Tree Physiology, 2024, **44**, tpae125
https://doi.org/10.1093/treephys/tpae125
Advance access publication date 4 October 2024
Research paper

Isotopic steady state or non-steady state transpiration? Insights from whole-tree chambers

Richard Harwood[1,*], Lucas A. Cernusak[2], John E. Drake[3], Craig V.M. Barton[4], Mark G. Tjoelker[4] and Margaret M. Barbour[1,5]

[1]School of Life and Environmental Sciences, The University of Sydney, Private Bag 4011, Narellan, NSW 2567, Australia
[2]College of Science and Engineering, James Cook University, Cairns, QLD 4879, Australia
[3]Department of Sustainable Resources Management, College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, NY 13210, USA
[4]Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia
[5]Te Aka Mātuatua - School of Science, The University of Waikato, Private Bag 3105, Hamilton 3216, New Zealand
*Corresponding author: (richardwilliamharwood@gmail.com)

Handling Editor: Daniel Epron

Unravelling the complexities of transpiration can be assisted by understanding the oxygen isotope composition of transpired water vapour (δ_E). It is often assumed that δ_E is at steady state, thereby mirroring the oxygen isotope composition of source water (δ_{source}), but this assumption has never been tested at the whole-tree scale. This study utilized the unique infrastructure of 12 whole-tree chambers enclosing *Eucalyptus parramattensis E.C.Hall* trees to measure δ_E along with concurrent temperature and gas exchange data. Six chambers tracked ambient air temperature and six were exposed to an ambient +3 °C warming treatment. Day time means for δ_E were within 1.2‰ of δ_{source} (−3.3‰) but varied considerably throughout the day. Our observations show that *E. parramattensis* trees are seldom transpiring at isotopic steady state over a diel period, but transpiration approaches source water isotopic composition over longer time periods.

Keywords: evapotranspiration, organic material, oxygen isotopes.

Introduction

Half to two-thirds of terrestrial evaporation of water occurs as transpiration (Salati et al. 1979), so regulation of water loss by leaves is critical to the global water cycle. Stable isotopes have proven to be a valuable tool in understanding local, regional and global biogeochemical cycles, and this is particularly true for water cycles (e.g. Gibson and Edwards 2002, Dutton et al. 2005). Leaves are the most active site of water isotope fractionation in plants. Lighter isotopes (^{16}O and ^{1}H) evaporate and diffuse more readily, resulting in leaf water being enriched in the heavier isotopes: ^{18}O and ^{2}H. These isotopic effects provide opportunities to understand physiological and environmental influences on transpiration at a range of spatial scales from leaf to globe (Cernusak et al. 2016), as well as temporal records in tree rings (Gessler et al. 2014) and ice cores (Casado et al. 2020). Of particular interest are the use of isotopes to validate estimates of the proportion of terrestrial evaporation derived from transpiration (Dubbert and Werner 2019), and reconstruction of past environmental and plant physiological conditions (e.g. Lorrey et al. 2016).

Owing to conservation of mass, the integrated oxygen isotope composition of transpired water (δ_E) must equal $\delta^{18}O$ of water taken up by the plant, but at any given point in time δ_E may depart significantly from $\delta^{18}O$ of soil water due to changes in leaf evaporative conditions and the time it takes for leaf water pools to turn over. Steady state δ_E is defined as the situation when the isotope composition of the transpired water vapour is identical to the source water (δ_{source}), and conversely non-steady state δ_E is defined as the isotope composition of the transpired water vapour deviating from that of the source water. Until recently, direct measurement of δ_E was technically challenging, and consequently datasets are relatively rare. Studies that directly measure δ_E at the leaf level suggest that non-steady state transpiration is common (Simonin et al. 2013, Dubbert et al. 2014, Song et al. 2015*a*), but larger spatial scale and temporal patterns remain relatively untested.

The emergence of high-resolution δ_E datasets is due to advancements in laser spectroscopy, with direct measurements at scales ranging from part of a leaf (e.g. Simonin et al. 2013) to a 200 L volume surrounding a branch (e.g. Dubbert et al. 2014). Measurements at each of these scales allow for different types of research questions to be addressed. For example, Simonin et al. (2013) measured a portion of a leaf in a gas exchange system coupled to a water vapour isotope analyser and Song et al. (2015*b*) measured whole leaves in a larger leaf chamber. The benefit of this approach is the ability to control and measure the isotopic composition of vapour entering the leaf chamber and to regulate the environmental conditions inside the chamber. The downside is that scaling to the ecosystem level is uncertain, as the information must be extrapolated from a small leaf area that may not be representative of entire canopies. Dubbert et al. (2014) employed custom branch

Received: December 6, 2023. **Accepted:** October 2, 2024

chambers to allow measurements in the field. This approach measures the atmosphere-biosphere interactions fundamental to understanding ecosystem function at close-to-relevant scales. These two different experimental approaches highlight that there are still scales at which we currently have significant knowledge gaps in understanding δ_E. For instance, unresolved questions regarding spatial and temporal variation in leaf water isotopic composition and current theoretical models to predict this variation (e.g. Craig and Gordon 1965) would benefit from tightly controlled environmental conditions like that in Simonin et al. (2013). Conversely an investigation using oxygen isotope ratios to partition ecosystem evapotranspiration fluxes would benefit from measurements at larger spatial scales like that in Dubbert et al. (2014).

One area of uncertainty in leaf water isotopic understanding is the proportion of time leaves spend at isotopic steady state, and consequently the proportion of time δ_E deviates from δ_{source}. Isotopic steady state is assumed in models used in most studies that interpret $\delta^{18}O$ measurements of carbon, water, oxygen and organic matter at spatial scales larger than leaves and temporal scales longer than a few minutes. However, if leaves are not actually at steady state, then interpretations can become inaccurate. For example, the fractional contribution of transpiration (F_T) to evapotranspiration can be estimated by an isotopic mass balance model:

$$F_T\,(\%) = \frac{\delta_{ET} - \delta_{Soil}}{\delta_E - \delta_{Soil}} \quad (1)$$

where δ_{ET} is the $\delta^{18}O$ of evapotranspiration, δ_{Soil} is the $\delta^{18}O$ of evaporation and δ_E is the $\delta^{18}O$ of transpiration. If δ_{Soil} is −15‰, δ_{ET} −7‰, δ_{source} of source water is −4‰ and δ_E is at steady state (−4‰), the contribution of transpiration to evapotranspiration is 72%. However, if δ_E is enriched relative to δ_{source} (e.g. δ_E = −1.5‰) the contribution of transpiration to evapotranspiration is much lower (59% in this example). Conversely, if δ_E is depleted relative to δ_{source} (e.g. δ_E = −6.5‰) the contribution of transpiration to evapotranspiration is higher (94% in this example). That is, when the degree of departure of δ_E from isotopic steady state is unknown, errors in partitioning transpiration from evapotranspiration can be substantial.

There is a growing body of research confirming $\delta^{18}O$ of leaf water and transpired vapour are not consistently at steady state over short timeframes (1 to 24 h e.g. Simonin et al. 2013, Dubbert et al. 2014, Cernusak et al. 2016, Farquhar et al. 2021, Kübert et al. 2023). Non-steady state isotopic composition of leaf water depends on the following key processes: evaporative enrichment during transpiration, spatial variation in enrichment across the leaf, leaf water turnover time and temporal variation in leaf water content. It is also important to acknowledge that spatial variation of environmental conditions within a canopy is likely and will have strong effects on the extent of evaporative enrichment of an individual leaf, and its contribution to δ_E at the whole-tree scale. Similarly, environmental conditions within a tree crown are highly dynamic over time, and this variation will contribute to variability in δ_E for the whole tree.

In this study, we addressed the knowledge gap concerning isotopic steady state during transpiration for whole trees by measuring the $\delta^{18}O$ of the atmosphere at the experimental site (δ_F), the $\delta^{18}O$ of the water vapour inside the whole-tree chamber (WTC) (δ_V) and the $\delta^{18}O$ of condensed water collected from the chamber air and climate control system (δ_C). From these measurements we were able to calculate the $\delta^{18}O$ of whole-tree transpiration (δ_E) using an isotopic mass balance approach.

To the best of our knowledge our WTC setup is the first of its kind to estimate diel δ_E for whole trees. The setup was designed to have environmental control similar to that of a leaf cuvette, but at a whole-tree scale, to facilitate scaling of δ_E between leaf and ecosystem. We use the results from this study to determine the deviation of δ_E from isotopic steady state over two diel periods and assess the impact of non-steady state transpiration on the partitioning of ecosystem fluxes.

Another implication for non-steady state transpiration is the interpretation of leaf-derived organic matter. If transpiration is not at isotopic steady state, a poor estimate of the $\delta^{18}O$ of leaf water at the site of evaporation (δ_e) might occur. Subsequently, the estimated isotopic signal that gets incorporated into plant organic matter may be incorrect. We address the assumption that isotopic steady state transpiration has little impact on the interpretation of the $\delta^{18}O$ of organic material because leaf derived organic matter is produced at periods of high assimilation rate when leaf water is most likely to be at steady state (Cernusak et al. 2016).

Materials and methods

Site description

WTCs accurately control ambient CO_2 concentration (C_a), relative humidity and air temperature (T_{air}), whilst continuously measuring net CO_2 and water vapour fluxes between entire tree crowns and the atmosphere in trees up to 9 m tall. The WTCs enclosed individual trees rooted in soil inside large cylindrical structures (3.25 m in diameter, 9 m in height and volume of ∼53 m^3). A detailed description of the WTC infrastructure and operations is documented in Barton et al. (2010). The site has a central refrigeration plant that delivers a glycol/water solution to each chamber at a temperature 1–2 °C below the ambient dewpoint. This chilled solution passes through a conditioning coil in each WTC for humidity and temperature control. The air within each WTC is continuously passed through a large heat exchange system (1 m × 2 m), and a dynamically controlled portion of the air passes over the cool conditioning coil via computer-controlled baffles. Some of the water vapour in the WTC air condenses on the conditioning coil and drips out of the WTC through a tipping bucket, where the condensation rate is measured. The humidity of the air within each chamber was thus influenced by the water vapour added by each tree via transpiration and the condensate removed by the heat exchange system (Barton et al. 2010, Drake et al. 2016, 2018).

Twelve WTCs were operational at the Hawkesbury Forest Experiment site in Richmond, New South Wales (Australia; 33°36′40″S, 150°44′26.5″E). WTCs compartmentalized the stem and crown portion of the trees from the soil and roots; this was achieved starting on 28 February 2016 by means of a suspended floor at 45 cm height above the soil and sealed around the stem. The soil was covered with a landscaping cloth to limit surface evaporation. In addition, a root exclusion barrier extended from the base of the chamber perimeter

vertically to 1 m deep. It is important to note that even with this barrier to lateral spread some tree roots may have penetrated deeper in the soil profile (Duursma et al. 2011, Drake et al. 2018).

Eucalyptus parramattensis E.C.Hall seeds were purchased from Harvest Seeds and Native Plants (Terry Hills, NSW, Australia) and germinated on site in a shade house. Three months after seed germination (28 October 2015) six potted plants were placed in each WTC and the temperature treatment (described below) was initiated. On 23 December 2015, one seedling ~60 cm in height was planted in the native soil in each chamber, and the other five seedlings removed.

Experimental description

A warming experiment began on 28 October 2015. Six chambers tracked the natural variation in air temperature (T_{air}) and relative humidity (RH) observed at the experiment site; these chambers are henceforth referred to as 'ambient' chambers. The remaining six chambers tracked the ambient T_{air} with +3 °C warming, while also tracking the ambient RH; these chambers are henceforth referred to as 'elevated' chambers. The warming treatment was implemented on both the aboveground and belowground compartments of the WTCs. The average warming was +2.9 °C (± SD of 0.6 across 265 d) for T_{air} in the upper compartment, +2.9 °C (± 0.8) for soil temperature (T_{soil}) at 5-cm depth, +3.0 °C (± 0.5) for T_{soil} at 10-cm depth and + 1.6 °C (± 0.2) for T_{soil} at 50-cm depth. Humidity was controlled to achieve equivalent RH between ambient and elevated chambers; this meant the absolute humidity was higher in the elevated chambers relative to the ambient chambers and the vapour pressure deficit (VPD) was also higher in the elevated chambers relative to the ambient chambers. Climate change models predict that RH will remain the same with increased future temperatures, so that VPD necessarily increases (Soden and Held 2006, Novick et al. 2016), and the experiment was designed to match these predictions. The WTCs calculated the rates of CO_2 and H_2O exchange between each tree and its canopy airspace every 15 min using a mass balance approach (Barton et al. 2010, Drake et al. 2016). Flux measurements began on 28 February 2016, after installation of the suspended floor to separate stem and canopy from soil and roots. To account for differences in tree size, canopy fluxes are expressed on a whole-tree leaf area basis. Leaf area measurements are described below. Atmospheric wind speed and direction were collected from a nearby weather station, and rain was recorded daily from an on-site rain gauge. The site was unattended on Saturday and Sunday so any rainfall on these days would be accounted for on the Monday reading.

Leaf temperature

Leaf temperature (T_{leaf}) was determined using continuous infrared measurements of the upper canopy ($T_{L\text{-}IR}$) along with automated thermocouple measurements ($T_{L\text{-}TC}$). An infrared radiometer designed to measure surface temperatures (SI-111; Apogee Instruments, Logan, UT, USA, emissivity set to 0.97) was installed inside the sun-facing northern side of each WTC and directed at an area of thick foliage in the upper third of the canopy for each tree; the sensors were raised over the measurement period to match tree height growth. These sensors covered an area of ~1 m^2 meaning they recorded a temperature averaged across numerous leaves. For a detailed explanation of leaf temperature methods see Drake et al. (2018, 2020).

Whole-tree leaf area and leaf water content

Total canopy leaf area was recorded for each tree with a destructive harvest on 23 November 2016. The canopy of each tree was separated into three equal heights (low-, mid- and top-canopy thirds) and all leaves were detached from branches. A random sample of 100 leaves per canopy layer was measured for total leaf area (LI3100C; Licor Environment, Lincoln, NE, USA), dry mass and specific leaf area (SLA, m^2 leaf area g^{-1} leaf dry mass). The total leaf area for each tree was calculated as the product of layer-specific SLA and leaf dry mass summed across the three canopy layers. It was assumed that total leaf area did not change substantially during the period for which the campaigns occurred. This assumption is based on observations of low rates of leaf formation and litter fall during the 3-month measurement period of this study. Leaf water content was acquired by weighing and measuring the area of 15 fresh leaves. The leaves were then oven-dried and dry mass was measured. From these data, an average leaf water content of 12.0 mol m^{-2} was calculated.

Stable isotope measurements

To complement this section, we provide a schematic diagram (Figure 1) which defines the isotope terms and illustrates the movement of $\delta^{18}O$ through the atmosphere, chambers and trees. The inclusion of the condenser within the WTC system differs significantly to the true flow-through systems used at the leaf and branch scale by Simonin et al. (2013) and Dubbert et al. (2014), respectively. The rate of condensation, and its oxygen isotope composition (δ_C), need to be measured in order to implement a stable isotope mass balance approach.

The stable isotope composition of water vapour of the external ambient air and air within each chamber was monitored over 85 days from late August to mid-November using a water vapour isotope analyser (TIWA-45EP; Los Gatos Inc., Mount View, CA, USA) plumbed into the chamber air sampling system. A valve system (Multiport Inlet Unit; Los Gatos Inc., Mount View, CA, USA) was used to sequentially sample each chamber for 4.5 min every hour, followed by the ambient air above the site for 6 min. The laser was calibrated once a week using the IAEA standards VSMOW, SLAP and GISP. To calibrate, the water vapour isotope analyser was coupled to a vapourizer (Water Vapour Isotope Standard Source; Los Gatos Inc., Mount View, CA, USA) and a dry air source (Dry Air Source; Los Gatos Inc., Mount View, CA, USA), and each standard analysed for at least 5 min after vapour concentrations had stabilized. We assume that there was no isotopic exchange between the chamber construction materials and the gas inside the chamber system. The transparent film of ultra-thin ethylene-tetrafluroethylene surrounding the chambers, which forms the majority of the surface area within the system, is known to have very low water absorption/desorption properties (0.03%). We also note the elevated temperature chambers had a subfloor compartment air exchange rate that was lower compared with the ambient chambers to maintain temperature control which may have caused differences in surface evaporation and soil water isotope composition.

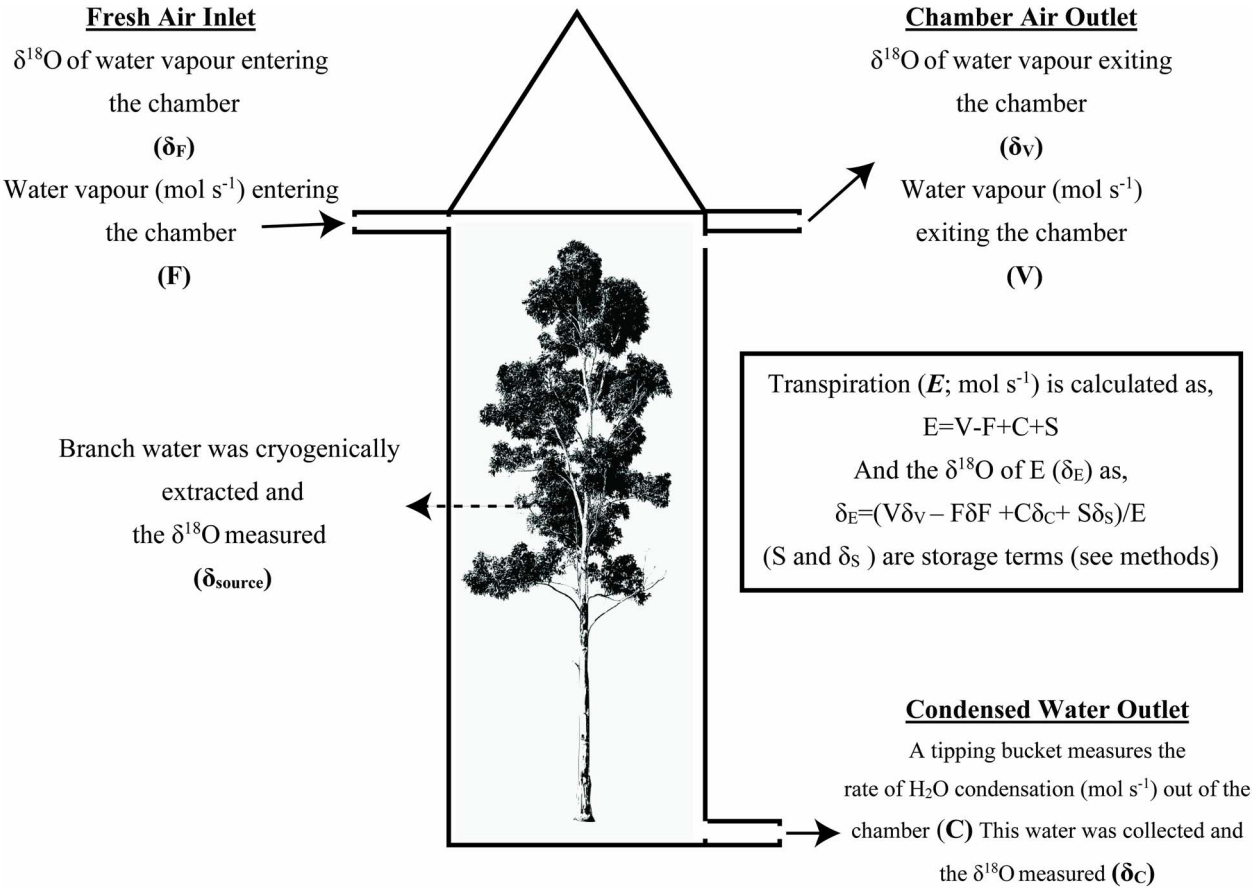

Figure 1. A schematic diagram of oxygen isotopes of water measured using the whole-tree chambers and their corresponding terms. The dashed arrow line indicates the destructive sampling technique to determine δ_{source}.

Condensed water was collected from the WTC's over two campaigns in order to estimate the $\delta^{18}O$ of transpiration via a mass balance approach (campaign one was 24 October and 25 October, campaign two was 20 November and 21 November). Condensed water was collected from the WTC condensation outlet in an exetainer and immediately wrapped in Parafilm. The samples were refrigerated and $\delta^{18}O$ measured (δ_C) after vapourisation as described above.

Source water $\delta^{18}O$ was determined from cryogenically extracted (Loucos et al. 2015) water from tree branches using the isotope analyser after vaporization as described above (we corrected for interference by organic compounds of the laser absorption method, see Figure S1, available as Supplementary data at *Tree Physiology* Online for a full description). There were no statistically significant differences in δ_{source} between trees across both temperature treatments, so an average value of −3.3‰ was used. This value aligns with long term $\delta^{18}O$ precipitation measurements of the Sydney Basin (Hughes and Crawford 2013), and dam reservoir water which was used to irrigate the trees. For condensed water and extracted branch water samples, the analyser was calibrated using three secondary standards with $\delta^{18}O$ values of −14.4 −1.5 and 34.1‰.

Leaf water turnover time

Leaf water turnover time is a measure of how long it will take to replace the entire leaf water reservoir and depends on transpiration rate and leaf water content. The leaf water turnover time constant (τ) was described by Dongmann et al. (1974) and Farquhar and Cernusak (2005) as:

$$\tau = \frac{W}{g_t\, w_i} \tag{2}$$

where W (mol water m^{-2}) is the leaf water content, g_t (mol m^{-2} s^{-1}) is total conductance to water vapour through the stomata (g_s) and leaf boundary layer (g_b) (i.e. $1/g_t = 1/g_s + 1/g_b$), and w_i is the leaf intercellular vapour concentration (mol water vapour mol^{-1} air).

Consideration of the isotopic turnover time of leaf water (τ_i) requires inclusion of the relevant isotopic fractionation effects, namely kinetic fractionation and equilibrium fractionation. Hence, Eq. (2) can be rewritten as (Farquhar and Cernusak 2005, Simonin et al. 2013, Song et al. 2015*b*):

$$\tau_i = \frac{W\alpha_k\alpha^+}{pg_t w_i} \tag{3}$$

where p (> 1) is a multiplier to account for the degree to which bulk leaf water is less enriched than evaporation site water. Also in Eq. (3), α_k and α^+ are the kinetic and equilibrium isotopic fractionation factors for diffusion from the leaf and the phase change at the liquid/vapour interface, respectively. Note that $\alpha_k = 1 + \varepsilon_k$ and $\alpha^+ = 1 + \varepsilon^+$; ε_k was assumed to be 27 ‰ and ε^+ was calculated as (Bottinga and Craig 1968):

$$\varepsilon^{+} = 2.644 - 3.206\left(\frac{10^3}{T_{leaf(k)}}\right) + 1.534\left(\frac{10^3}{T_{leaf(k)^2}}\right) \quad (4)$$

where $T_{leaf(k)}$ is leaf temperature expressed in Kelvin.

The simple formulation described in Eq. (3) is complicated by the possibility that not all leaf water is hydraulically well connected to the transpiration stream. The complexity of leaf anatomy and crown architecture means one single pool of leaf water is unlikely. More likely is that a leaf has multiple pools of water, and each pool will be positioned uniquely in relation to the transpiration stream, resulting in each pool having a unique turnover time (Zwieniecki et al. 2007, Barbour et al. 2021). At present we have no understanding of differences in the turnover time of the hypothesized differences in leaf water pools, so that the most parsimonious assumption remains that leaf water is comprised of a single, well-mixed water pool. We recently assessed the relevance of the Péclet effect (p) in a range of species and found that for *E. parramattensis* bulk leaf water was less enriched than Craig-Gordon-predicted evaporation site water, but the fractional difference in enrichment between bulk leaf water and evaporation site water was not dependent on transpiration rate (Barbour et al. 2021). This implies that the Péclet effect is not a strong determinant of leaf water isotopic enrichment in *E. parramattensis*, and that a two-pool model may adequately predict enrichment with an estimate of p of 1.20 used here to calculate τ_i (Figure S2, available as Supplementary data at *Tree Physiology* Online).

The incorporation of Eq. (3) into models of leaf water enrichment has been inconsistent to date. Farquhar and Cernusak (2005) derived a model for non-steady state conditions for leaves in the open air, while Song et al. (2015*b*) and Farquhar et al. (2021) provided a formulation for the special case of a leaf inside a gas exchange chamber. None has attempted to derive expressions for the case of multiple pools of water in the leaf, although Simonin et al. (2013) do discuss the implications of multiple pools on τ_i.

Estimating the $\delta^{18}O$ of transpired water vapour

We used the unique infrastructure of WTCs to investigate the diel patterns of whole-tree transpiration and address the isotopic steady state assumption. The WTCs are designed to function as quasi null-balance systems. Thus, CO_2 is injected into the inflowing air with the target of replacing the CO_2 in the chamber consumed by photosynthesis, and there is a condenser to remove water vapour from the air within the chamber with the target of maintaining the vapour pressure inside the chamber similar to that of the reference air at the inlet (Medhurst et al. 2006; Barton et al. 2010). Transpiration (E; mol s^{-1}) is calculated as:

$$E = V - F + C + S \quad (5)$$

where V is the venting of water vapour out of the chamber (mol s^{-1}), calculated as the product of the flow rate out of the chamber and the water vapour mole fraction of outflowing air; F is the flow of water vapour into the chamber (mol s^{-1}), calculated as the product of the flow rate into the chamber and the water vapour mole fraction of inflowing air; C is the rate of water removal from the chamber air by the condenser (mol s^{-1}); and S is the change in storage of water vapour in the chamber air from one time step to the next (mol s^{-1}). The change in storage, S, is calculated as:

$$S = \frac{\left(w^{(t)} - w^{(t-1)}\right)v}{\Delta t} \quad (6)$$

where $w^{(t)}$ is the water vapour mole fraction of air in the chamber at time t, $w^{(t-1)}$ is that at the previous time step, v is the molar volume of air in the chamber and Δt is the number of seconds from time $t-1$ to time t.

The $\delta^{18}O$ of transpiration, δ_E, can be calculated as:

$$\delta_E = \frac{V\delta_V - F\delta_F + C\delta_C + S\delta_S}{E} \quad (7)$$

where δ_V is the $\delta^{18}O$ of the outflowing air, measured by sampling the air inside the WTC; δ_F is the $\delta^{18}O$ of inflowing air, measured by sampling the atmosphere outside the chamber from which air is drawn into the chamber; δ_C is the $\delta^{18}O$ of the liquid water captured by the condenser; and δ_S is the $\delta^{18}O$ of the water vapour added to or removed from the water vapour in the chamber from one time step to the next. The δ_S can be calculated as:

$$\delta_S = \frac{w^{(t)}\delta^{(t)} - w^{(t-1)}\delta^{(t-1)}}{w^{(t)}} \quad (8)$$

where $\delta_{(t)}$ is the $\delta^{18}O$ of chamber air measured at time t, and $\delta_{(t\text{-}1)}$ is the $\delta^{18}O$ of chamber air measured at the previous time step. We note that if C and δ_C were constant an attempt would have been made to estimate δ_E over a seasonal scale. However, given the variation, especially in δ_C, we were not confident in estimating δ_E without accounting for δ_C.

Estimating the $\delta^{18}O$ of leaf water at the site of evaporation

The $\delta^{18}O$ of leaf water at the site of evaporation (δ_e) was calculated as:

$$\delta_e = \left(1+\varepsilon^{+}\right)\left[\left(1+\varepsilon_k\right)\left(1+\delta_E\right)\left(1-\frac{e_v}{e_i}\right)+\frac{e_v}{e_i}\left(1+\delta_v\right)\right]-1 \quad (9)$$

e_v/e_i is the ratio of the water vapour mole fraction in the air relative to that in the intercellular air spaces.

To further investigate the influence of δ_E on δ_V we arranged Eq. (7) to solve for δ_V and assumed δ_E was at steady-state ($\delta_E = \delta_{source}$), the calculation for the $\delta^{18}O$ of chamber air assuming steady state δ_E ($\delta_{V\text{–}ss}$) is:

$$\delta_{V-ss} = \frac{E\delta_{Source} + F\delta_F - C\,\varepsilon^{+}}{V + C} \quad (10)$$

The above equation incorporates a steady state estimate for δ_C ($\delta_{C\text{–}ss}$), calculated as:

$$\delta_{C-ss} = \delta_{V+}\varepsilon^{+} \quad (11)$$

which is integrated into the estimate of δ_{V-ss}. Furthermore, in this context transpiration is assumed in steady state, as such the storage term is removed from the calculation.

To further investigate the error that the assumption of steady state δ_E adds to the interpretation of $\delta^{18}O$ of organic material, we investigated how steady state δ_E influences the

Table 1. Determination of $\delta^{18}O$ of water vapour fluxes at canopy scale of trees grown under ambient and elevated (+ 3 °C) air temperatures and measured in two diel campaigns. Average day-time means for $\delta^{18}O$ for chamber water vapour (δ_V), atmospheric water vapour (δ_F) and transpired water vapour (δ_E), isotopic leaf water turnover time (Ti, h). Source water is −3.28‰. 'Day-time' refers to an observation where photosynthetically active radiation is greater than or equal to 0.01 μmol m^{-2} s^{-1} (± SE, n = 6 chambers)

Treatment	Campaign	δ_V	δ_F	δ_E	τ_i
Ambient	1	−12.23 (1.34)	−12.43 (1.38)	−3.75 (2.17)	1.38 (0.91)
Ambient	2	−13.52 (0.9)	−11.59 (0.31)	−4.47 (2.45)	1.60 (1.40)
Elevated	1	−11.84 (1.17)	−12.33 (1.36)	−4.30 (2.73)	1.74 (1.86)
Elevated	2	−12.60 (1.00)	−11.59 (0.30)	−3.91 (1.68)	1.42 (1.04)

$\delta^{18}O$ of leaf water at the site of evaporation (δ_e). The $\delta^{18}O$ of leaf water is transferred into the organic material created in the leaf. Firstly, we calculated δ_e assuming steady state δ_E ($\delta_{e\text{-}ss}$) by modifying Eq. (9)

$$\delta_{e-ss} = \left(1+\varepsilon^{+}\right)\left[\left(1+\varepsilon_k\right)\left(1+\delta_{source}\right)\left(1-\frac{e_v}{e_i}\right)+\frac{e_v}{e_i}\left(1+\delta_v\right)\right]-1 \quad (12)$$

Given that more organic material is produced at periods of high photosynthesis we calculated an assimilation weighted mean for δ_e (assimilation weighted δ_e) and δ_{e-ss} (assimilation weighted δ_{e-ss}) using only daytime values (photosynthetically active radiation is greater than or equal to 0.01 μmol m^{-2} s^{-1}). The 'assimilation weighted' qualifier describes the δ_e (or δ_{e-ss}) being weighted by net CO_2 flux, and thus represents the δ_e (or $\delta_{e\text{-}ss}$) at which most of the photosynthesis occurred.

Results

We focused on two diel campaigns of condensed water collection from chamber air and transpired water to allow us to apply the mass balance approach and calculate δ_E. During these campaigns we were able to determine the proportion of time the canopy was at isotopic steady state. Air temperature and VPD were typical of a normal diel cycle as was canopy transpiration rate (Figure 2a–f). Calculated leaf total conductance was higher at night-time for ambient (Figure 2g) compared with elevated temperature chambers (Figure 2h). However, these leaf total conductance values were calculated when the difference between chamber air temperature and chamber dew point temperature was negative or within 1 °C (identifiable by black *symbols in Figure 2). In such humid conditions, we expect transpiration rates to be low and errors in conductance estimates to be high (Ewers and Oren 2000). Thus, these observations were removed from the analyses.

The two campaigns resulted in distinct diel cycles for the ambient and elevated temperature treatments. Campaign one had an average daytime δ_E of −3.8‰ ± 2.2 in the ambient treatment and an average daytime δ_E of −4.3‰ ± 2.7 in the elevated treatment. Campaign two had an average daytime δ_E of −4.5‰ ± 2.4 in the ambient treatment and an average daytime δ_E of −3.9‰ ± 1.7 in the elevated treatment (Table 1). There was a significant difference between elevated and ambient temperature chambers for δ_E for campaign 1, but not campaign 2.

All daytime averages for isotopic leaf water turnover were under 1.8 h (Table 1). The time constants of isotopic leaf water turnover time (Figure 3a–d) varied by orders of magnitude over the diel measurement periods, with larger values at night when transpiration was negligible compared with the day-light hours (note that total time for leaf water to completely turnover is roughly three times the leaf water turnover time constant).

The measured WTC isotopes, that is, the $\delta^{18}O$ of the atmosphere (δ_F, Figure 3m and n) inside the WTC (δ_V, Figure 3e–h) and the condensed water (δ_C, Figure 3i–l), do not have clearly defined temporal trends. As water condenses, the lighter isotopologue $H_2{}^{16}O$ is preferentially left in the chamber, meaning the water that exits the chamber via the condenser (δ_C) is enriched relative to (δ_V). If δ_E were at steady state throughout a 24-h period, δ_F was constant, the condenser fractionation effect was constant and source water was in equilibrium with the vapour in the chamber, we would expect that transpiration would add heavier water (^{18}O) and the condenser would remove heavier water at equal rates. In this idealized case all isotopic effects are steady. However, if δ_F is variable and δ_E is not at steady state, patterns of δ_V and δ_C enrichment and depletion will not be clear. Essentially, δ_V and δ_C are influenced by δ_F and the relative deviation of δ_E from steady state, as such it is difficult to see trends in the measured WTC isotopes (Figure 3). The relationship between δ_V, δ_C, δ_F and δ_E is disentangled with the mass balance approach.

In general, δ_E was depleted relative to source water from midday until nighttime and relatively enriched from sunrise until mid-morning. For the ambient chambers in campaign one δ_E trended towards enrichment during the afternoon and was enriched relative to steady state during the night (Figure 4i). Conversely, the ambient chambers in campaign two (Figure 4k) became more depleted in the afternoon and rarely were enriched relative to source water during the evening. The elevated chambers mirror this trend and were also rarely enriched relative to source water during the evening. It is worth highlighting that when transpiration is low, it becomes harder to estimate δ_E, as shown in larger deviation in the nighttime values. In summary, δ_E is enriched relative to source water in the morning and depleted in the afternoon, and when a daytime average is taken δ_E roughly equals δ_{source}.

The assumption of isotopic steady state is important when considering the partitioning of ecosystem fluxes (e.g. evapotranspiration into its evaporation and transpiration components). To test this, we arranged Eq. (7) to solve for δ_V for all daytime observations assuming δ_E and δ_C were at steady state. As δ_V is influenced by δ_E, comparing the observed values (which incorporate non steady state δ_E) to the steady state estimate highlights the consequences of assuming steady state. As shown in Figure 5, assuming steady state δ_E results in modest differences between δ_V and $\delta_{V\text{-}ss}$. On average $\delta_{V\text{-}ss}$ was ±0.76‰ different from δ_V with a maximum difference of 3.43‰. In other words, transpired water vapour has the

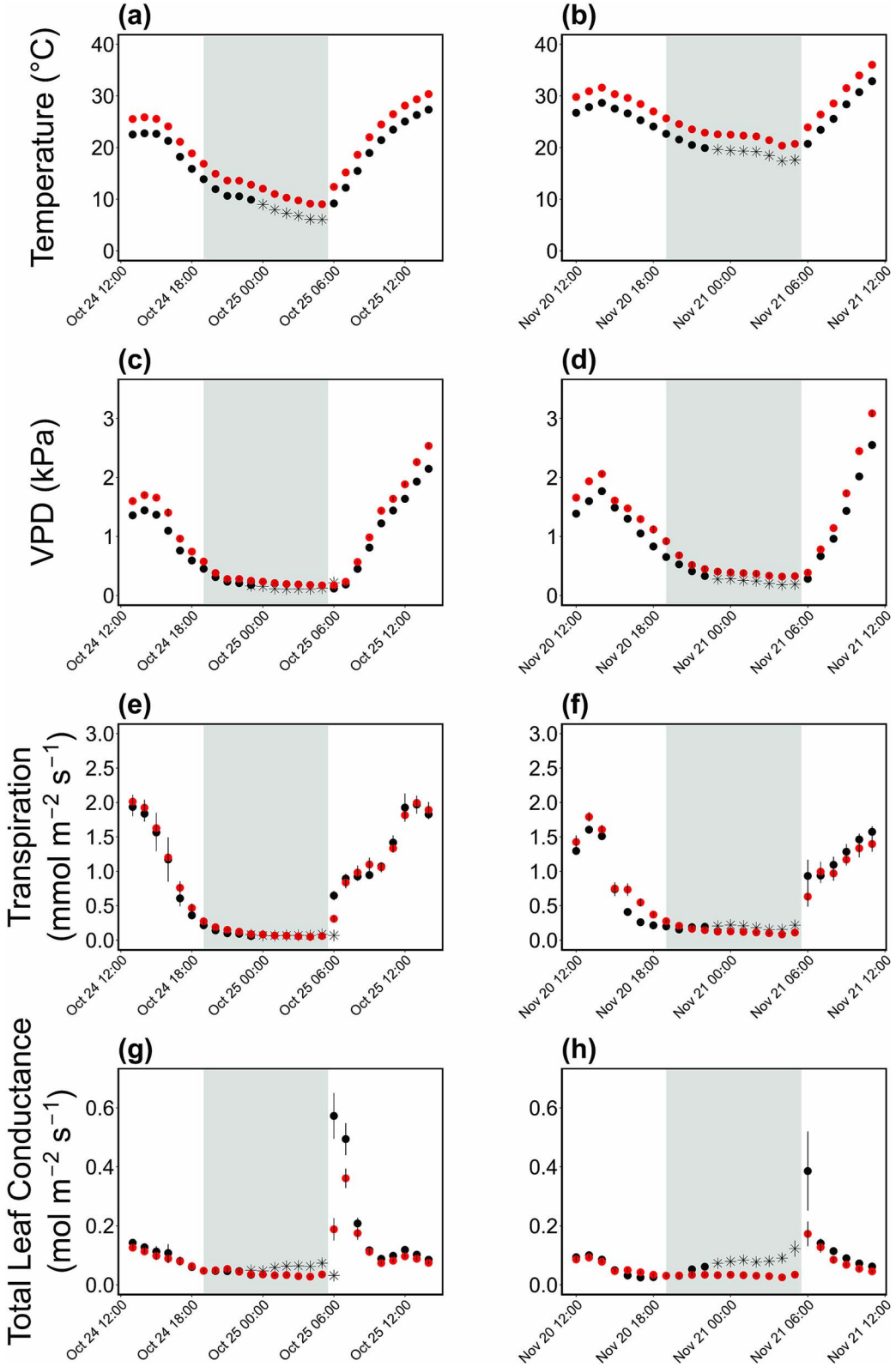

Figure 2. Diel data from two campaigns to measure whole-tree canopy transpiration under ambient and elevated (+ 3 °C) air temperatures: chamber air temperature (a, b), vapour pressure deficit (VPD) (c,d), transpiration per leaf area (e,f) and leaf total conductance to water vapour (g,h).The shaded grey area is night time. Black circles represent ambient temperature chambers. Red circles represent elevated temperature (+ 3 °C) chambers. The black * symbol represents observations in the ambient chambers where the difference between chamber air temperature and chamber dew point temperature were within 1 °C or negative (error bars are ± SE, n = 6 chambers).

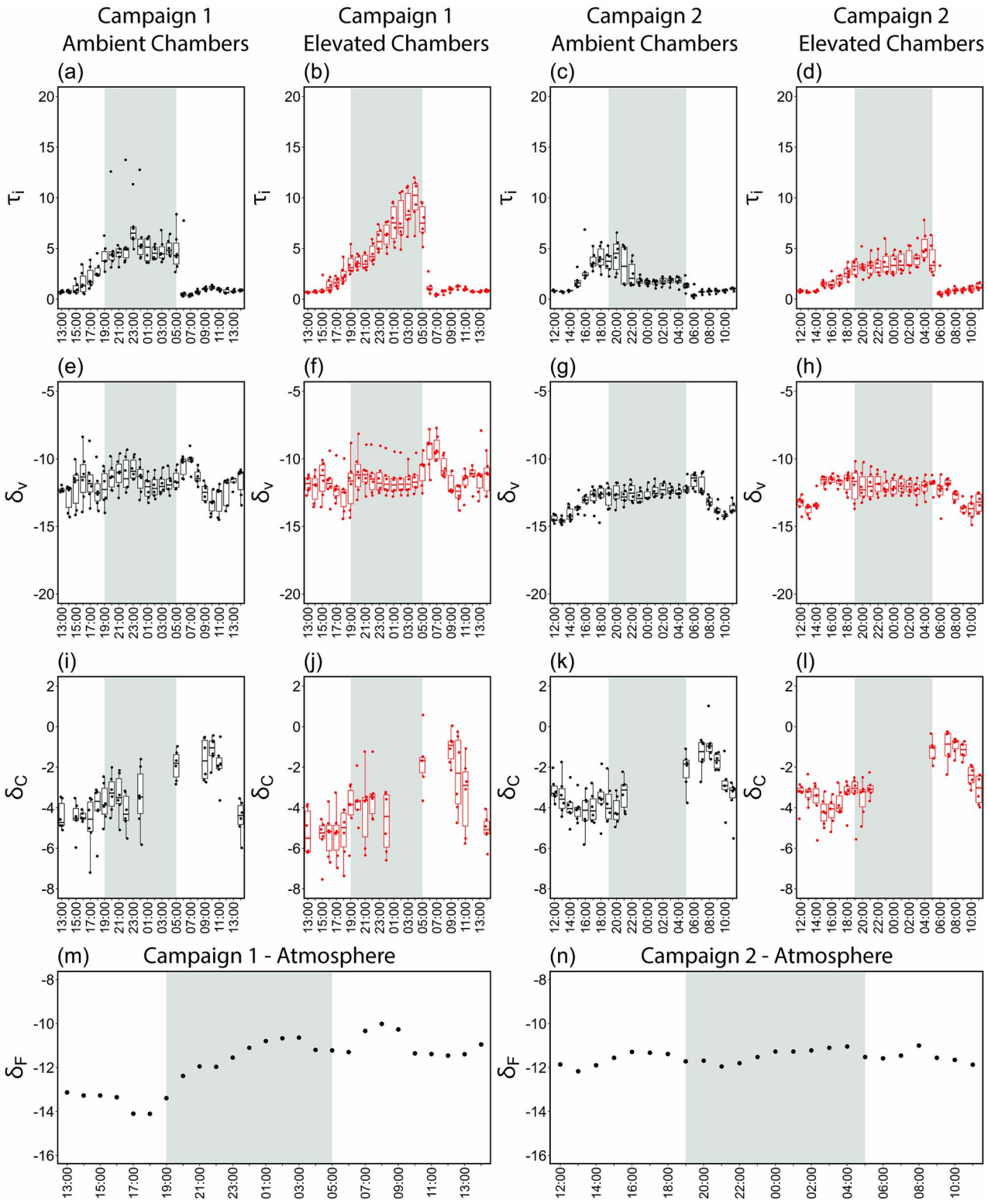

Figure 3. Determination of $\delta^{18}O$ of water fluxes at canopy scale of trees grown under ambient (black symbols) and elevated (+ 3 °C; red symbols) air temperatures, measured in two diel campaigns. Diel data from two campaigns for: Isotopic turnover time (h) of leaf water (a–d). $\delta^{18}O$ of water vapour in the chamber (δ_V, e–h), condensed chamber water (δ_C, i–l), the atmosphere (δ_F, m, n). Black boxplots represent ambient temperature chambers. Red boxplots represent elevated temperature chambers. For m and n black circles represent the atmosphere. The box plots indicate the median and quartiles and the error bars represent the 1.5 interquartile range. Observations (n = 6 chambers) are overlayed as circles.

capacity to alter the $\delta^{18}O$ of chamber water vapour. The observation that the differences are quite small when calculated over a diel period implies that the effects of non-steady state are not overly consequential to partitioning ecosystem fluxes. However, given that the difference can be large at specific timepoints implies that the effects of non-steady

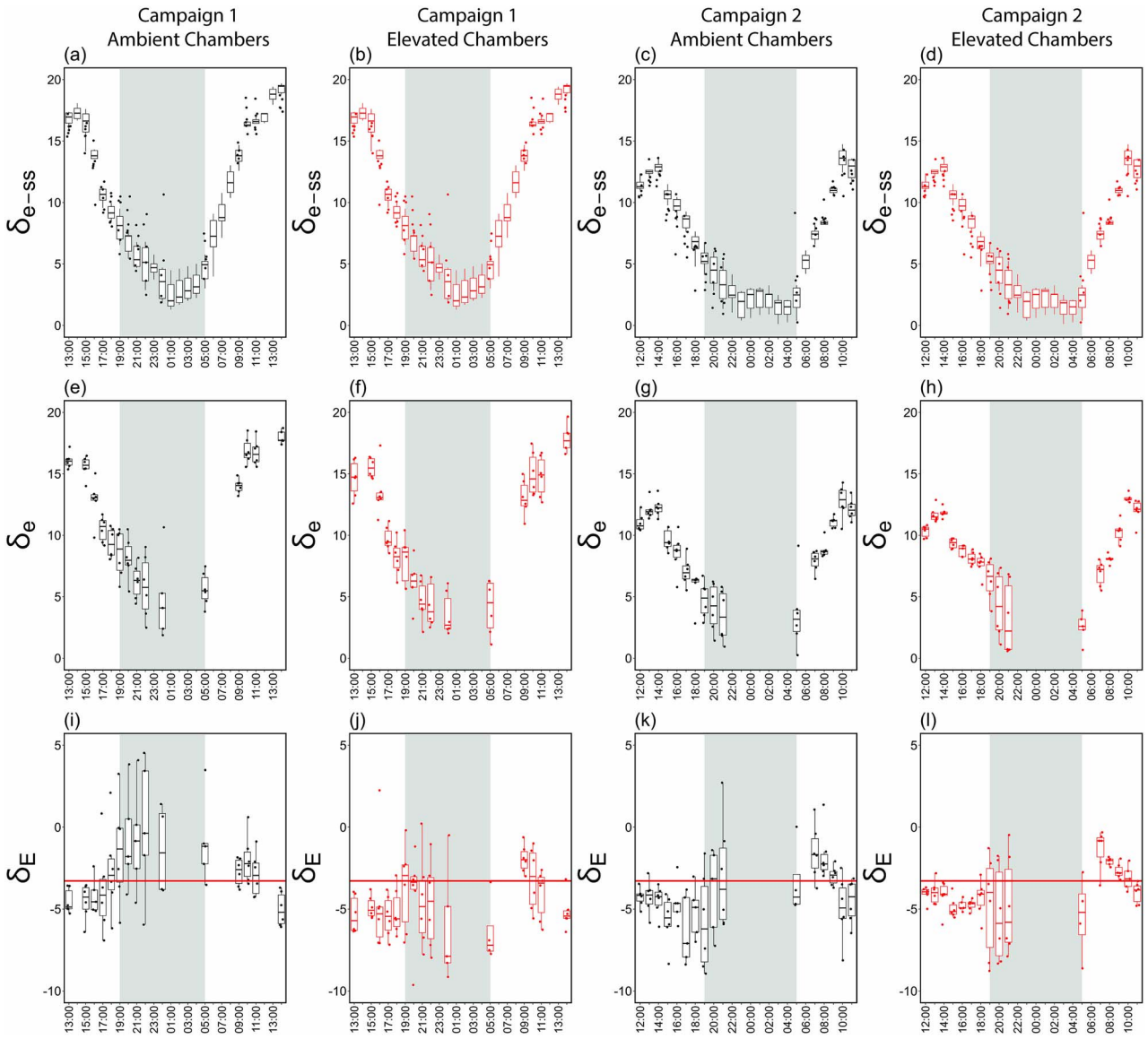

Figure 4. Calculated $\delta^{18}O$ estimates of leaf water at the evaporating site and transpiration at canopy scale of trees grown under ambient (black symbols) and elevated (+ 3 °C; red symbols) air temperatures, measured in two diel campaigns. Diel data from two campaigns for: $\delta^{18}O$ of water at the site of evaporation assuming steady state ($\delta_{e\text{-}ss}$, a–d), $\delta^{18}O$ of water at the site of evaporation calculated with observed values (δ_e, e–h), $\delta^{18}O$ of transpiration (δ_E, i–l). The red line in i–l is source water (−3.28‰). Red boxplots represent elevated chambers, black boxplots represent ambient chambers. The box plots indicate the median and quartiles and the error bars represent the 1.5 interquartile range, for each time point each observation (n = 6) is overlayed as a circle.

state cannot be a priori ignored when partitioning ecosystem fluxes.

We assessed the error in assuming isotopic steady state when interpreting the $\delta^{18}O$ of organic material by comparing assimilation weighted δ_e to assimilation weighted $\delta_{e\text{-}ss}$ (Figure 6). In Figure 6, deviations from the 1:1 line reflect error when assuming δ_E is at steady state. δ_e is the primary determinant of $\delta^{18}O$ of leaf water, and is reflected in $\delta^{18}O$ of organic molecules synthesized in leaves. Therefore, if estimates of leaf water $\delta^{18}O$ are incorrect, interpretation of the $\delta^{18}O$ of a given organic material will be incorrect. Whilst there was some influence of non-steady state, the error from assuming isotopic steady state did not exceed 1‰. That is, assimilation weighted $\delta_{e\text{-}ss}$ and assimilation weighted δ_e were always within 1‰, suggesting that the effect of isotopic non-steady state has negligible influence on δ_e. Our data imply that research questions relating to the $\delta^{18}O$ of organic material do not need to consider non-steady δ_E.

Discussion

Understanding why transpiration is not at steady state

Average daytime δ_E did deviate slightly (up to ±1.2‰) from source water. During the day δ_E was relatively depleted compared with source water in the afternoon and relatively enriched compared with source water in the early morning and although there are few observations it appears that δ_E is enriched in the predawn period particularly for elevated chambers. Our two 24-h campaigns were 28 days apart and, with the +3 °C warming treatment, we captured four unique

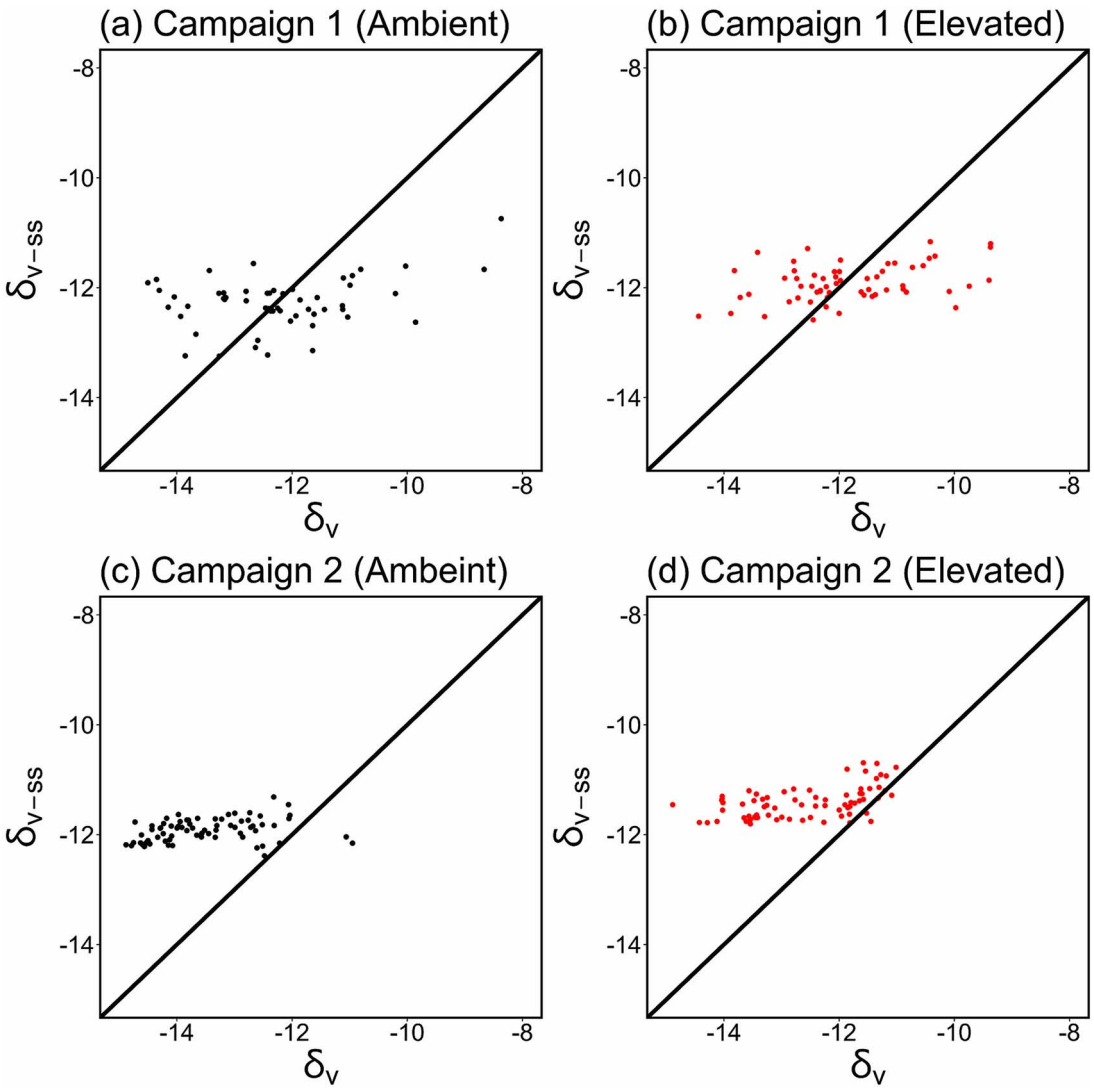

Figure 5. The relationships between $\delta^{18}O$ of water vapour in the chamber (δ_V) and when Eq. (5) is rearranged to solve for δ_V assuming transpiration is at isotopic steady state (δ_V-ss). The 1:1 line is included.

diurnal periods. For both campaigns transpiration became isotopically depleted in ^{18}O as the day progressed (from sunrise to mid-day/early afternoon). This was because the leaf water was slowly becoming enriched due to vapour pressure deficit increasing. As transpiration drastically decreased in the late afternoon and throughout the night, the leaf water was left enriched. At first light, when transpiration increased, the heavier ^{18}O isotopes were a part of the transpiration stream and δ_E was typically enriched compared with δ_{source} in the early hours of the morning. As VPD and transpiration increased throughout the day, the heavier ^{18}O isotopes started to make up a lower proportion of the transpired water vapour and by around mid-day δ_E was typically depleted compared with δ_{source}.

One-way transpiration flux and leaf water content determine leaf water turnover time (Eq. 2), which can vary from minutes to hours. A longer leaf water turnover time increases the time to reach steady state $\delta^{18}O$ of both leaf water and transpiration. Average daytime estimates of leaf water isotopic turnover time constants were all under 1.8 h. These turnover times compare with 15 to 120 min estimated in cotton by Song et al. (2015*b*), whereas Dubbert et al. (2014) predicted leaf water turnover times between 3.6 and 5.4 h for *Quercus suber*, depending on the time of year. The daytime turnover times observed here align with observations of δ_E being close to, but seldom at, isotopic steady state. If the water supplying transpiration comes from multiple pools within the leaf, which vary in size, our simple assumption of two pools (an evaporatively enriched pool and an unenriched pool), which both have the same turnover time constant, is inappropriate.

Comparing species differences in the $\delta^{18}O$ of transpiration

As different plants reach physiologically stable conditions (e.g. stomatal conductance response to light or temperature) over different time periods, the time for δ_E to reach steady state can vary considerably (Simonin et al. 2013, Dubbert et al. 2014). Given that VPD and air temperature change over a diurnal cycle (Figure 2), isotopic steady state is unlikely to be achieved at transitional parts of the day, such as morning and late afternoon. Lai et al. (2006) monitored water fluxes and micrometeorological conditions with eddy covariance towers in old-growth coniferous forest in the Pacific Northwest of the USA and found differences between δ_E and δ_{source} of up to 13‰ in the morning, with an increase towards steady state in the afternoon. Yepez et al. (2007) also used eddy covariance

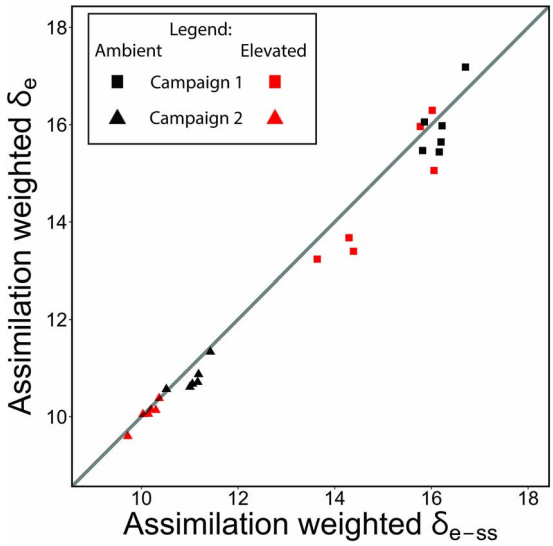

Figure 6. The relationship between assimilation-weighted $\delta^{18}O$ of water at the site of evaporation assuming steady state ($\delta_{e\text{-}ss}$) and non-steady state (δ_e) the 1:1 line is included.

towers in a similar fashion and observed differences between δ_E and δ_{source} of up to 9‰ in morning, with differences diminishing in the afternoon in semiarid riparian woodland in south-eastern Arizona USA. Dubbert et al. (2014) monitored δ_E using custom branch chambers and observed that δ_E never reached δ_{source} and varied throughout the day, ranging between −26.1‰ and −6.2‰ for the woodland site in autumn, spring and summer. In a diurnal comparison of δ_E between cork oak trees and a grassland (Dubbert and Werner 2019) found that the grassland reached isotopic steady-state quickly, whereas as described above the cork trees never reach isotopic steady-state. To better understand isotopic steady-state and when it should and should not be used it would be valuable to measure contrasting species using different techniques (e.g. compare five contrasting species (from grasses to trees) using the same technique at multiple spatial resolutions: leaf cuvette, custom branch chamber, WTCs and eddy covariance towers).

Implications of non-steady state transpiration

If a canopy has a large transpiration flux it is likely to contribute a significant amount of water vapour to the canopy boundary layer. Therefore, canopy transpiration has the capacity to change the composition of the immediately adjacent atmosphere. For example, a high transpiration flux could increase the water content and alter the $\delta^{18}O$ of water vapour in the atmosphere compared with an area of land that had no vegetation. If steady state between transpiration and source water isotopic composition is assumed when non-steady state is occurring, another process (e.g. evaporation) could be erroneously assumed responsible for variation in $\delta^{18}O$ of the ecosystem flux. For example, the Dubbert et al. (2013) study tested the degree that steady state assumptions influence evapotranspiration partitioning between plant transpiration and soil evaporation. They observed that the difference between non-steady state and steady state estimates of the proportion of evapotranspiration comprised of plant transpiration ranged from negligible in the late afternoon, up to a 50% difference in the morning. Similarly, Yepez et al. (2007) reported errors in the proportion of evapotranspiration comprised of plant transpiration of up to 25% when comparing steady state and non-steady state techniques.

Whilst our results show that δ_E daytime means across both campaigns are roughly equal to δ_{source} (within 1.2‰) there are clearly specific times of the day when δ_E deviates from δ_{source}. It is these transitional parts of the day that can be important for splitting evapotranspiration into its components. That is, δ_E observations above or below the red line (δ_{source}) in Figure 4i and j reflect the extent of isotopic non-steady state. This implies that if *E. parramattensis* was the dominant species in an ecosystem, calculations of the proportional contribution of transpiration to ecosystem evapotranspiration would be incorrect if steady state δ_E was assumed. The extent of how poor the estimate would be and whether it over or underestimates the fraction of transpiration depends on the time of day. Given that δ_E exhibits trends (e.g. enriched in the morning and depleted in the afternoon) suggests that with a growing body of isotopic transpiration data, a time of day correction may be appropriate to improve the accuracy when assuming isotopic steady state. Kübert et al. (2023) assessed δ_E deviations from steady-state at the leaf scale over several months. They observed that at any given time δ_E may be far from steady state, but, when looking at averages, δ_E will roughly equal δ_{source}. Kübert et al. (2023) state that at time periods longer than 3 days isotopic non-steady state effects will even out. Our data, along with that from Kübert et al. (2023), suggest there is less need to account for non-steady transpiration when partitioning ecosystem fluxes on time scales greater than 3 days (e.g. comparing the proportion of evaporation with evapotranspiration across a whole summer vs winter period) compared with shorter time scales (e.g. comparing a mid-morning observation with a mid-day observation).

As δ_E deviates from steady state, a consequence could be changes in assimilation-weighted δ_e. If this is the case, the interpretation of $\delta^{18}O$ of organic matter could be erroneous. Oxygen isotopic composition of organic matter is expected to be weighted towards periods of high photosynthesis, when production of assimilates occurs (Helliker and Richter 2008). Of secondary importance is isotopic exchange between carbonyl oxygen and local water during remobilization of non-structural carbohydrates (e.g. transitory starch) prior to transport for biomass growth (see Song et al. 2014 for discussion). The local water with which organic oxygen exchanges may be leaf water (potentially with a different isotope composition than that at the time of initial carbon fixation), water in a shoot or root meristem or cambium (perhaps closer to source water $\delta^{18}O$; see Ogee et al. 2009). Given the uncertainty regarding exchange of organic oxygen with water within a plant, a steady state model is the most parsimonious and appropriate assumption (Cernusak et al. 2016). Our results show that isotopic steady state and non-steady state assimilation-weighted δ_e estimates were within 1‰ of each other, implying that investigations into the $\delta^{18}O$ of leaf derived organic matter will not greatly benefit from accounting for non-steady state in the first instance.

Conclusion

Utilizing the unique infrastructure of WTCs to implement a + 3 °C warming treatment in large trees, we investigated the $\delta^{18}O$ of canopy transpiration (δ_E) over two diel campaigns.

We observed that average daytime δ_E was within 1.2‰ of δ_{source}, but varied considerably over a diel period, meaning that a steady-state assumption cannot be assumed for whole trees over such a time course. For example, we demonstrate that using the steady state assumption to partition evapotranspiration into its components could produce poor estimates at high temporal resolution. Alternatively, when interpreting the $\delta^{18}O$ of organic matter, accounting for non-steady state has little influence on assimilation-weighted δ_e.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Acknowledgments

We thank Burhan Amiji (Western Sydney University) for maintaining the site and for his excellent research support. We thank Kristine Crous and David Ellsworth for the use of leaf thermocouples and gallium-arsenide photodiodes, and Wenbo Yang for stable isotope mass spectrometry. An anonymous reviewer is thanked for their insightful comments that greatly improved the manuscript. This experiment was made possible through collaboration with Sune Linder and the Swedish University of Agricultural Sciences, who designed, built and generously provided the whole tree chambers.

Conflict of interest

None declared.

Funding

The Australian Research Council DP170104267 and DP140103415.

Data availability

All data and code used to produce the results is hosted on github: https://github.com/RichardHarwood/Whole-Tree-Chamber-Isotope

References

Barbour MM, Loucos KE, Lockhart E, Shrestha A, McCallum D, Simonin KA, Song X, Griffani DS, Farquhar GD (2021) Can hydraulic design explain patterns of leaf water isotopic enrichment in C3 plants? Plant Cell Environ 44:432–444. https://doi.org/10.1111/pce.13943.

Barton CVM, Ellsworth DS, Medlyn BE et al. (2010) Whole-tree chambers for elevated atmospheric CO_2 experimentation and tree scale flux measurements in south-eastern Australia: the Hawkesbury Forest experiment. Agric For Meteorol 150:941–951. https://doi.org/10.1016/j.agrformet.2010.03.001.

Bottinga Y, Craig H (1968) Oxygen isotope fractionation between CO2 and water, and the isotopic composition of marine atmospheric CO_2. Earth Planet Sci Lett 5:285–295. https://doi.org/10.1016/S0012-821X(68)80054-8.

Casado M, Münch T, Laepple T (2020) Climatic information archived in ice cores: impact of intermittency and diffusion on the recorded isotopic signal in Antarctica. Clim Past 16:1581–1598. https://doi.org/10.5194/cp-16-1581-2020.

Cernusak LA, Barbour MM, Arndt SK et al. (2016) Stable isotopes in leaf water of terrestrial plants. Plant Cell Environ 39:1087–1102. https://doi.org/10.1111/pce.12703.

Craig H, Gordon L (1965) Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. In Tongiorgi E (ed), Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Palaeotemperatures. Lischi and Figli, Pisa, pp. 9–130.

Dongmann G, Nürnberg HW, Förstel H, Wagener K (1974) On the enrichment of $H_2{}^{18}O$ in the leaves of transpiring plants. Radiat Environ Biophys 11:41–52. https://doi.org/10.1007/BF01323099.

Drake JE, Tjoelker MG, Aspinwall MJ, Reich PB, Barton CVM, Medlyn BE, Duursma RA (2016) Does physiological acclimation to climate warming stabilize the ratio of canopy respiration to photosynthesis? New Phytol 211:850–863. https://doi.org/10.1111/nph.13978.

Drake JE, Tjoelker MG, Vårhammar A, et al. (2018) Trees tolerate an extreme heatwave via sustained transpirational cooling and increased leaf thermal tolerance. Glob Chang Biol 24:2390–2402. https://doi.org/10.1111/gcb.14037.

Drake JE, Harwood R, Vårhammar A, Barbour MM, Reich PB, Barton CV, Tjoelker MG (2020) No evidence of homeostatic regulation of leaf temperature in *Eucalyptus parramattensis* trees: integration of CO_2 flux and oxygen isotope methodologies. New Phytol 228:1511–1523. https://doi.org/10.1111/nph.16733.

Dubbert M, Werner C (2019) Water fluxes mediated by vegetation: emerging isotopic insights at the soil and atmosphere interfaces. New Phytol 221:1754–1763. https://doi.org/10.1111/nph.15547.

Dubbert M, Cuntz M, Piayda A, Maguás C, Werner C (2013) Partitioning evapotranspiration–testing the Craig and Gordon model with field measurements of oxygen isotope ratios of evaporative fluxes. J Hydrol 496:142–153. https://doi.org/10.1016/j.jhydrol.2013.05.033.

Dubbert M, Cuntz M, Piayda A, Werner C (2014) Oxygen isotope signatures of transpired water vapor: the role of isotopic non-steady-state transpiration under natural conditions. New Phytol 203:1242–1252. https://doi.org/10.1111/nph.12878.

Dutton A, Wilkinson BH, Welker JM, Bowen GJ, Lohmann KC (2005) Spatial distribution and seasonal variation in $^{18}O/^{16}O$ of modern precipitation and river water across conterminous USA. Hydrol Process 19:4121–4146. https://doi.org/10.1002/hyp.5876.

Duursma RA, Barton CV, Eamus D, Medlyn BE, Ellsworth DS, Forster MA, Tissue DT, Linder S, McMurtrie RE (2011) Rooting depth explains $CO_2 \times$ drought interaction in *Eucalyptus saligna*. Tree Physiol 31:922–931. https://doi.org/10.1093/treephys/tpr030.

Ewers BE, Oren R (2000) Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements. Tree Phys siol 20:579–589. https://doi.org/10.1093/treephys/20.9.579.

Farquhar GD, Cernusak LA (2005) On the isotopic composition of leaf water in the non-steady state. Funct Plant Biol 32:293–303. https://doi.org/10.1071/FP04232.

Farquhar GD, Griffani DS, Barbour MM (2021) The effects on isotopic composition of leaf water and transpiration of adding a gas-exchange cuvette. Plant Cell Environ 44:2844–2857. https://doi.org/10.1111/pce.14076.

Gessler A, Ferrio JP, Hommel R, Treydte K, Werner RA, Monson RK (2014) Stable isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood. Tree Physiol 34:796–818. https://doi.org/10.1093/treephys/tpu040.

Gibson JJ, Edwards TWD (2002) Regional water balance trends and evaporation-transpiration partitioning from a stable isotope survey of lakes in northern Canada. Global Biogeochem Cycles 16:1–14.

Helliker BR, Richter SL (2008) Subtropical to boreal convergence of tree-leaf temperatures. Nature 454:511–514. https://doi.org/10.1038/nature07031.

Hughes CE, Crawford J (2013) Spatial and temporal variation in precipitation isotopes in the Sydney Basin, Australia. J Hydrol 489:42–55. https://doi.org/10.1016/j.jhydrol.2013.02.036.

Kübert A, Dubbert M, Bamberger I et al. (2023) Tracing plant source water dynamics during drought by continuous transpiration measurements: an in-situ stable isotope approach. Plant Cell Environ 46:133–149. https://doi.org/10.1111/pce.14475.

Lai C, Ehleringer JR, Bond BJ, Paw UKT (2006) Contributions of evaporation, isotopic non-steady state transpiration and atmospheric mixing on the $\delta^{18}O$ of water vapour in Pacific northwest coniferous forests. Plant Cell Environ 29:77–94. https://doi.org/10.1111/j.1365-3040.2005.01402.x.

Lorrey AM, Brookman TH, Evans MN et al. (2016) Stable oxygen isotope signatures of early season wood in New Zealand kauri (*Agathis australis*) tree rings: prospects for palaeoclimatic reconstruction. Dendrochronologia 40:50–63. https://doi.org/10.1016/j.dendro.2016.03.012.

Loucos KE, Simonin KA, Song X, Barbour MM (2015) Observed relationships between leaf H218O Peclet effective length and leaf hydraulic conductance reflect assumptions in Craig-Gordon model calculations. Tree Phys siol 35:16–26. https://doi.org/10.1093/treephys/tpu110.

Medhurst J, Parsby JAN, Linder S, Wallin G, Ceschia, E, Slaney M (2006) A whole-tree chamber system for examining tree-level physiological responses of field-grown trees to environmental variation and climate change. Plant Cell Environ 29:1853–1869.

Novick KA, Ficklin DL, Stoy PC et al. (2016) The increasing importance of atmospheric demand for ecosystem water and carbon fluxes. Nat Clim Change 6:1023–1027. https://doi.org/10.1038/nclimate3114.

Ogee J, Barbour MM, Wingate L et al. (2009) A single-substrate model to interpret intra-annual stable isotope signals in tree-ring cellulose. Plant Cell Environ 32:1071–1090. https://doi.org/10.1111/j.1365-3040.2009.01989.x.

Salati E, Dall'Olio A, Matsui E, Gat JR, Dall'Olio A, Matsui E, Gat JR (1979) Recycling of water in the Amazon basin: an isotopic study. Water Resour Res 15:1250–1258. https://doi.org/10.1029/WR015i005p01250.

Simonin KA, Roddy AB, Link P, Apodaca R, Tu KP, Hu JIA, Dawson TE, Barbour MM (2013) Isotopic composition of transpiration and rates of change in leaf water isotopologue storage in response to environmental variables. Plant Cell Environ 36:2190–2206. https://doi.org/10.1111/pce.12129.

Soden BJ, Held IM (2006) An assessment of climate feedbacks in coupled atmosphere–ocean models. J Clim 19:3354–3360. https://doi.org/10.1175/JCLI3799.1.

Song X, Farquhar GD, Gessler A, Barbour MM (2014) Turnover time of the non-structural carbohydrate pool influences $\delta^{18}O$ of leaf cellulose. Plant Cell Environ 37:2500–2507. https://doi.org/10.1111/pce.12309.

Song X, Loucos KE, Simonin KA, Farquhar GD, Barbour MM (2015*a*) Measurements of transpiration isotopologues and leaf water to assess enrichment models in cotton. New Phytol 206:637–646. https://doi.org/10.1111/nph.13296.

Song X, Simonin KA, Loucos KE, Barbour MM (2015*b*) Modelling non-steady-state isotope enrichment of leaf water in a gas-exchange cuvette environment. Plant Cell Environ 38:2618–2628. https://doi.org/10.1111/pce.12571.

Yepez EA, Scott RL, Cable WL, Williams DG (2007) Intraseasonal variation in water and carbon dioxide flux components in a semi-arid riparian woodland. Ecosystems 10:1100–1115. https://doi.org/10.1007/s10021-007-9079-y.

Zwieniecki MA, Brodribb TJ, Holbrook NM (2007) Hydraulic design of leaves: insights from rehydration kinetics. Plant Cell Environ 30:910–921. https://doi.org/10.1111/j.1365-3040.2007.001681.x.