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Do \(^2\)H and \(^{18}\)O in leaf water reflect environmental drivers differently?

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Summary

We compiled hydrogen and oxygen stable isotope compositions (\(\delta^2\)H and \(\delta^{18}\)O) of leaf water from multiple biomes to examine variations with environmental drivers. Leaf water \(\delta^2\)H was more closely correlated with \(\delta^2\)H of xylem water or atmospheric vapour, whereas leaf water \(\delta^{18}\)O was more closely correlated with air relative humidity. This resulted from the larger proportional range for \(\delta^2\)H of meteoric waters relative to the extent of leaf water evaporative enrichment compared with \(\delta^{18}\)O. We next expressed leaf water as isotopic enrichment above xylem water (\(\Delta^2\)H and \(\Delta^{18}\)O) to remove the impact of xylem water isotopic variation. For \(\Delta^2\)H, leaf water still correlated with atmospheric vapour, whereas \(\Delta^{18}\)O showed no such correlation. This was explained by covariance between air relative humidity and the \(\Delta^{18}\)O of atmospheric vapour. This is consistent with a previously observed diurnal correlation between air relative humidity and the deuterium excess of atmospheric vapour across a range of ecosystems. We
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Introduction

The stable isotope composition of hydrogen and oxygen in leaf water varies throughout the day, among plants within a site and across environmental gradients (Zundel et al., 1978; Flanagan et al., 1991a; Cernusak et al., 2002, 2016; Lai et al., 2008; West et al., 2008). Leaf water becomes enriched in the heavy isotopes $^{2}$H and $^{18}$O compared with the water entering the roots as a result of evaporative isotopic fractionation during transpiration (Gonfiantini et al., 1965). There is also isotopic exchange between water vapour in the atmosphere and that in the leaf (Craig & Gordon, 1965); notably, this continues even if transpiration has ceased under a saturated atmosphere (Welp et al., 2008; Kim & Lee, 2011; Hellieker, 2014; Goldsmith et al., 2017). Furthermore, the distribution of isotope enrichment within the leaf can vary as a function of leaf anatomy and physiology (Yakir et al., 1989; Gan et al., 2002; Holloway-Phillips et al., 2016; Barbour et al., 2021).

Therefore, the stable isotope composition of leaf water provides an information-rich isotopic signal that can be applied across a broad range of disciplines (Yakir, 1998). Interest in understanding leaf water stable isotope composition has been further motivated by recognition that leaf water is the starting point for isotope signals in plant organic compounds such as sucrose, starch, cellulose, lignin, leaf waxes (Yakir, 1992; Farquhar et al., 1998; Barbour, 2007; Lehmann et al., 2020). Leaf water isotopic signals can even be reflected in the bones and teeth of herbivores, such as kangaroos (Ayliffe & Chivas, 1990; Faith, 2018).

Models of leaf water stable isotope composition have been developed over several decades and typically perform reasonably well at explaining observed leaf water isotopic variation (Dongmann et al., 1974; Flanagan et al., 1991b; Roden & Ehleringer, 1999; Farquhar & Cernusak, 2005; Cuntz et al., 2007; Ogée et al., 2007). However, some questions about subtler aspects of leaf water isotopic composition remain (Cernusak et al., 2016). One such question is whether stable isotopes of hydrogen and oxygen reflect differently the balance of environmental and physiological drivers that lead to variation in leaf water stable isotopes.

Models of leaf water isotopic composition do not differentiate between hydrogen and oxygen in their general formulation; the major mechanisms that cause leaf water to change isotopically are common to both elements. However, the magnitudes of the fractionation factors associated with the mechanisms do differ. This is also true for meteoric waters, such that the relative extent of variation in the isotopic composition of plant source water and atmospheric vapour across the landscape is different between $^{2}$H and $^{18}$O; on average, there is a c. 8% change in $^{2}$H for a given 1% change in $^{18}$O (Craig, 1961; Rozanski et al., 1993). Movement of the two isotopologues $^{2}$H$^{16}$O and $^{2}$H$^{18}$O within the leaf may also vary, for example due to different diffusivities in water (Cuntz et al., 2007), in air (Barbour et al., 2017), and potentially across membranes (Mamonov et al., 2007) and there can be different extents of exchange with organic molecules (Yakir, 1992; Chen et al., 2020). Here, we aimed to assess whether hydrogen and oxygen stable isotopes in leaf water respond differently to the environment, to better understand whether $\delta^{2}$H and $\delta^{18}$O in organic matter proxies capture environmental signals differently.

To do this, we compiled datasets that provided measurements under natural conditions of both $\delta^{2}$H and $\delta^{18}$O in leaf water, xylem water and atmospheric vapour, along with concurrent measurements of air temperature and relative humidity. Table 1 provides a summary of data sources. Within each dataset, we averaged individual observations, such that each row of data in the compiled dataset represents a mean value for a given species by site by time combination. In total, the dataset contained 546 such rows. The geographic range of the combined dataset covered more than 100° of latitude and more than 3000 m of elevation (Table 1). We limited the dataset to daytime observations, as it is primarily during photosynthesis that leaf water signals are incorporated into organic compounds. This also helped to avoid issues of nonsteady state leaf water enrichment at night (Cernusak et al., 2002, 2005; Seibt et al., 2006). We note that it has recently been shown that extraction of stem xylem water for isotopic analysis can be accompanied by an offset in $\delta^{2}$H from the water that is likely to have been taken up by the roots (Zhao et al., 2016; Chen et al., 2020; Barbetta et al., 2022). We did not attempt to apply a correction for this offset as we lacked a basis on which to make the correction that could be applied across the compiled dataset.

$\delta^{2}$H and $\delta^{18}$O of leaf water, xylem water and vapour

The Craig–Gordon equation (Craig & Gordon, 1965) forms the basic building block for models of leaf water isotopic composition and provides a convenient entry point for examining the environmental drivers of leaf water $\delta^{2}$H and $\delta^{18}$O. The Craig–Gordon equation can be approximated as:

$$\delta_i \approx \delta_s + \epsilon^e + \epsilon_k + (\delta_i - \delta_s - \epsilon_k) b$$  \hspace{1cm} \text{Eqn 1}$$

($\delta_i$, predicted $\delta^{2}$H or $\delta^{18}$O at the evaporative sites within leaves; $\delta_s$, $\delta^{2}$H or $\delta^{18}$O of source water, which we equated in our dataset to xylem water; $e^e$, equilibrium fractionation between liquid and vapour; $\epsilon_k$, kinetic fractionation during diffusion through the stomata and boundary layer; $\delta_i$, $\delta^{2}$H or $\delta^{18}$O of atmospheric vapour and $b$, $w_a/w_i$, the water vapour mole fraction in the air outside the leaf boundary layer divided by that at the evaporative sites inside the leaf substomatal cavity). The $w_i$ is typically assumed to be saturated at leaf temperature, although recent evidence has suggested that it may be less than saturated at times (Cernusak et al., 2018, 2019; Buckley & Sack, 2019; Holloway-Phillips et al., 2021). If $w_i$ is saturated and leaf temperature is equal to air...
Table 1 Datasets and associated site information for the data compilation presented in this paper.

| Dataset                      | Site                          | Latitude | Longitude | Elevation (m) | MAP (mm) | MAT (°C) | Vegetation type | References                     |
|------------------------------|-------------------------------|----------|-----------|---------------|----------|----------|-----------------|--------------------------------|
| Western_USA_Roden            | Cascade_Heads                 | 45.03    | −123.91   | 14            | 2410     | 10.7     | Forest          | (Roden & Ehleringer, 2000a,b)  |
|                              | Bill_Williams_River           | 34.26    | −114.03   | 150           | 97       | 23.8     | Woodland        |                                |
|                              | Weber_River                   | 41.13    | −111.90   | 1450          | 510      | 10.6     | Woodland        |                                |
|                              | Red_Butte_Canyon              | 40.78    | −111.80   | 1790          | 700      | 10.1     | Woodland        |                                |
|                              | Big_Cottonwood                | 40.62    | −111.73   | 1987          | 840      | 9.4      | Woodland        |                                |
| Washington_USA_Lai           | Wind_River                    | 45.82    | −121.95   | 371           | 2467     | 8.7      | Forest          | (Lai & Ehleringer, 2011)       |
| Utah_USA_Flanagan            | Coral_Pink                    | 37.04    | −112.72   | 1855          | 380      | 10.5     | Woodland        | (Flanagan et al., 1993)        |
| Tibetan_Plateau_Yu           | Lhasa                         | 29.65    | 91.03     | 3658          | 460      | 8.4      | Grassland       | W. Yu, unpublished             |
|                             | Cairns                        | −16.79   | 145.69    | 30            | 2000     | 25.0     | Forest/Woodland | (Munksgaard et al., 2017)      |
|                             | Tinaroo                       | −17.17   | 145.54    | 680           | 1400     | 22.0     | Forest/Woodland |                                |
|                             | Herberton                     | −17.34   | 145.42    | 918           | 1150     | 19.0     | Woodland        |                                |
|                             | Wild_River                    | −17.65   | 145.28    | 860           | 950      | 21.0     | Woodland        |                                |
|                             | Mount_Garnet                  | −17.67   | 145.10    | 660           | 800      | 24.0     | Woodland        |                                |
| NW_China_Zhao                | Pailugou_2900                 | 38.54    | 100.30    | 2900          | 369.2    | 0.7      | Forest          | (Zhao et al., 2014)            |
|                             | Pailugou_2700                 | 38.55    | 100.29    | 2780          | 369.2    | 0.7      | Forest          |                                |
|                             | Riparian                      | 42.02    | 101.23    | 930           | 34.9     | 8.9      | Woodland        |                                |
|                             | Gobi                          | 42.27    | 101.12    | 906           | 34.9     | 8.9      | Woodland        |                                |
| NT_Aus_Cernusak              | Alice_Springs                 | −23.70   | 133.82    | 688           | 276      | 21.0     | Woodland        | (Kahmen et al., 2013a; Cernusak et al., 2016) |
|                             | Tennant_Creek                 | −19.65   | 134.16    | 365           | 454      | 25.9     | Woodland        |                                |
|                             | Elliot                        | −17.50   | 133.51    | 234           | 604      | 26.8     | Woodland        |                                |
|                             | Katherine                     | −14.48   | 132.36    | 143           | 1140     | 27.2     | Woodland        |                                |
|                             | Darwin                        | −12.44   | 130.88    | 33            | 1736     | 27.6     | Woodland        |                                |
| NSW_Aus_Twinning             | Tumburumba                    | −35.66   | 148.15    | 1249          | 1900     | 9.6      | Forest          | (Twining et al., 2006)         |
| Hawaii_USA_Kahmen            | MLM_1                         | 19.69    | 155.20    | 683           | 5676     | 18.4     | Forest          | (Kahmen et al., 2011)          |
|                             | MLM_3                         | 19.66    | 155.47    | 2061          | 2000     | 11.3     | Forest/Woodland |                                |
|                             | MLM_4                         | 19.59    | 155.45    | 2465          | 1500     | 9.9      | Forest/Woodland |                                |
|                             | MLM_5                         | 19.83    | 155.82    | 694           | 500      | 20.0     | Forest/Woodland |                                |
| Greenland_Bush              | Kangerflussuaq                | 67.02    | −50.70    | 50            | 140      | −5.7     | Grassland       | (Bush et al., 2017)            |
| Germany_Hirl                 | Grünschwaige                  | 48.40    | 11.75     | 448           | 743      | 9.3      | Grassland       | (Hirl et al., 2019)            |
| Germany_Bögelein            | Palatinate                    | 49.28    | 7.81      | 550           | 1067     | 7.9      | Forest          | (Bögelein et al., 2017)        |
| France_Wingate               | LeBray                        | 44.71    | −0.77     | 62            | 900      | 13.0     | Forest          | L. Wingate & J. Ogé, unpublished |
| France_Barbeta               | Ciron                         | 44.38    | −0.31     | 60            | 813      | 12.9     | Forest          | A. Barbeta, unpublished        |
| Canada_Flanagan              | Lethbridge                    | 49.69    | −112.83   | 910           | 380      | 5.8      | Grassland       | (Flanagan et al., 1991a)       |

Egn 1 assumes isotopic steady state, in which the water leaving the leaf through transpiration has the same isotopic composition as that entering the leaf from the xylem. Furthermore, it makes a prediction for the evaporative sites, while the unit of measure in our dataset is bulk leaf water (δL), the total sum of water extracted from the leaf. Bulk leaf water can be expected to be somewhat less enriched than the evaporative sites, due to the influx of unenriched xylem water in the veins (Roden & Ehleringer, 1999; Farquhar & Gan, 2003; Farquhar et al., 2007; Holloway-Phillips et al., 2016). Whereas the mechanisms in the Craig–Gordon equation are identical for δ2H and δ18O, the relative magnitudes of the equilibrium and kinetic fractionation factors differ. For δ2H, the ε+ is relatively large and εk relatively small, whereas the converse is true for δ18O (Merlivat, 1978; Horita & Wesolowski, 1994; Cernusak et al., 2016). The ratio ε+ : εk is c. 3 : 1 for δ2H and 1 : 3 for δ18O (Dataset S1).

We plotted the Craig–Gordon predicted leaf water isotopic compositions against observations for our dataset, to determine whether the Craig–Gordon equation could provide a reasonable framework for guiding analyses of different drivers. Fig. 1 shows the observed bulk leaf water δ2H and δ18O plotted against that predicted by the Craig–Gordon equation, using the measured air temperature, relative humidity, isotopic composition of xylem water and atmospheric vapour. Overall, the Craig–Gordon equation explained 89% of observed variation in leaf water δ2H and 67% of observed variation in leaf water δ18O. As anticipated, the slopes of the relationships were less than unity, as would be the case if some fraction of bulk leaf water represented unenriched xylem water. The generally good predictive ability of the Craig–Gordon equation for daytime leaf water isotopic composition suggests that it can provide a framework for evaluating whether...
different environmental drivers predominate for hydrogen vs oxygen.

Some additional sources of unexplained variation in Fig. 1 could include departures from isotopic steady state (Dongmann et al., 1974; Farquhar & Cernusak, 2005), variation in the fraction of unenriched water in leaves associated with differences in leaf anatomy and physiology (Holloway-Phillips et al., 2016; Barbour et al., 2021) and unaccounted for variation in boundary layer conductance (Buhay et al., 1996). The detailed data required to test for each of these possibilities were not available across the compiled dataset. However, we did repeat our analyses with observations limited to the middle of the day (from 11:00 h to 14:00 h), when isotopic steady state is most likely to be achieved (Harwood et al., 1998). This yielded very similar results to those shown in Fig. 1. The same was also true for subsequent figures and we therefore present analyses with all daytime observations included.

The environmental drivers that are used in the Craig–Gordon equation are air temperature, which impacts $\delta^2$H (Horita & Wesolowski, 1994); relative humidity, which is assumed equal to $w_i/w_v$ if leaf temperature has not deviated from air temperature and $w_i$ is saturated; and isotopic composition of source water entering the leaf, assumed equal to the measured xylem water in our analyses; and the isotopic composition of atmospheric water vapour. Fig. 2 shows observed leaf water $\delta^2$H and $\delta^{18}$O plotted against each of these four environmental drivers. For $\delta^2$H, xylem water $\delta^2$H and atmospheric vapour $\delta^2$H were much more strongly correlated with it than air temperature or relative humidity. For $\delta^{18}$O, conversely, air relative humidity was much more strongly correlated than any of the other drivers. For $\delta^2$H, either xylem water or atmospheric vapour $\delta^2$H explained more than two-thirds of its variation, whereas for $\delta^{18}$O the air relative humidity explained about half of its variation.

The reason that xylem water is a much stronger driver of leaf water for $\delta^2$H than for $\delta^{18}$O is because the range of variation in meteoric water isotopic composition compared with that in leaf water evaporative enrichment is larger for $\delta^2$H than for $\delta^{18}$O. This

![Fig. 1 Observed leaf water isotopic composition for $\delta^2$H (a) and $\delta^{18}$O (b) plotted against values predicted by the Craig–Gordon equation. Symbols with different colours refer to the different datasets that have been compiled for this paper. The dotted lines show one-to-one lines and solid lines show least-squares linear regressions with fitted coefficients shown in the panels along with the coefficient of determination, $R^2$.](image1)

![Fig. 2 Observed isotopic composition for leaf water $\delta^2$H (a–d) and $\delta^{18}$O (e–h) plotted against the four environmental drivers in the Craig–Gordon equation: air temperature (a, e), air relative humidity (b, f), the corresponding isotopic composition of atmospheric vapour (c, g) and the corresponding isotopic composition of xylem water (d, h). The symbol colours show the different datasets compiled for this paper. Solid lines are least-squares linear regressions, with fitted coefficients shown in the panels, along with the coefficient of determination, $R^2$.](image2)
Fig. 3 The isotopic composition of xylem water, leaf water and atmospheric vapour plotted in δ2H–δ18O dual-isotope space. The black line shows the meteoric water line, defined as δ2H = 8 × δ18O + 10. The coloured lines show the evaporation lines for leaf water, in which the intercept with the meteoric water line is the mean for each site and the slope is calculated as (δ2H – δ2Hl)/(δ18Ol – δ18Ox) using the mean quantities for each site, where subscript ‘l’ refers to leaf water and ‘x’ to xylem water. The colours of the lines refer to the individual datasets compiled for this paper. The range of δ18O observed for each site defines the length of the coloured lines.

can be seen through inspection of Fig. 3, which shows the evaporation lines for leaf water for each site in the dataset and their extrapolation to the meteoric water line. The range in the y-axis over which the evaporation lines intersect the meteoric water line for δ2H is c. 120‰ and the corresponding range on the x-axis for δ18O is c. 15‰, for a ratio of c. 8 : 1, consistent with the slope of the meteoric water line. Conversely, the range for leaf water isotopic composition beginning at the meteoric water line and moving right along the evaporation lines is c. 100‰ on the y-axis for δ2H and c. 30‰ on the x-axis for δ18O, for a ratio of c. 3 : 1. Therefore the point at which the evaporation line intersects the meteoric water line can exert a much stronger influence on leaf water for δ2H than for δ18O, because its range is relatively large compared with the range over which evaporation can enrich the leaf water above source water. Another way to understand this conceptually is to consider that the slope of the meteoric water line, defining source water variation in δ2H–δ18O space, corresponds approximately to the ratio of the equilibrium fractionations for δ2H and δ18O (mean = 8.6 in our dataset). Conversely, the slopes of the evaporation lines corresponded approximately to the ratio of the sum of equilibrium and kinetic fractionations (mean = 2.9 in our dataset).

This difference between leaf water dynamics for δ2H and δ18O, driven by source water isotopic composition, is important for interpreting organic material signals. For example, leaf water proxies based on δ2H, such as the δ2H of n-alkanes derived from leaf waxes, if sampled across a large geographic range, could be expected to be strongly influenced by a widely varying δ2H of source water (Liu & Yang, 2008; Sachse et al., 2012; Ladd et al., 2021). Conversely, only if there is little variation in source water δ2H, will the variation in n-alkane δ2H of leaf waxes reliably record the extent of leaf water evaporative enrichment (Kahmen et al., 2013b). For an organic matter proxy such as cellulose δ18O, we would expect the geographic variation in source water isotopic composition to have less influence compared with the dynamics of leaf water enrichment above source water, driven primarily by relative humidity (Barbour & Farquhar, 2000; Kahmen et al., 2011). To the extent that such geographic variation can provide a space for time substitution, our results also have implications for interpreting changes through time within a site. For example, δ2H of n-alkanes from leaf waxes has been combined with δ18O of hemicellulose sugars for reconstructing paleoclimate from sedimentary records (Zech et al., 2013; Hepp et al., 2021). Our results suggest that δ2H of n-alkanes should be better suited to detecting changes in δ2H of precipitation and δ18O of hemicellulose sugars to detecting changes in relative humidity. We note, however, that the extent of transfer of the leaf water signal to the biomarker will also be important; for example, for cellulose δ18O, our analysis of leaf water δ18O may be more relevant to leaf cellulose than to stem wood cellulose, as the latter is subject to partial exchange with unenriched xylem water (Rodent et al., 2000; Kahmen et al., 2011; Cheesman & Cernusak, 2017), with the same caveat also applicable for grasses (Helliker & Ehleringer, 2000; Liu et al., 2017).

Isotopic enrichment of leaf water above xylem water

In addition to xylem water δ2H having a close correlation with leaf water δ2H, atmospheric vapour δ2H also had a close correlation. The next question we asked in our analysis was whether the relationship between leaf water and vapour for δ2H would still remain stronger than that for δ18O when variation in xylem water isotopic composition was removed. To answer this question, we expressed leaf water as enrichment above xylem water (Δl), calculated as Δl = (δl – δx)/(1 + δx), where the subscript ‘l’ refers to leaf water and ‘x’ to xylem water. Again, we use the approximate form of the Craig–Gordon equation here for ease of interpretation to guide our analysis, but used the more precise form in our calculations. With leaf water expressed as enrichment above source water, the Craig–Gordon equation becomes (Farquhar et al., 1989):
\[ \Delta_x \approx \epsilon^+ + \epsilon_k + (\Delta_v - \epsilon_k)h \]  
Eqn 2

\( \Delta_x \) (predicted enrichment at the evaporative sites in leaves and \( \Delta_v \), enrichment of atmospheric vapour relative to source water). Note that this latter term is generally negative; that is, atmospheric vapour is generally depleted in heavier isotopes compared with source water. In our analysis, we calculated \( \Delta_x \) as \( \Delta_x = (\delta_v - \delta_h)/(1 + \delta_h) \), where \( \delta_v \) is \( \delta^2\text{H} \) or \( \delta^{18}\text{O} \) of atmospheric vapour and \( \delta_h \) is that of xylem water. In Fig. 4, we show the observed bulk leaf water enrichment plotted against the three environmental drivers remaining in Eqn 2.

Fig. 4 shows that the correlation between leaf water and relative humidity for hydrogen has been markedly improved by removing source water variation, with relative humidity now explaining 41% of the variation in \( \Delta^2\text{H} \). Therefore, after calculating \( \Delta^2\text{H} \) to remove the source water signal, the sensitivity to relative humidity became more apparent. For oxygen, there was also a strengthening of the correlation between leaf water enrichment and relative humidity, with the \( R^2 \) increasing from 0.49 to 0.57. Interestingly, however, the correlation between leaf water enrichment and atmospheric vapour enrichment was still relatively strong for hydrogen, but weak for oxygen, which stands out as a point of difference between \( \Delta^2\text{H} \) and \( \Delta^{18}\text{O} \) in Fig. 4. Stronger relationships with atmospheric vapour for \( \Delta^2\text{H} \) than for \( \Delta^{18}\text{O} \) have also been observed previously in some of the individual datasets that have now been compiled for this paper (Cernusak et al., 2016; Bögelein et al., 2017; Munksgaard et al., 2017).

**Role of atmospheric vapour isotopic composition**

Can we identify further the underlying cause of the stronger correlation between leaf water and atmospheric vapour for \( \Delta^2\text{H} \) compared with \( \Delta^{18}\text{O} \)? To explore this, we turned again to the Craig–Gordon equation, taking the derivative of Eqn 2 with respect to \( \Delta_v \). This provides a mathematical description of predicted drivers of the change in \( \Delta_v \) for a given change in \( \Delta_x \):

\[ \frac{d\Delta_l}{d\Delta_v} = \frac{d\epsilon^+}{d\Delta_v} + \frac{d\epsilon_k}{d\Delta_v} + h \left( \frac{1 - d\epsilon_k}{d\Delta_v} \right) + (\Delta_v - \epsilon_k) \frac{dh}{d\Delta_v} \]  
Eqn 3

We used our dataset to estimate the terms in Eqn 3 by taking regression slopes for the derivative terms and mean values for \( h \) and \( (\Delta_v - \epsilon_k) \). These estimates are shown in Table 2 for \( \Delta^2\text{H} \) and \( \Delta^{18}\text{O} \). From Table 2, it can be seen that the first two terms on the right side of Eqn 3 are small in magnitude for both \( \Delta^2\text{H} \) and \( \Delta^{18}\text{O} \) and unlikely to have a strong influence on \( d\Delta_l/d\Delta_v \) for either. Because \( d\epsilon_k/d\Delta_v \) is small, it means that the third term on the right side will approach the value of \( h \), which is larger by comparison, having a mean value in our dataset of 0.5, or an air relative humidity of \( c \), 50%. The largest term in Eqn 3 by far for both \( \Delta^2\text{H} \) and \( \Delta^{18}\text{O} \) is \( (\Delta_v - \epsilon_k) \), having mean values of \(-79\%\) and \(-35\%\) for \( \Delta^2\text{H} \) and \( \Delta^{18}\text{O} \), respectively. This is then multiplied by a much smaller term, \( dh/d\Delta_v \). Importantly, \( (\Delta_v - \epsilon_k) \) is negative, setting up the possibility that the interplay between the third and fourth terms on the right side of the equation could be important, with the third term, \( h(1 - d\epsilon_k/d\Delta_v) \), being positive and the fourth term,
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According to Eqn 3, the dependence of leaf water isotopic enrichment on the atmospheric vapour isotopic composition, \( \Delta \), is equal to the sum of the shaded columns, which is shown in the final column. As can be seen, the primary difference for \( \Delta^2H \) compared with \( \Delta^{18}O \) results from the term \( dh/d\Delta_v \); that is, a correlation between atmospheric humidity and the isotopic composition of atmospheric vapour, which is much stronger for \( \Delta^{18}O \) than for \( \Delta^2H \).

\( (\Delta_v - \epsilon_v) \frac{dh}{d\Delta_v}, \) potentially counteracting it with a negative value.

The interaction between the third and fourth terms in Eqn 3 does indeed appear to be pivotal in explaining why leaf water correlates more strongly with atmospheric vapour for \( \Delta^{18}O \) than for \( \Delta^2H \). For \( \Delta^2H \), the linear regression between \( h \) and \( \Delta^2H \) was not significant (\( P = 0.32, n = 546 \)) and had a slope of \(-0.0004\). This gives a value for the fourth term in Eqn 3 for \( \Delta^2H \) of 0.03, which therefore adds slightly to the positive value of the third term, again having a value of c. 0.5. Conversely, for \( \Delta^{18}O \), the regression between \( h \) and \( \Delta^{18}O \) was significant (\( P < 0.001, n = 546 \)) and had a positive slope of 0.0102. Because this slope was positive, the fourth term on the right side of Eqn 3 for \( \Delta^{18}O \) takes on an overall negative value of \(-0.36\). Therefore, for \( \Delta^{18}O \), the fourth term on the right side of Eqn 3 largely cancels the influence of the third term and, as a result, there is little change in \( \Delta^{18}O \) for a given change in \( \Delta^{18}O \). This manifests in our dataset as a weakened correlation between leaf water and atmospheric vapour for \( \Delta^{18}O \) as seen in Fig. 4f whereas, for \( \Delta^2H \), there is a stronger correlation between leaf water and atmospheric vapour, as seen in Fig. 4c.

The analysis above shows that there is a positive correlation between relative humidity and the \( \Delta^{18}O \) of atmospheric vapour in our dataset, whereas such a correlation does not exist between relative humidity and the \( \Delta^2H \) of atmospheric vapour. Through application of Eqn 3, we showed that this difference partly explains why leaf water \( \delta^2H \) more strongly correlates with atmospheric vapour \( \delta^2H \) than is the case for \( \delta^{18}O \). Another way to approach the underlying issue of this apparent difference in behaviour of atmospheric vapour for the two isotopologues with respect to relative humidity is to calculate the deuterium excess or the deuterium excess of water vapour, \( d_v \), as \( d_v = \delta^2H_v - 8 \times \delta^{18}O_v \) (Dansgaard, 1964). We also made the same calculation for atmospheric vapour composition with respect to xylem water, \( \Delta d_v = \Delta^2H_v - 8 \times \Delta^{18}O_v \). We then tested for correlations between these parameters and the air relative humidity in our dataset. Both showed significant negative correlations with relative humidity, with the relationship stronger for \( \Delta d_v \) (\( R^2 = 0.17, P < 0.001, n = 546 \)) than for \( d_v \) (\( R^2 = 0.08, P < 0.001, n = 546 \)).

A relationship between the deuterium excess of atmospheric vapour and relative humidity has also been observed on diurnal timescales at six sites in the northern hemisphere (Welp et al., 2012) and at a tropical site in Cairns, Australia (Munksgaard et al., 2020). This pronounced, general pattern is thought to be driven by the diurnal pattern of plant transpiration and the contribution of transpired water to atmospheric vapour and by entrainment of the free atmosphere into the planetary boundary layer with increased convective mixing during the day. The result is a general midday decrease in the \( \delta^{18}O \) of atmospheric vapour, but little to no change in \( \delta^2H \). This leads to the diurnal variability of \( d_v \), which is anticorrelated with the diurnal pattern of relative humidity (Welp et al., 2012; Munksgaard et al., 2020). A shorter time series of 3 d at the Wind River Experimental Forest (Washington, USA) showed a similar pattern (Lai & Ehleringer, 2011). The strength of this pattern suggests that such diurnal variation could be driving the overall relationship between \( d_v \) and relative humidity in our dataset. Welp et al. 2012 also observed negative correlations between day-to-day variation in \( d_v \) and relative humidity throughout the summer months at sites located near large bodies of water, with such patterns also previously reported for sites in marine-type settings (Uemura et al., 2008; Gat et al., 2011). Such dynamics related to marine air sources may also have been relevant at some sites within our dataset. When we restricted our analysis from daytime observations to only midday observations (between 11:00 h and 13:00 h) to minimise diurnality, we observed a weak, but still significant, relationship between \( d_v \) and relative humidity (\( R^2 = 0.02, P < 0.05, n = 200 \)), showing the importance of diurnal effects. What is clear overall is that covariation between \( \delta^{18}O \), and relative humidity, but not \( \delta^2H \), and relative humidity, plays an important role in modulating leaf water isotope dynamics, leading to the result highlighted in our dataset of \( \Delta^2H \) of leaf water showing stronger correlation with \( \Delta^2H \) of atmospheric vapour than is the case for \( \Delta^{18}O \).

**Are two isotopes better than one?**

Some organic matter proxies, such as plant cellulose, allow both \( \delta^{18}O \) and \( \delta^2H \) to be measured. In such cases, it is possible to estimate leaf water isotopic composition for both \( \delta^2H \) and \( \delta^{18}O \) and therefore to reconstruct \( d_v \), the deuterium excess for leaf water. As seen in Fig. 3, the slopes of the evaporation lines tend to be uniform and independent of the source water and the position

| \( \Delta^2H \) | \( \Delta^{18}O \) |
|---|---|
| \( \frac{dh}{d\Delta_v} \) | \( \frac{dh}{d\Delta_v} \) |
| \( \Delta_v - \epsilon_v \) | \( \Delta_v - \epsilon_v \) |
| \( \Delta \) | \( \Delta \) |

Table 2 Values for the terms in Eqn 3 calculated from the combined dataset.
Review of the correlation between
Lehmann et al., 2014). Our dataset gave us an opportunity to test this idea across a diverse range of sites and conditions.

Fig. 5 shows $d_l$ for the dataset, calculated as $d_l = \delta^2H - 8 \times \delta^{18}O_l$, plotted against potential drivers, including relative humidity. Because $d_l$ was calculated from $\delta^{18}O_l$ and $\delta^2H_l$, we can compare the relationships with relative humidity among the three. The $d_l$ does indeed have the strongest relationship; however, it is not very much stronger than that for $\delta^{18}O_l$. The $R^2$ for $d_l$ vs relative humidity is 0.54 (Fig. 5b), whereas that for $\delta^{18}O_l$ is 0.49 (Fig. 2b), and that for $\delta^2H_l$ is 0.07 (Fig. 2b). Therefore, surprisingly, leaf water $\delta^{18}O$ on its own would be nearly as good a predictor of air relative humidity as $d_l$ calculated from both the $\delta^{18}O_l$ and $\delta^2H_l$ of leaf water. The explanation for this is the relatively constrained variation in $\delta^{18}O_l$ of xylem water, especially in comparison with $\delta^2H$ (Fig. 3) and the relative insensitivity of $\Delta^{18}O$ to atmospheric vapour $\Delta^{18}O_v$, as discussed above. Given these considerations, we suggest that if applying a dual-isotope approach in this way, one should weigh up carefully the uncertainty associated with estimating leaf water $\delta^2H$ from cellulose $\delta^2H$, given the relative complexity in the signal transfer pathway from leaf water to cellulose for $\delta^2H$ (Cormier et al., 2018; Lehmann et al., 2021). The cost in uncertainty associated with this may not be worth the relatively modest improvement in strength of the correlation between $d_l$ and relative humidity compared with that for $\delta^{18}O_l$ alone.

Conclusions

We compiled a dataset for $\delta^2H$ and $\delta^{18}O$ of leaf water, xylem water, atmospheric vapour, air temperature and relative humidity from a diverse range of sites and used the dataset to test for differences in how leaf water $\delta^2H$ and $\delta^{18}O$ reflect environmental drivers. We conducted the analysis in the context of asking which drivers could be best reconstructed from leaf water proxies based on organic material. Xylem water $\delta^2H$ was a much stronger driver of variation in leaf water $\delta^2H$ than was the case for xylem water $\delta^{18}O$ as a driver of variation in leaf water $\delta^{18}O$. Conversely, relative humidity showed a considerably stronger relationship with leaf water $\delta^{18}O$ than it did with leaf water $\delta^2H$. This pattern persisted when we removed xylem water isotopic variation from the leaf water signal by expressing the leaf water isotopic composition as an enrichment above xylem water. We identified the underlying reason for this pattern as a correlation between relative humidity and the $\delta^{18}O$ of atmospheric vapour. Such a correlation has also been observed in time series of vapour isotopic composition measurements, and manifests most clearly as an anticorrelation between the deuterium excess of atmospheric vapour and relative humidity on diurnal timescales. While we did not have sufficient resolution of sampling within sites to tease this apart in our dataset, we suspect that this diurnal pattern is likely to underlie the correlation between relative humidity and atmospheric vapour $\Delta^{18}O$, that we observed. We conclude that leaf water $\delta^2H$ and $\delta^{18}O$ do indeed reflect the balance of potential environmental drivers differently: leaf water $\delta^2H$ reflects more strongly xylem water $\delta^2H$ and atmospheric vapour $\delta^2H$, whereas leaf water $\delta^{18}O$ reflects more strongly air relative humidity.

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Author contributions

LAC and MC initiated the review and conducted initial analyses. All authors contributed to further development of ideas and writing of the manuscript.
Data availability

All data used in the analyses presented in this paper are available in Supporting Information Dataset S1.

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**Supporting Information**

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Dataset S1** An Excel file containing the compiled dataset that was analysed for this paper.

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