $\overline{\in_a}$ in (A26) and gathering the resulting terms in $\overline{\in_L}$

$$\left(s - \frac{v.s.b.s}{\left(\mathrm{s} + \frac{1}{\tau_a}\right)} + \frac{1}{\tau}\right)\overline{\in_L} - \; \in_{L0} + \frac{v.s}{\left(\mathrm{s} + \frac{1}{\tau_a}\right)}\left(\in_{a0} + b.\in_{L0} + \frac{\in_{as}}{\tau_a.s}\right) \; - v.\in_{ao} = \frac{\in_{Ls}}{\tau.s} \qquad \text{(A29)}$$

from which

$$\overline{\in_L} = - \; \frac{\in_{L0} - \frac{v.s}{\left(\mathrm{s} + \frac{1}{\tau_a}\right)}\left(\in_{a0} + b.\in_{L0} + \frac{\in_{as}}{\tau_a.s}\right) + v.\in_{ao} + \frac{\in_{Ls}}{\tau.s}}{\left(s - \frac{v.s.b.s}{\left(\mathrm{s} + \frac{1}{\tau_a}\right)} + \frac{1}{\tau}\right)} \qquad \text{(A30)}$$

We see that we are creating the product $\left(s + \frac{1}{\tau_a}\right)\left(s - \frac{v.s.b.s}{\left(\mathrm{s} + \frac{1}{\tau_a}\right)} + \frac{1}{\tau}\right)$ in the denominator of the right-hand side. The product can be rewritten as $\left(s + \frac{1}{\tau_a}\right)\left(s + \frac{1}{\tau}\right) - v.b.s^2$ which creates a quadratic form with the coefficient of s^2 being less than unity, which is a nuisance in Laplace transforms. We overcome the problem by further rewriting the product as

$(1 - v.b)(s - s_1)(s - s_2)$ where s_1 and s_2 are the roots of the quadratic equation

$$s^2 + \frac{1/\tau + 1/\tau_a}{(1 - v.b)}s + \frac{1}{\tau.\tau_a(1 - v.b)} = 0. \qquad \text{(A31)}$$

Note that s_1 and s_2 correspond to negative, inverse time constants -1/τ_1 and -1/τ_2 which are perturbations, depending on the magnitude of $v.b = \frac{a/u_a}{\Sigma\alpha r}$, from the unperturbed -1/τ and -1/τ_a. In other words

$$(-s_1)(-s_2) = \frac{1}{\tau_1.\tau_2} = \frac{1}{\tau.\tau_a(1-v.b)} = \frac{1}{\tau.\tau_a\left(1-\frac{a/u_a}{\Sigma\alpha r}\right)}. \qquad (\text{A}32a)$$

We note that (A31) implies

$$\tau_1 = \left[\tau_a + \tau - \sqrt{(\tau_a+\tau)^2 - 4\tau_a\tau(1-v.b)}\right]/2 \qquad \text{(A32b)}$$

and

$$\tau_2 = \left[\tau_a + \tau + \sqrt{(\tau_a+\tau)^2 - 4\tau_a\tau(1-v.b)}\right]/2 \qquad \text{(A32c).}$$

Thus (A30) becomes

$$\overline{\in_L} = \frac{\left(s+\frac{1}{\tau_a}\right)\left(\in_{L0}+v.\in_{ao}+\frac{\in_{Ls}}{\tau.s}\right) - v.s\left(\in_{a0}+b.\in_{L0}+\frac{\in_{as}}{\tau_a.s}\right)}{(1-v.b)(s-s_1)(s-s_2)} \qquad . \qquad \text{(A33)}$$

We see that the limit as s→∞ of $s.\,\overline{\in_L} = \frac{(\in_{L0}+v.\in_{ao}) - v.(\in_{a0}+b.\in_{L0})}{(1-v.b)} = \in_{L0}$, consistent with it being the limit as t→0 of $\in_L$ (t). Similarly, the limit as s→0 of $s.\,\overline{\in_L} = \frac{\left(\frac{1}{\tau_a}\right)\left(\frac{\in_{Ls}}{\tau}\right)}{(1-v.b)(-s_1)(-s_2)}$ which, using (A32) = $\in_{Ls}$, consistent with it being the limit as t→∞ of $\in_L$ (t).

We rewrite (A33) for convenient application of the inverse Laplace transform:

$$\overline{\in_L} = \frac{(\in_{L0}+v.\in_{ao})}{(1-v.b)} \cdot \frac{\left(s+\frac{1}{\tau_a}\right)}{(s-s_1)(s-s_2)} + \frac{\frac{\in_{Ls}}{\tau}}{(1-v.b)} \cdot \frac{\frac{1}{s}\left(s+\frac{1}{\tau_a}\right)}{(s-s_1)(s-s_2)} - \frac{v.(\in_{a0}+b.\in_{L0})}{(1-v.b)} \cdot \frac{s}{(s-s_1)(s-s_2)} -$$

$$\frac{v.\left(\frac{\in_{as}}{\tau_a}\right)}{(1-v.b)} \cdot \frac{1}{(s-s_1)(s-s_2)} \qquad \text{(A34)}$$

We make use of the following elementary Laplace transformations:

$$\frac{1}{(s-s_1)(s-s_2)} \rightarrow \frac{e^{s_2 t}-e^{s_1 t}}{(s_2-s_1)} \qquad \text{(A35a)}$$

$$(\frac{s}{(s-s_1)(s-s_2)} \rightarrow \frac{s_2 e^{s_2 t}-s_1 e^{s_1 t}}{(s_2-s_1)} \qquad \text{(A35b)}$$

$$\frac{1}{s(s-s_1)(s-s_2)} \rightarrow \int_0^t \quad \frac{e^{s_2 u}-e^{s_1 u}}{(s_2-s_1)} du = \left[\frac{\frac{1}{s_2}e^{s_2 u}-\frac{1}{s_1}e^{s_1 u}}{(s_2-s_1)}\right]_0^t = \frac{\frac{1}{s_2}e^{s_2 t}-\frac{1}{s_1}e^{s_1 t}-(\frac{1}{s_2} \quad -\frac{1}{s_1})}{(s_2-s_1)} \quad \text{(A35c)} .$$

And so after transformation back to the time domain (A34) becomes

$$\in_L\ (t) = \frac{(\in_{L0}+v.\in_{ao})}{(1-v.b)} \cdot \frac{s_2 e^{s_2 t}-s_1 e^{s_1 t}+\frac{1}{\tau_a}\left(e^{s_2 t}-e^{s_1 t}\right)}{(s_2-s_1)}$$

$$+\frac{\frac{\in_{Ls}}{\tau}}{(1-v.b)} . \frac{e^{s_2 t}-e^{s_1 t}+\left(\frac{1}{\tau_a}\right)\left[\frac{1}{s_2}e^{s_2 t}-\frac{1}{s_1}e^{s_1 t}-(\frac{1}{s_2} \quad -\frac{1}{s_1})\right]}{(s_2-s_1)} - \frac{v.(\in_{a0}+b.\in_{L0})}{(1-v.b)} . \frac{s_2 e^{s_2 t}-s_1 e^{s_1 t}}{(s_2-s_1)} - \frac{v.\left(\frac{\in_{as}}{\tau_a}\right)}{(1-v.b)} . \frac{e^{s_2 t}-e^{s_1 t}}{(s_2-s_1)} .$$

(A36)

The terms in $\in_{L0}$and $\in_{ao}$ can be simplified

$$\in_L\ (t) = \in_{L0}\ .\ \frac{s_2 e^{s_2 t}-s_1 e^{s_1 t}}{(s_2-s_1)} +$$

$$\frac{(\in_{L0}+v.\in_{ao})\frac{1}{\tau_a}\left(e^{s_2 t}-e^{s_1 t}\right)}{(1-v.b)(s_2-s_1)} + \frac{\frac{\in_{Ls}}{\tau}}{(1-v.b)} . \frac{e^{s_2 t}-e^{s_1 t}+\left(\frac{1}{\tau_a}\right)\left[\frac{1}{s_2}e^{s_2 t}-\frac{1}{s_1}e^{s_1 t}-(\frac{1}{s_2} \quad -\frac{1}{s_1})\right]}{(s_2-s_1)} - \frac{v.\left(\frac{\in_{as}}{\tau_a}\right)}{(1-v.b)} . \frac{e^{s_2 t}-e^{s_1 t}}{(s_2-s_1)} .$$

And one term in $\in_{Ls}$ can be combined with $\in_{as}$

$$\in_L\ (t) = \in_{L0}\ .\ \frac{s_2 e^{s_2 t}-s_1 e^{s_1 t}}{(s_2-s_1)} + \frac{(\in_{L0}+v.\in_{ao})\frac{1}{\tau_a}\left(e^{s_2 t}-e^{s_1 t}\right)}{(1-v.b)(s_2-s_1)} + \frac{\frac{\in_{Ls}}{\tau.\tau_a}}{(1-v.b)} . \frac{\left[\frac{1}{s_2}e^{s_2 t}-\frac{1}{s_1}e^{s_1 t}-(\frac{1}{s_2} \quad -\frac{1}{s_1})\right]}{(s_2-s_1)} +$$

$$\frac{\frac{\in_{Ls}}{\tau}-v.\left(\frac{\in_{as}}{\tau_a}\right)}{(1-v.b)} . \frac{e^{s_2 t}-e^{s_1 t}}{(s_2-s_1)} .$$

Converting s_1 to -1/τ_1 and s_2 to -1/τ_2 ,

$$\in_L\ (t) = \in_{L0}\ .\ \frac{1/\tau_1 e^{-\mathrm{t}/\tau_1} - 1/\tau_2 e^{-\mathrm{t}/\tau_2}}{(1/\tau_1 - 1/\tau_2)}$$

$$+ \frac{(\in_{L0} + v.\in_{ao})\frac{1}{\tau_a}\left(e^{-\mathrm{t}/\tau_2} - e^{-\mathrm{t}/\tau_1}\right)}{(1-v.b)(1/\tau_1 - 1/\tau_2)} + \frac{\frac{\in_{Ls}}{\tau.\tau_a}}{(1-v.b)} . \frac{\left[\frac{1}{-1/\tau_2}e^{-\mathrm{t}/\tau_2} - \frac{1}{-1/\tau_1}e^{-t/\tau_1} - \left(\frac{1}{-1/\tau_2} - \frac{1}{-1/\tau_1}\right)\right]}{(1/\tau_1 - 1/\tau_2)} +$$

$$\frac{\frac{\in_{Ls}}{\tau} - v.\left(\frac{\in_{as}}{\tau_a}\right)}{(1-v.b)} . \frac{e^{-\mathrm{t}/\tau_2} - e^{-\mathrm{t}/\tau_1}}{(1/\tau_1 - 1/\tau_2)} .$$

Multiplying above and below by $\tau_1\tau_2$

$$\in_L\ (t) = \in_{L0}\ .\ \frac{\tau_2 e^{-\mathrm{t}/\tau_1} - \tau_1 e^{-\mathrm{t}/\tau_2}}{(\tau_2 - \tau_1)} + \frac{(\in_{L0} + v.\in_{ao})\frac{\tau_1\tau_2}{\tau_a}\left(e^{-\mathrm{t}/\tau_2} - e^{-\mathrm{t}/\tau_1}\right)}{(1-v.b)(\tau_2 - \tau_1)} +$$

$$\frac{\frac{\in_{Ls}.\tau_1\tau_2}{\tau.\tau_a}}{(1-v.b)} . \frac{\left[-\tau_2 e^{-\mathrm{t}/\tau_2} + \tau_1 e^{-t/\tau_1} - (-\tau_2 + \tau_1)\right]}{(\tau_2 - \tau_1)} + \frac{\tau_1\tau_2\left(\frac{\in_{Ls}}{\tau} - v.\left(\frac{\in_{as}}{\tau_a}\right)\right)}{(1-v.b)} . \frac{e^{-\mathrm{t}/\tau_2} - e^{-\mathrm{t}/\tau_1}}{(\tau_2 - \tau_1)} . \qquad \text{(A37)}$$

Making use of (A32) and for the sake of argument, let us assume that τ_2 is the modified τ, and τ_1 the modified τ_a, so that usually $\tau_2 \geq \tau_1$

$$\in_L\ (t) = \in_{L0}\ .\ \frac{\tau_2 e^{-\mathrm{t}/\tau_1} - \tau_1 e^{-\mathrm{t}/\tau_2}}{(\tau_2 - \tau_1)} + \frac{(\in_{L0} + v.\in_{ao})\tau\left(e^{-\mathrm{t}/\tau_2} - e^{-\mathrm{t}/\tau_1}\right)}{(\tau_2 - \tau_1)} + \in_{Ls}.\left(1 - \frac{\tau_2 e^{-\frac{\mathrm{t}}{\tau_2}} - \tau_1 e^{-\frac{t}{\tau_1}}}{(\tau_2 - \tau_1)}\right) +$$

$$\tau.\tau_a\left(\frac{\in_{Ls}}{\tau} - v.\left(\frac{\in_{as}}{\tau_a}\right)\right).\frac{e^{-\mathrm{t}/\tau_2} - e^{-\mathrm{t}/\tau_1}}{(\tau_2 - \tau_1)} . \qquad \text{(A38)}$$

It is interesting to compare (A38) to the simpler form we considered earlier. To that end we rewrite (A38) as

$$\in_L\ (t) - \in_{Ls} = \in_{L0}\ .\ \frac{\tau_2 e^{-\mathrm{t}/\tau_1} - \tau_1 e^{-\mathrm{t}/\tau_2} + \tau\left(e^{-\mathrm{t}/\tau_2} - e^{-\mathrm{t}/\tau_1}\right)}{(\tau_2 - \tau_1)} - \in_{Ls}.\left(\frac{\tau_2 e^{-\mathrm{t}/\tau_2} - \tau_1 e^{-\mathrm{t}/\tau_1}}{(\tau_2 - \tau_1)}\right) +$$

$$\tau.\tau_a\left(\frac{\in_{Ls}}{\tau} - v.\left(\frac{\in_{as}}{\tau_a}\right)\right).\frac{e^{-\mathrm{t}/\tau_2} - e^{-\mathrm{t}/\tau_1}}{(\tau_2 - \tau_1)} + \frac{(v.\in_{ao})\tau\left(e^{-\mathrm{t}/\tau_2} - e^{-\mathrm{t}/\tau_1}\right)}{(\tau_2 - \tau_1)}. \qquad \text{(A39)}$$

Making use of (A32a)

$$\in_L\ (t) - \in_{Ls} = \in_{L0}\ .\ \frac{\tau_2 e^{-t/\tau_1} - \tau_1 e^{-t/\tau_2} + \tau\left(e^{-t/\tau_2} - e^{-t/\tau_1}\right)}{(\tau_2 - \tau_1)}$$

$$- \in_{Ls}.\left(\frac{\tau_2 e^{-t/\tau_2} - \tau_1 e^{-t/\tau_1} - \tau_a(e^{-t/\tau_2} - e^{-t/\tau_1})}{(\tau_2 - \tau_1)}\right) - \frac{v(\in_{as} - \in_{a0})\tau\left(e^{-t/\tau_2} - e^{-t/\tau_1}\right)}{(\tau_2 - \tau_1)}. \qquad \text{(A40)}$$

As volume *V* approaches 0, $v \to 0, \tau_a \to 0,\ \tau_1 \to 0, \tau_2 \to \tau, b \to \infty$ and $b\tau_a$ remains equal to $\frac{aW}{u_a w_a}$.

In this limit (A40) degenerates to the simpler case (Eq 25):

$$\in_L\ (t) - \in_{Ls} = \ (\in_{L0} - \in_{Ls}).\, e^{-t/\tau}. \qquad \text{(A41)}$$

The solution for $\in_a\ (t)$may be found in a manner analogous to that for $\in_L$ (Eqs (A25) to (A40)) and is

$$\in_a\ (t) - \in_{as} = \in_{a0}.\frac{\tau_2 e^{-\frac{t}{\tau_1}} - \tau_1 e^{-\frac{t}{\tau_2}} + \tau_a\ \left(e^{-\frac{t}{\tau_2}} - e^{-\frac{t}{\tau_1}}\right)}{(\tau_2 - \tau_1)}$$

$$- \in_{as}.\left(\frac{\tau_2 e^{-\frac{t}{\tau_2}} - \tau_1 e^{-\frac{t}{\tau_1}} - \tau(e^{-\frac{t}{\tau_2}} - e^{-\frac{t}{\tau_1}})}{(\tau_2 - \tau_1)}\right)$$

$$-(\in_{Ls} - \in_{L0})\,\frac{b\tau_a\left(e^{-t/\tau_2} - e^{-t/\tau_1}\right)}{(\tau_2 - \tau_1)}. \qquad \text{(A42)}$$

Eqs (A22) and (A25) are symmetrical, with the following correspondences going from $\in_a$ to $\in_L$: $\in_a \to \in_L, \in_{as} \to \in_{Ls}, \in_{a0} \to \in_{L0}, \tau_a \to \tau, b \to v$. Making these substitutions in (A42), but keeping the same notation of τ_1 and τ_2, we obtain (A40) (and vice versa), which is a convenient check on the derivations.

Eq (A42) seems rather complicated but can be written as $\in_{as}$ minus the sum of two exponentials:

$$\in_a(t) = \in_{as} - \left\{\beta e^{-\frac{t}{\tau_1}} + \gamma e^{-\frac{t}{\tau_2}}\right\} / (\tau_2 - \tau_1) \qquad \text{(A43a)}$$

where,

$$\beta = \in_{as}(\tau - \tau_1) - \in_{a0}(\tau_2 - \tau_a) - (\in_{Ls} - \in_{L0}) b\tau_a \qquad \text{(A43b)}$$

$$\gamma = \in_{as}(\tau - \tau_2) - \in_{a0}(\tau_1 - \tau_a) - (\in_{Ls} - \in_{L0}) b\tau_a \qquad \text{(A43c)}$$

Similarly, the expression for $\in_L(t)$ may be rewritten as $\in_{Ls}$ minus the sum of two exponentials:

$$\in_L(t) = \in_{Ls} - \left\{\omega e^{-\frac{t}{\tau_1}} + \theta e^{-\frac{t}{\tau_2}}\right\} / (\tau_2 - \tau_1) \qquad \text{(A44a)}$$

where

$$\omega = \in_{Ls}(\tau_a - \tau_1) - \in_{L0}(\tau - \tau_2) - (\in_{as} - \in_{a0}) \upsilon\tau \qquad \text{(A44b)}$$

$$\theta = \in_{Ls}(\tau_2 - \tau_a) - \in_{L0}(\tau_1 - \tau) - (\in_{a0} - \in_{as}) \upsilon\tau \qquad \text{(A44c)}$$

As volume V approaches 0, $v \to 0$, $\tau_a \to 0$, $\tau_1 \to 0$, $\tau_2 \to \tau$, $b \to \infty$ and $b\tau_a$ remains equal to $\frac{aW}{u_a w_a}$.

In this limit (A44) recovers the simple case in Eq (18) of the main text when volume V is zero and (A43) becomes

$$\in_a(t) = \in_{as} + \left[(\in_{L0} - \in_{Ls}) \frac{b\tau_a}{\tau}\right] e^{-\frac{t}{\tau}}, \qquad \text{(A45)}$$

and we need to know the relationship between $\in_{L0} - \in_{Ls}$ and $\in_{a0} - \in_{as}$.

From (A45) at *t*=0

$$\epsilon_{a0}-\epsilon_{as}=(\epsilon_{L0}-\epsilon_{Ls})\frac{b\tau_a}{\tau}$$

Thus, as volume *V* approaches 0, (A43) becomes

$$\epsilon_a(t)=\epsilon_{as}+(\epsilon_{a0}-\epsilon_{as})e^{-\frac{t}{\tau}}.$$

identical to the simple case in Eq (23) of the main text when volume *V* is zero.

SUMMARY STATEMENT

The transient responses of isotopic composition of water in leaves and transpiration depends on the leaf water content and the one way flux of water vapour out of the leaves, not the net transpiration rate.