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Identifying drivers of leaf water and cellulose stable isotope enrichment in *Eucalyptus* in northern Australia

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Abstract Several previous studies have investigated the use of the stable hydrogen and oxygen isotope compositions in plant materials as indicators of palaeoclimate. However, accurate interpretation relies on a detailed understanding of both physiological and environmental drivers of the variations in isotopic enrichments that occur in leaf water and associated organic compounds. To progress this aim we measured \(\delta^{18}O\) and \(\delta^{2}H\) values in eucalypt leaf and stem water and \(\delta^{18}O\) values in leaf cellulose, along with the isotopic compositions of water vapour, across a north-eastern Australian aridity gradient. Here we compare observed leaf water enrichment, along with previously published enrichment data from a similar north Australian transect, to Craig–Gordon modelled predictions of leaf water isotopic enrichment. Our investigation of model parameters shows that observed \(\delta^{18}O\) enrichment across the aridity gradients is dominated by the relationship between atmospheric and internal leaf water vapour pressure while \(\delta^{2}H\) enrichment is driven mainly by variation in the water vapour–source water isotopic disequilibrium. During exceptionally dry and hot conditions (RH < 21%, \(T > 37^\circ C\)) we observed strong deviations from Craig–Gordon predicted isotope enrichments caused by partial stomatal closure. The atmospheric–leaf vapour pressure relationship is also a strong predictor of the observed leaf cellulose \(\delta^{18}O\) values across one aridity gradient. Our finding supports a wider applicability of leaf cellulose \(\delta^{18}O\) composition as a climate proxy for atmospheric humidity conditions during the leaf growing season than previously documented.

Keywords Isotope modelling · Aridity gradient · Source water · Water vapour · Climate proxy

Introduction

Water within leaves of terrestrial plants becomes enriched in heavy isotopes of oxygen and hydrogen during the process of transpiration, with the extent of enrichment being controlled by both the plant’s environment and its physiology (Dongmann et al. 1974; Flanagan et al. 1991; Roden and Ehleringer 1999; Farquhar and Cernusak 2005; Cernusak et al. 2016). A record of the leaf water isotopic composition is integrated into plant organic materials, such as cellulose, along with the source water signal. Analyses of isotope ratios in these organic materials can therefore be useful for reconstructing features of the environment in which the plant grew, and in some cases, physiological characteristics of the plant. Some potential applications are palaeoclimate reconstruction (McCarroll and Loader...
2004), reconstructing physiological traits for plant breeding (Barbour et al. 2000), and assigning geographic origins to plant-derived products (Gori et al. 2015).

Analysis of plant cellulose for its $^{18}\text{O}/^{16}\text{O}$ composition for the purpose of climate reconstruction has most frequently focussed on wood in tree rings (e.g. Treydte et al. 2006). However, in Australia and other tropical climates palaeoclimate studies using tree rings are often hampered by non-ideal ring structures (Zuidema et al. 2013; Haines et al. 2016). Applications in ecophysiology and agriculture have also taken advantage of leaf cellulose (e.g. Barbour et al. 2000; Cernusak et al. 2004) and in some cases grain material (e.g. Ferrio et al. 2007). Although leaf organic material is not preserved in a time-structured way to the same extent that tree rings are, it could still prove helpful for understanding climatic variations through time and space. For example, herbarium samples can be analysed to represent leaves that have grown over the last one to two centuries (Bonal et al. 2011), and leaf macrofossils could yield organic material amenable to $^{18}\text{O}$ analysis from much older deposits (Kennedy et al. 2002; Holmgren et al. 2003). In terms of its relationship to leaf water, leaf cellulose may be a more faithful recorder than wood cellulose, because processes associated with trafficking and translocation of sugars which could impart isotopic fractionations are less prominent (Offermann et al. 2011). For example, Kahmen et al. (2011) found that leaf cellulose had a stronger relationship with climatic conditions than did stem wood cellulose for *Meterosideros polymorpha* trees growing in Hawaii.

Here, we tested how well bulk leaf water isotopic enrichment in eucalypts, a wide-spread group of Australian trees, could be predicted with the steady-state Craig–Gordon model across aridity gradients in northern Australia; and we investigated relationships between observed bulk leaf water isotopic enrichment and relative humidity, VPD, and the source water/air vapour isotopic disequilibrium. We also investigated how well observed and predicted leaf cellulose $^{18}\text{O}$ values agreed across one aridity gradient and tested whether the relationship observed by Kahmen et al. (2011) between leaf cellulose enrichment in $^{18}\text{O}$ and VPD on an oceanic island would also hold for climate gradients on the continental margin of Australia.

**Modelling of leaf water isotope enrichment**

The leaf water isotopic composition is influenced firstly by the plant’s source water, and secondly by the enrichment associated with transpiration. During transpiration, liquid water evaporates from the cell walls that line the sub-stomatal cavities, and the vapour diffuses through the stomatal pores to the atmosphere. There are isotopic fractionations associated with the phase change from liquid to vapour and with the diffusion of vapour. The leaf water isotopic composition is also influenced by the isotopic composition of the vapour in the atmosphere outside the leaf, because this vapour diffuses into the leaf through the same pathway as that diffusing out of the leaf. Under steady-state conditions, the influence of the above processes on leaf water stable isotope enrichment can be predicted using the Craig–Gordon model (Craig and Gordon 1965). This model can therefore also serve as a useful guide for interpreting variations in the $^{18}\text{O}$ and $^{2}H$ of plant organic materials (Saurer et al. 1997; Roden et al. 2000; Barbour 2007).

The Craig–Gordon model, originally written to describe evaporative enrichment of a freely evaporating water body, has been subsequently modified for application to leaves (Dongmann et al. 1974; Flanagan et al. 1991; Farquhar and Lloyd 1993). It can be expressed as

$$
\Delta_{e} \approx \varepsilon + \varepsilon_{k} + (\Delta_{vapour-source}) \frac{e_{a}}{e_{i}},
$$

(1a)

where $\Delta_{e}$ is the enrichment of evaporative site water above source water, $\varepsilon$ is the equilibrium fractionation that occurs between liquid water and water vapour, $\varepsilon_{k}$ is the kinetic fractionation that occurs during diffusion of vapour through the stomata and the boundary layer, $\Delta_{vapour-source}$ is the isotope enrichment of vapour in the atmosphere surrounding the leaf compared to source water, and $(\frac{e_{a}}{e_{i}})$ is the ratio of the atmospheric vapour pressure to the vapour pressure inside the leaf. The $\Delta_{e}$ and $\Delta_{vapour-source}$ are calculated with respect to source water as $\Delta_{s} = (x_{s} - x_{source})(1 + x_{source})$, where subscript $s$ refers to $^{18}\text{O}$ or $^{2}H$ of evaporative site water ($e_{s}$) or atmospheric vapour, respectively. Because the $^{18}\text{O}$ and $^{2}H$ of atmospheric vapour are generally lower than corresponding values for source water, $\Delta_{vapour-source}$ typically has a negative value, whereas $\Delta_{e}$ typically has a positive value indicating isotopic enrichment of leaf water compared to source water.

Equation 1 can be rewritten to isolate the effect of the isotopic disequilibrium between atmospheric vapour and source water. The term $\varepsilon + \Delta_{vapour-source}$ in Eq. 1b below represents this disequilibrium:

$$
\Delta_{e} \approx (\varepsilon + \varepsilon_{k}) (1 - \frac{e_{a}}{e_{i}}) + (\varepsilon + \Delta_{vapour-source}) \frac{e_{a}}{e_{i}}
$$

(1b)

When atmospheric vapour is in equilibrium with source water, the term $\varepsilon + \Delta_{vapour-source}$ will be equal to zero. In this case, the leaf water isotopic enrichment will be primarily controlled by the relative humidity term $(\frac{e_{a}}{e_{i}})$. Kahmen et al. (2011) suggested that the leaf-to-air vapour pressure...
difference (VPD) could be a parameter that would be particularly useful to retrieve from the plant cellulose isotopic composition, because it plays an important role in controlling stomatal conductance and evapotranspiration (Oren et al. 1999). The VPD is equal to \((e_i - e_v)\), and when leaf temperature is equal to air temperature, VPD is equivalent to the vapour pressure deficit of the air. The VPD can be explicitly denoted in Eq. 1 as follows:

\[
\Delta e \approx \left( \epsilon^+ + \epsilon_k \right) \left( \frac{V_{\text{PD}}}{e_i} \right) + \left( \epsilon^+ + \Delta_{\text{vapour-source}} \right) \frac{e_i}{e_i} \quad (1c)
\]

The intercellular vapour pressure, \(e_i\), is controlled by leaf temperature, which itself is largely controlled by air temperature (Campbell and Norman 1998). Thus, Eq. 1c shows that when atmospheric vapour is in equilibrium with source water and when temperature is relatively invariant, leaf water isotopic enrichment should relate closely to VPD, when in the steady state.

We hypothesised that the source water/air vapour isotopic disequilibrium would play a more prominent role in determining leaf water \(\delta^2\text{H}\) than \(\delta^{18}\text{O}\), because \(\epsilon^+\) is large compared to \(\epsilon_k\) for \(\delta^2\text{H}\), whereas the opposite is true for \(\delta^{18}\text{O}\). This suggests that steady-state leaf water enrichment should be driven more by the equilibrium isotope effect for \(\delta^2\text{H}\); in contrast, it should be driven more by the kinetic isotope effect for \(\delta^{18}\text{O}\).

Materials and methods

Field sites

Five sampling sites were situated along a 120-km-long NE to SW transect stretching from the coastline across the Great Dividing Range (maximum elevation \(\approx 1600\) m) to the inland savanna country dominated by eucalypt woodlands (ESM Fig. 1). The local climate is humid tropical along the coast, transitioning rapidly inland to wet/dry tropical. There is a pronounced wet season between December and May and mean annual rainfall decreases from 2001 mm year\(^{-1}\) at Cairns to 818 mm year\(^{-1}\) at Mt Garnet (BOM 2015). The annual mean maximum/minimum \(T\) ranges from \(\approx 29/21\) °C at Cairns to \(\approx 31/16\) °C at Mt Garnet, while the 9 am/3 pm RH ranges from \(\approx 72/62\)% at Cairns to \(\approx 62/37\)% at Mt Garnet (BOM 2015).

Sampling

Leaf and stem samples were collected from the same trees during two field campaigns in the 2014 wet (April) and dry (October) seasons (Table 1). In April \(T_{\text{air}}\) (25 to 32 °C) and RH (72 to 52%) varied less along the Cairns to Mt Garnet transect than in October when stronger gradients were recorded (\(T_{\text{air}} = 28\) to 40 °C; RH = 71 to 14%) (Table 2). There were also strong gradients in the 3-month antecedent rainfall from the coast inland (1138 to 408 mm in April; 46 to 22 mm in October). The weather conditions during sampling were dry and sunny with 1–2 okta cloud cover with the exception of the Tinaroo October sampling when cloud cover increased from 1 okta in the morning to 8 okta by mid-afternoon. All selected trees were confirmed to belong to the genus *Eucalyptus* based on leaf and bark morphology; availability of flower buds and seed capsules at two sites allowed for species determination. Small branches were removed from a height of 3–5 m. Leaves were sampled with the mid-vein included and weighed (0.5–0.7 g). Sections of stems of approx. 3–5 mm diameter and weighing 0.5–0.7 g with bark and phloem tissue included were sampled from 5 to 10 cm below the first leaf of each branch. All samples were quickly placed in double zip-lock plastic bags with most air excluded and frozen within 3 h. A total of 144 leaf and 44 stem samples were sampled.

Leaf traits

Thawed leaf samples were photographed and leaf surface area was determined using ImageJ 1 software (Rasband 1997–2016). As part of the water isotope analysis (see below) leaf and stem water contents were determined by weighing samples before and after water extraction. Specific leaf area (SLA) was calculated as the surface area-to-dry weight ratio.

Leaf and stem water \(\delta^{18}\text{O}\) and \(\delta^2\text{H}\) values

Leaf and stem water isotope analysis was carried out using microwave extraction—isotope ratio infrared spectroscopy (ME-IRIS, Munksgaard et al. (2014)). This system extracts water from leaf or stem samples within a flow-through vessel placed within a microwave oven, with the resulting air stream then passed to a Picarro L2130-i wavelength-scanned cavity ring-down spectrometer for real-time isotopic analysis. Calibration of \(\delta^{18}\text{O}\) and \(\delta^2\text{H}\) values to the VSMOW scale was carried out by analysis of secondary water standards. Isotope data precision was typically 0.3‰ for \(\delta^{18}\text{O}\) and 2‰ for \(\delta^2\text{H}\) (1SD). A Micro Combustion Module (MCM) was added to the ME-IRIS system to reduce possible organic interference of the isotope measurements (details in ESM-1). Examination of spectral interference measures (Munksgaard et al. 2014) indicated that all analyses were interference free.
Atmospheric water vapour $\delta^{18}O$ and $\delta^2H$ values

A Picarro L2130-i water isotope analyser was used to obtain continuous water vapour $\delta^{18}O$ and $\delta^2H$ data over 24-h periods during both sampling campaigns at Cairns, Tinaroo and Mt Garnet. Fluorinated ethylene propylene tubing was used to convey ambient air from a sheltered location 2 m above the ground to the analyser. The isotope data was scaled to VSMOW using vapour derived from the same secondary water standards used to scale the leaf and stem water isotope data. The water standards were quantitatively converted to water vapour using an LGR Water Vapour Isotope Standard Source connected to the analyser. Isotope data precision when analysing a constant vapour source was typically $<0.1‰$ for $\delta^{18}O$ and $<0.2‰$ for $\delta^2H$ (1SD) at a 30-s integration time.

Leaf cellulose $\delta^{18}O$ values

Nine (April) and six (October) microwave dried leaf samples from each tree at Cairns, Tinaroo and Mt Garnet were combined and pulsed and cellulose was extracted using the standard Brendel method modified for small samples (Brendel et al. 2000). Values for $\delta^{18}O$ of cellulose were measured using a ThermoScientific High Temperature Conversion Elemental Analyser coupled via a ConFloIV to a ThermoScientific Delta V PLUS IRMS at James Cook University. Repeat analyses of a cellulose secondary standard was used to monitor the accuracy of repeated analyses of the cellulose standard.

Table 1  Field site and sampling details

<table>
<thead>
<tr>
<th>Site, elevation coordinates</th>
<th>Wet season Date, time (h)</th>
<th>Dry season Date, time (h)</th>
<th>Tree species, height</th>
<th>No of trees sampled</th>
<th>No leaf/stem samples each time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cairns, 10 m 16°47.41’ 145°41.65’</td>
<td>April 6 2014 11,13,15</td>
<td>Oct 7 2014 11,15</td>
<td>Eucalyptus spp. 12–15 m</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tinaroo, 680 m 17°10.32’ 145°32.61’</td>
<td>April 7 2014 11,13,15</td>
<td>Oct 8 2014 13,15</td>
<td>E. tindaliae 8–10 m</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Herberton, 870 m 17°20.47’ 145°25.21’</td>
<td>April 8 2014 11</td>
<td>Oct 9 2014 11</td>
<td>E. tindaliae 12 m</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Wild River, 650 m 17°38.96’ 145°16.94’</td>
<td>April 8 2014 14</td>
<td>Oct 9 2014 12</td>
<td>Eucalyptus spp. 6 m</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Mt Garnet, 670 m 17°40.19’ 145°06.29’</td>
<td>April 9 2014 11,13,15</td>
<td>Oct 9 2014 13,15</td>
<td>Eucalyptus spp. 6–10 m</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2  Atmospheric water vapour $\delta^{18}O$ and $\delta^2H$ values, air temperature (T), relative humidity (RH) and 3-month antecedent rainfall during sampling campaigns in April and October 2014

<table>
<thead>
<tr>
<th>Site</th>
<th>$\delta^{18}O_{vapour}$ (‰)</th>
<th>$\delta^2H_{vapour}$ (‰)</th>
<th>Air T (°C)</th>
<th>RH (%)</th>
<th>Antecedent rainfall (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cairns</td>
<td>−11.0 to −11.6</td>
<td>−71.5 to −74.8</td>
<td>29</td>
<td>67 to 72</td>
<td>1138</td>
</tr>
<tr>
<td>Tinaroo</td>
<td>−11.7 to −12.7</td>
<td>−76.0 to −81.0</td>
<td>29 to 31</td>
<td>59 to 62</td>
<td>526</td>
</tr>
<tr>
<td>Herberton</td>
<td>No data</td>
<td>No data</td>
<td>25</td>
<td>63</td>
<td>510</td>
</tr>
<tr>
<td>Wild River</td>
<td>No data</td>
<td>No data</td>
<td>31</td>
<td>52</td>
<td>No data</td>
</tr>
<tr>
<td>Mt Garnet</td>
<td>−12.5 to −12.6</td>
<td>−81.4 to −83.2</td>
<td>27 to 28</td>
<td>64 to 67</td>
<td>408</td>
</tr>
<tr>
<td>October 2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cairns</td>
<td>−10.8 to −11.0</td>
<td>−73.8 to −74.3</td>
<td>28</td>
<td>63 to 64</td>
<td>46</td>
</tr>
<tr>
<td>Tinaroo</td>
<td>−11.1 to −11.2</td>
<td>−74.3 to −74.7</td>
<td>26 to 33</td>
<td>45 to 71</td>
<td>36</td>
</tr>
<tr>
<td>Herberton</td>
<td>No data</td>
<td>No data</td>
<td>34</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>Wild River</td>
<td>No data</td>
<td>No data</td>
<td>38</td>
<td>24</td>
<td>No data</td>
</tr>
<tr>
<td>Mt Garnet</td>
<td>−12.2 to −13.8</td>
<td>−84.1 to −94.2</td>
<td>37 to 40</td>
<td>14 to 21</td>
<td>22</td>
</tr>
</tbody>
</table>

and reference material IAEA 601 yielded a precision better than 0.4‰.

Air temperature (T\text{air}), relative humidity (RH) and rainfall

T\text{air} and RH were logged continuously throughout both field campaigns at 5 min intervals using an Easylog EL-USB-2-LCD. Rainfall data for 2014 was obtained from Cairns, Tinaroo, Herberton and Mt Garnet meteorological observation stations (BOM 2015).

Data treatment

Statistical comparisons of leaf traits and leaf water isotope ratios between different sites and seasons were carried out for sites where multiple trees were sampled (Cairns, Tinaroo and Mt Garnet) using single factor analysis of variance (ANOVA).

Craig–Gordon isotope modelling

To calculate predicted leaf isotopic enrichment values Δε (Eq. 1b) we used RH, T\text{air} and vapour δH and δ18O values at the time of sampling. During most sampling days T and RH were relatively stable (±2 °C and ±5% RH) between the hours of 10 am and 4 pm (sampling occurred between 11 am and 3 pm). In the absence of leaf temperature data, T\text{leaf} was estimated from T\text{air} using an empirical relationship between air and leaf temperatures (T\text{leaf} = 0.94 T\text{air} + 0.816, Adj. r² = 0.98), derived from daytime measurements by thermocouple of 222 leaves of both Corymbia and Eucalyptus species in northern Australia (Kahmen et al. 2013; Cernusak et al. 2016). Air vapour pressure (e\text{a}) was calculated from RH and T\text{air}, and leaf vapour pressure (e\text{L}) was calculated assuming saturation at the estimated T\text{leaf}. Values for stomatal (0.49 mol m⁻² s⁻¹) and boundary layer conductance (2.85 mol m⁻² s⁻¹) were derived from Kahmen et al. (2013; Cernusak et al. 2016), resulting in e\text{a} of 26.7 and 23.8 for 18O and 2H, respectively. The e\text{L} was calculated using the temperature-dependent equations of Majoube (1971). To provide a more comprehensive assessment of how well leaf water isotopic enrichment in Eucalyptus in northern Australia can be predicted with the model, we include data from a similar North Australian aridity gradient from Darwin to Alice Springs. This data was obtained in April and September 2010 (Kahmen et al. 2013), but first published in full by Cernusak et al. (2016) and includes environmental, physiological and isotope data for the genera Eucalyptus and Corymbia which are often referred to as ‘eucalypts’ and are morphologically and ecologically indistinct (Parra-O et al. 2006; Kahmen et al. 2013). The Darwin to Alice Springs transect has been relatively well characterised with respect to its climate, vegetation, and stable isotope dynamics (Williams et al. 1997; Schulze et al. 1998; Miller et al. 2001; Hutley et al. 2011; Kahmen et al. 2013; Soper et al. 2015). Mean annual precipitation decreases from about 1700 mm in Darwin to about 300 mm in Alice Springs over a distance of about 1300 km.

Cellulose isotope modelling

The δ18O enrichment of cellulose above source water (Δ18O_{cell}) has been described by Barbour et al. (2004):

\[
\Delta^{18}O_{\text{cell}} = \Delta^{18}O_{\text{air}}(1 - p_{\text{ex}} p_{x}) + \varepsilon_{\text{wc}},
\]

where Δ18O_{\text{c}} is derived from Eq. 1b, p_{\text{ex}} is the proportion of exchangeable oxygen in cellulose formed from sucrose, p_{x} is the proportion of unriched (xylem) water in the cell forming the cellulose and ε_{wc} is the equilibrium fractionation factor between carbonyl oxygen and water (+27‰; Sternberg et al. 1986; Sternberg and Ellsworth 2011). The Δ18O_{\text{c}} values were calculated as detailed above using the means of monthly T\text{air} to calculate T\text{leaf} and the means of RH at 9 am and 3 pm at the nearest meteorological observation sites (Cairns, Atherton and Mt Surprise, BOM 2015) in the assumed leaf flush season of October to December (Williams et al. 1997; Kahmen et al. 2013). Estimates of atmospheric water vapour δ18O values were derived from a nearby parallel transect measured 3 times during 2014 (Cairns to Chillagoe, unpublished data by C. Zwart). These data show a linear relationship between δ18O_{\text{vapour}} values and elevation (δ18O_{\text{vapour}} = −0.0038 (m) −12.81, r² = 0.64−0.92 for individual transects) which was used to derive δ18O_{\text{vapour}} values for Cairns (−12.86‰), Tinaroo (−15.21‰) and Mt Garnet (−15.17‰) based on elevation (Table 1). The mean October stem water data were used to represent source water δ18O values at Cairns (−4.00‰) and Tinaroo (−5.67‰), but for Mt Garnet we used the mean April δ18O stem water value (−6.03‰) for source water as the October data were likely affected by evaporative enrichment (see “Discussion”). To complete the calculation of predicted 18O enrichment of leaf cellulose (Δ18O_{\text{cell}}, Eq. 2) ε_{wc} was set at +27‰ (Barbour et al. 2004) and a p_{\text{ex}} value of 0.38 was derived from Eucalyptus globulus data (Cernusak et al. 2005). Finally, predicted δ18O_{\text{cell}} values were derived from Δ18O_{\text{cell}} values by accounting for source water δ18O values at each sampling site.
Results and discussion

Isotopic composition of atmospheric water vapour

During the two leaf-sampling campaigns, daily average water vapour $\delta^{18}O$ and $\delta^2H$ values decreased inland from the coast by up to 1.6 and 11.7‰, respectively, in April and by up to 3.0 and 20.4‰, respectively, in October. These trends are similar to gradients in water vapour isotopic compositions recorded along the nearby Cairns to Chilla-goe transect, which have been shown to correlate significantly with land elevation (C. Zwart, unpublished data).

Isotopic composition of precipitation and source water

During 2014 the prevailing weather patterns in the study area influenced $\delta^{18}O$ and $\delta^2H$ values in rainfall mirroring the systematic relationship between air mass trajectories and the isotopic composition of precipitation previously identified by (Munksgaard et al. 2012). This relationship, combined with a diminishing amount of south-easterly derived rainfall in the rain shadow west of the coastal mountain range (ESM Fig. 1) means that north-westerly sourced rainfall increasingly dominate the isotopic composition of rainfall along the transect from Cairns to Mt Garnet.

Daily $\delta^{18}O$ and $\delta^2H$ values in rainfall were measured at Cairns as part of an International Atomic Energy Agency Cooperative Research Program (N.C. Munksgaard and C. Zwart unpublished data) throughout 2014. The 848 mm of rainfall originating mainly from the southeast over 43 rain days had amount-weighted $\delta^{18}O$ and $\delta^2H$ values of $-2.9$ and $-6.4$‰, respectively, while the 397 mm of rainfall (13 rain days) arriving from the northwest had corresponding values of $-6.1$ and $-33.8$‰, respectively. In comparison, during the 3 months prior to the October leaf sampling (46 mm of rainfall from the southeast over 14 rain days), the amount-weighted mean isotopic values for $\delta^{18}O$ and $\delta^2H$ were $-1.4$ and $+9.5$‰, respectively, with no recorded rainfall from north-westerly directions.

The isotopic composition of stem water (xylem water) of most plants matches that of the available soil water, indicating that there is little to no isotope fractionation associated with absorption of water by roots (Ehleringer and Dawson 1992; Dawson 1993). Consequently, stem water is often used to represent plant source water ultimately derived from precipitation. The regional east–west trend of decreasing isotopic values in precipitation is reflected in the majority of $\delta^{18}O$ and $\delta^2H$ values of stem waters measured in the present study (Fig. 1). Stem water $\delta$-values at Cairns, Tinaroo and Herberton were shifted towards lower $\delta^{18}O$ and $\delta^2H$ values in the dry season (October) compared to the wet season (April). We interpret this seasonal change as a change in water source from near-surface soil water in April, dominated by recent south-easterly rainfall with relatively high $\delta$-values, to a deeper and older ground water source in October with lower $\delta$-values. In most wet seasons the mean annual rainfall includes a significant contribution of isotopically depleted monsoonal rainfall from north-westerly directions, but this component was uncharacteristically diminished in coastal areas in early 2014. The higher elevation and further inland position of Tinaroo and Herberton explains their lower stem water $\delta$-values compared to Cairns.

The isotopically depleted stem water compositions recorded in April at Wild River and Mt Garnet on the western slopes of the coastal range are consistent with soil water being derived mainly from isotopically depleted monsoonal rainfall at these two sites. In contrast to the seasonal trends recorded at the three sites closest to the coast, the Wild River and Mt Garnet stem water $\delta$-values increased from April to October. There was no significant precipitation between April and October and it is possible that stem transpiration effects (Dawson and Ehleringer 1993) or evaporative enrichment of soil water (Allison et al. 1983) was responsible for the isotopic shift in stem waters from April to October.

Fig. 1 Mean leaf and stem water $\delta^{18}O$ and $\delta^2H$ values for each tree at each sampling time across the Cairns to Mt Garnet aridity gradient. The amount-weighted local meteoric water line (LMWL) for rainfall in Cairns was calculated according to Crawford et al. (2014) for Feb 2014 to Feb 2015 and is shown along with the mean isotope values in rainfall arriving from the southeast (plus sign) and the northwest (cross sign) during the wet season Jan–April 2014.
Leaf traits and isotopic composition of leaf and stem water

Given the climatic variation along the Cairns to Mt Gar- net transect, it is not surprising that the leaf and stem traits and water stable isotope compositions showed variation among sites and in some cases between seasons (Table 3; Fig. 1, data provided in ESM Table 1). Analyses of vari- ance (ANOVA, ESM Tables 2 and 3) show that leaves varied significantly (P < 0.05) between sites in both April and October with respect to water content, SLA, δ¹⁸O and δ²H values. Comparisons of seasonal data for each site show that leaf water content varied significantly at Cairns and Tinaroo but not at Mt Garnet, δ¹⁸O values and SLA varied significantly at all three sites, but δ²H values were signifi- cantly different only at Mt Garnet. Stem water content var- ied significantly between seasons only at Tinaroo, but δ¹⁸O and δ²H values in stem water varied significantly between the two seasons at all three sites.

Although substantial differences exist between species and contrasting environments, physiological responses by euca- lypts to water deficit can include reduction in leaf water con- tent and SLA (Ngugi et al. 2003; Merchant et al. 2007). In the present study, the most significant decreases in leaf water content between the April (wet season) and October (dry sea- son) samples were recorded for trees at Cairns and Tinaroo; whereas SLA at all sites, with the exception of Wild River, decreased from April to October. This seasonal decrease in SLA is consistent with previous observations (Prior et al. 2004; Nouvellon et al. 2010), and provides further support for a general trend toward decreasing SLA in response to water deficit in eucalypts. Seasonal decreases in SLA could involve accumulation of dry matter, potentially as a result of osmotic adjustment, under dry conditions; changes in leaf structural properties, such as cell wall thickness, in leaves produced under dry conditions; or a shift in leaf demography under dry conditions toward a larger proportion of older, low SLA leaves in the canopy due to limited flushing of new leaves.

Table 3 Leaf and stem traits (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Site</th>
<th>Leaf water content (%wt)</th>
<th>Stem water content (%wt)</th>
<th>SLA (cm² g⁻¹ dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cairns (n = 27)</td>
<td>51.5 ± 7.1</td>
<td>42.6 ± 2.3</td>
<td>76.7 ± 16.3</td>
</tr>
<tr>
<td>Tinaroo (n = 27)</td>
<td>50.3 ± 3.0</td>
<td>47.3 ± 3.1</td>
<td>60.2 ± 7.2</td>
</tr>
<tr>
<td>Herberton (n = 3)</td>
<td>44.2 ± 3.6</td>
<td>40.4 ± 1.4</td>
<td>59.5 ± 2.2</td>
</tr>
<tr>
<td>Wild River (n = 3)</td>
<td>46.4 ± 1.1</td>
<td>47.1 ± 4.1</td>
<td>62.4 ± 3.7</td>
</tr>
<tr>
<td>Mt Garnet (n = 27)</td>
<td>48.0 ± 4.6</td>
<td>42.2 ± 3.6</td>
<td>86.5 ± 14.2</td>
</tr>
<tr>
<td>October 2014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cairns (n = 18)</td>
<td>44.5 ± 4.6</td>
<td>40.6 ± 1.6</td>
<td>59.1 ± 12.0</td>
</tr>
<tr>
<td>Tinaroo (n = 18)</td>
<td>46.1 ± 3.1</td>
<td>41.3 ± 2.9</td>
<td>55.5 ± 7.0</td>
</tr>
<tr>
<td>Herberton (n = 3)</td>
<td>41.9 ± 6.4</td>
<td>40.9 ± 3.4</td>
<td>53.5 ± 3.3</td>
</tr>
<tr>
<td>Wild River (n = 3)</td>
<td>53.8 ± 2.3</td>
<td>48.8 ± 2.8</td>
<td>76.2 ± 8.4</td>
</tr>
<tr>
<td>Mt Garnet (n = 18)</td>
<td>50.0 ± 3.7</td>
<td>43.0 ± 2.6</td>
<td>74.6 ± 9.0</td>
</tr>
</tbody>
</table>

Three leaf samples were combined from each tree for analysis of the composite sample.

Fig. 2 Predicted and observed a δ¹⁸O and b δ²H enrichment of leaf water in eucalypts across the Cairns to Mt Garnet aridity gradient. Background data represent previously published data from a similar Eucalypt transect from Darwin to Alice Springs (Cernusak et al. 2016).
Leaf water $^{18}$O and $^2$H enrichment

The coefficients of determination (adjusted $r^2$ values) for Craig–Gordon-modelled enrichment values of $^{18}$O and $^2$H ($\Delta_{v}$) with respect to observed enrichment values ($\Delta_{e}$) for the combined datasets (Fig. 2) were 0.80 and 0.90, respectively (Table 4). Although the linear fit is reduced if the Cairns to Mt Garnet transect data is assessed on its own (mainly for $\Delta^{18}$O, less so for $\Delta^2$H), it is noteworthy that the model captures a reasonable amount of the observed variation in spite our simple parameterisation. However, the model mostly overestimated $^{18}$O and $^2$H enrichment for the Cairns to Mt Garnet transect (mean difference between modelled and observed $^{18}$O and $^2$H was 4.0 and 16.3‰, respectively) and in particular for the Mt Garnet data presented (0.5‰ in $^{18}$O and 12.0‰ in $^2$H) or a manifestation of the 'Péclet' pools’ model, (e.g. Yakir et al. 1990; Roden and Ehleringer 1999; Song et al. 2015) or a manifestation of the ‘Péclet’ model where advection of less enriched water by the transpiration stream opposes the back-diffusion of isotopically enriched water from the evaporative sites (Farquhar and Lloyd 1993; Farquhar and Gan 2003; Barnes et al. 2004).

The large degree of overestimation of isotopic enrichment in the Mt Garnet leaf samples in October suggest a departure from steady-state conditions. Similar large departures (by 10 to 15‰) from Craig–Gordon predicted $^{18}$O enrichment has been observed by Saurer et al. (2016) in Siberian larch trees at low relative humidity ($\approx$ 20%). Steady-state conditions require free gas exchange between the sub-stomatal sites of evaporation and the atmosphere and is an underlying assumption of the Craig–Gordon model in the form expressed by Eqs. 1b and 1c (Cernusak et al. 2016). Very low relative humidity (RH = 14 to 21%) and high temperature ($T$ = 37 to 40 $^\circ$C) at Mt Garnet in October is likely to have induced varying degrees of stomata closure to limit water loss, thereby constraining free gas exchange and reducing water isotope enrichment. An empirical examination of the data collected for this paper suggests a threshold value for relative humidity of about 25%, below which notable departures from steady state might be expected (Fig. 3). However, a more detailed consideration of the transition from steady state to non-steady state would take into account the openness of the stomata, the leaf water content (degree of succulence), the vapour concentration inside the leaf, and the rate at which environmental conditions are changing (Farquhar and Cernusak 2005; Cernusak et al. 2008). Therefore, the possibility of a general threshold of relative humidity that could be expected to result in marked non-steady-state behaviour requires further empirical testing.

In order to identify drivers of leaf water isotopic enrichment we investigate linear correlations between observed enrichment values and the main parameters of the

### Table 4 Linear coefficients between observed leaf water isotopic enrichment and both modelled values and various environmental factors and between vapour–source disequilibrium coefficients and both $\delta_{\text{source}}$ and $\delta_{\text{vapour}}$ values

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Slope</th>
<th>Intercept</th>
<th>Adj. $r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta^{18}$O$_{e}$</td>
<td>1.05 ± 0.03</td>
<td>−1.32 ± 0.62</td>
<td>0.80</td>
</tr>
<tr>
<td>(\text{CAR})</td>
<td>−37.6 ± 1.3</td>
<td>36.8 ± 0.7</td>
<td>0.74</td>
</tr>
<tr>
<td>VPD</td>
<td>4.66 ± 0.31</td>
<td>7.51 ± 0.73</td>
<td>0.46</td>
</tr>
<tr>
<td>$\Delta^{18}$O$_{vapour-source}$</td>
<td>1.21 ± 0.15</td>
<td>26.7 ± 1.2</td>
<td>0.20</td>
</tr>
<tr>
<td>$\delta^{18}$O$_{vapour}$</td>
<td>1.09 ± 0.15</td>
<td>16.0 ± 0.5</td>
<td>0.17</td>
</tr>
<tr>
<td>$\Delta^{2}$H$_{e}$</td>
<td>1.12 ± 0.02</td>
<td>−19.4 ± 1.5</td>
<td>0.90</td>
</tr>
<tr>
<td>(\text{CAR})</td>
<td>−94.0 ± 5.8</td>
<td>99.3 ± 3.2</td>
<td>0.49</td>
</tr>
<tr>
<td>VPD</td>
<td>10.3 ± 1.1</td>
<td>29.1 ± 2.7</td>
<td>0.24</td>
</tr>
<tr>
<td>$\Delta^{2}$H$_{vapour-source}$</td>
<td>0.89 ± 0.04</td>
<td>89.7 ± 1.8</td>
<td>0.68</td>
</tr>
<tr>
<td>$\delta^{2}$H$_{vapour}$</td>
<td>0.83 ± 0.04</td>
<td>24.9 ± 1.47</td>
<td>0.64</td>
</tr>
<tr>
<td>$\delta^{18}$O$_{source}$</td>
<td>0.79 ± 0.03</td>
<td>12.7 ± 0.5</td>
<td>0.70</td>
</tr>
<tr>
<td>$\delta^{2}$H$_{source}$</td>
<td>−0.22 ± 0.10</td>
<td>−0.01 ± 0.71</td>
<td>0.01</td>
</tr>
<tr>
<td>$\delta^{2}$H$_{vapour}$</td>
<td>0.81 ± 0.03</td>
<td>110 ± 3</td>
<td>0.67</td>
</tr>
</tbody>
</table>

The significance ($P$) of adjusted $r^2$ values are indicated by bold type for $P < 0.001$ and italicised type for $P < 0.05$.
Craig–Gordon model for the Cairns to Mt Garnet and Darwin to Alice Springs datasets: (1) the ratio of atmospheric 
vapour pressure to internal leaf vapour pressure \((e_a/e_i)\); (2) leaf-to-air vapour pressure difference (VPD) and (3) 
the isotopic disequilibrium between atmospheric water 
vapour and source water \((\Delta (vapour-source))\). Figure 3 and 
Table 4 show that the influence of atmospheric conditions 
upon bulk leaf water isotopic enrichment, while significant 
for both \(\Delta^{18}O_L\) and \(\Delta^{2}H_L\), is substantially stronger for 
\(\Delta^{18}O_L\) \((r^2 = 0.74\) for \((e_a/e_i), r^2 = 0.47\) for VPD) than for 
\(\Delta^{2}H_L\) \((r^2 = 0.49\) for \((e_a/e_i), r^2 = 0.24\) for VPD). The dif-
ference in observed \(\Delta^{18}O_L\) and \(\Delta^{2}H_L\) values between the 
two transects at lower \((e_a/e_i)\) and higher VPD values can be 
explained by the non-steady state of the Mt Garnet October 
samples at low RH (14–21%). In contrast, RH were higher 
during sampling at the two driest sites along the Darwin
to Alice Springs transect (average RH = 27 and 36% at Elliot and Tennant Creek, respectively), and measured stomatal conductance (Cernusak et al. 2016) remained high at all sites indicating that the trees had ample soil water as they continued to transpire freely. This implies that atmospheric vapour conditions as well as source water availability determines whether non-steady-state conditions occur in arid environment.

In Fig. 4 we show the influence of \( \varepsilon^+ + \Delta_{(vapour-source)} \) conditions upon bulk leaf water isotopic enrichment. The inclusion here of a temperature-dependent fractionation factor \( \varepsilon^+ \) is convenient to assess equilibrium conditions \( \varepsilon^+ + \Delta_{(vapour-source)} = 0 \) at isotopic equilibrium. Coefficients of determination are significant for both \( \Delta^{18}O_L \) and \( \Delta^2H_L \) but are substantially stronger for \( \Delta^2H_L \) \( (r^2 = 0.64) \) than for \( \Delta^{18}O_L \) \( (r^2 = 0.17) \). The \( \varepsilon_{18O}^+ + \Delta^{18}O_{(vapour-source)} \) while significantly \( (t_{(269)} = 8.3, P < 0.001) \) different from 0, averages just 1.4‰ while \( \varepsilon_{2H}^+ + \Delta^2H_{(vapour-source)} \) is both significantly \( (t_{(269)} = 23.5, P < 0.001) \) and substantially (32‰) different from zero. Thus, the \( \Delta^{18}O \) observations are approximately balanced around the vapour–source equilibrium conditions, whereas \( \Delta^2H \) values are predominantly more positive than the vapour–source equilibrium conditions. Figure 5 shows that \( \varepsilon^+ + \Delta_{(vapour-source)} \) values are mainly driven by variation in vapour rather than source water isotopic compositions.

The assessment of leaf water isotopes in eucalypts along the aridity transects shows that \( ^{18}O \) enrichment is dominated by the atmospheric water vapour conditions \( (\varepsilon_{18O}^+) \) is the strongest predictor). In contrast, \( ^2H \) enrichment is dominated by variation in vapour–source disequilibrium \( (\Delta_{(vapour-source)} \) is the strongest predictor), which is driven

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**Table 5** Leaf cellulose \( \delta^{18}O \) values (%e) from Cairns, Tinaroo and Mt Garnet (mean ± standard deviation of three trees at each site)

<table>
<thead>
<tr>
<th></th>
<th>April 2014</th>
<th>October 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cairns</td>
<td>29.5 ± 0.9</td>
<td>30.6 ± 1.8</td>
</tr>
<tr>
<td>Tinaroo</td>
<td>27.5 ± 0.6</td>
<td>27.5 ± 0.2</td>
</tr>
<tr>
<td>Mt Garnet</td>
<td>34.5 ± 1.3</td>
<td>35.6 ± 2.3</td>
</tr>
</tbody>
</table>

---

Fig. 5 Partitioning of isotopic disequilibrium \( (\varepsilon^+ + \Delta_{(vapour-source)} \) in enrichment of a, b \( ^2H \) and c, d \( ^{18}O \) between source and water vapour signatures. Background data represent previously published data from a similar transect from Darwin to Alice Spring (Cernusak et al. 2016)
mainly by the isotopic composition of atmospheric vapour
whilst \( \frac{\delta}{\delta_{a}} \) plays a subordinate role. As we hypothesised
this contrasting behaviour of \( ^{18} \)O and \( ^{2} \)H can be explained
by \( e^+ \) being large compared to \( \delta_a \) for \( ^{2} \)H, whereas the oppo-
site is true for \( ^{18} \)O. In turn, this leads to different weight-
ing for \( ^{2} \)H and \( ^{18} \)O of the addends in Eqs. 1b and 1c.

Leaf cellulose \( ^{18} \)O enrichment

Cellulose \( ^{18} \)O data are provided in Table 5, and show a significant difference between sites (\( P < 0.001 \)) but not
between seasons (\( P = 0.66 \)). While predicted and observed
\( ^{18} \)Ocell values across the Cairns to Mt Garnet aridity gra-
dient agree reasonably well (within \( \approx \)) (Fig. 6a) Cairns
and Tinaroo values are under-predicted while Mt Garnet
values are over-predicted suggesting a systematic discrep-
ancy in model parameters. This discrepancy is likely related
to inaccuracies in the estimated values of model parameters
which could not be measured in situ during leaf growth.
Investigation of Eqs. 1b, 1c and 2 shows that humidity \( \frac{\delta}{\delta_{a}} \)
or VPD, the proportion of exchangeable oxygen during cel-
lulose formation from sucrose (\( p_{ex} \)) and the proportion of
unenriched (xylem) water in the cell forming the cellulose
(\( p_{a} \)) exert the most influence on predicted \( ^{18} \)Ocell values
(Fig. 6b). In particular, the values for \( p_{a} \) and \( p_{ex} \) are often
variable and subject to uncertainty (Ellsworth and Stern-
berg 2014; Song et al. 2014). In contrast, temperature and
\( ^{18} \)O values in source water and atmospheric water vapour
are relatively well constrained and/or influence model out-
comes to a lesser degree.

Song et al. (2014) recently argued that a correlation may
exist between \( p_{ex} \) and rainfall amount as water stressed
plants have a fast carbon pool turnover resulting in a lower
probability for hexose molecules to undergo triose cycling
during cellulose synthesis (Barbour and Farquhar 2000).
This means that \( p_{ex} \) values could be lower in the arid condi-
tions prevailing at Mt Garnet compared to the more humid
Cairns and Tinaroo sites. A reduction of the combined terms
\( p_{ex}p_{a} \) from 0.38 (as used to calculate predicted values shown
in Fig. 6a) to \( \approx 0.30 \) would align predicted and observed
\( ^{18} \)Ocell values for the Mt Garnet samples. A \( p_{ex}p_{a} \) value
of 0.30 is within the range of 0.1–0.9 estimated for a range of
plant species by Song et al. (2014).

Our finding that \( ^{18} \)O enrichment in leaf water in north
Australian eucalypts is dominated by the atmospheric water
vapour conditions, suggests that \( ^{18} \)O values in leaf cellulose
may similarly reflect atmospheric water vapour conditions
as leaf water isotope signals are incorporated during leaf
and stem growth. Such a relationship has previously been
observed in myrtle trees along a steep elevation gradient on
an oceanic island where both leaf and stem cellulose \( ^{18} \)O
values were strongly correlated with time-averaged VPD
which integrates the effects of air temperature and atmos-
pheric vapour pressure on the isotopic composition (Kah-
men et al. 2011). Figure 6b shows that VPD is also a strong
predictor \( (r^2 = 0.88) \) of \( ^{18} \)O values in eucalypt leaf cel-
lulose across the Cairns to Mt Garnet aridity gradient. Our
finding shows that in leaves the applicability of cellulose
\( ^{18} \)O values to be used as proxies for past VPD conditions,
as suggested by Kahmen et al. (2011), can be broadened
to eucalypts on the continental margin of north-eastern
Australia.

Conclusions

Leaf and stem water isotopic compositions in eucalypts
along with isotopic measurement of atmospheric water
vapour have been measured across a strong aridity grad-
ient in north-eastern Australia and compared to previously
published data along a similar, but longer, transect in
northern Australia. Our investigation of the drivers of leaf
water isotopic enrichment, as modelled by the Craig–Gor-
don equations, shows that observed \( ^{18} \)O enrichment across
the two aridity gradients is dominated by the relationship between atmospheric and internal leaf water vapour pressure, while $^2$H enrichment is driven mainly by variation in the water vapour–source water isotopic disequilibrium. Atmospheric–leaf vapour conditions are also a strong predictor of $^{18}$O values in eucalypt leaf cellulose across the north-eastern Australian aridity gradient (cellulose data was not available for the north Australian transect). This finding supports a wider applicability of leaf cellulose $^{18}$O data as proxies for atmospheric humidity conditions during the leaf growing season than previously documented.

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Author contribution statement NCM, AWC, LAC conceived and designed the study. NCM performed field work. NCM, NBE performed analytical work. CZ carried out meteorological analyses. NCM, AWC, NBE, CZ, AK, LAC analysed the data. NCM, AWC, LAC wrote the manuscript; other authors provided editorial advice.

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Declaration The authors declare that they have no conflict of interest.

Statement of human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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