Cavitron extraction of xylem water suggests cryogenic extraction biases vary across species but are independent of tree water stress

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Abstract

Cryogenic vacuum distillation (CVD) is a widely used technique for extracting plant water from stems for isotopic analysis, but concerns about potential isotopic biases have emerged. Here, we leverage the Cavitron centrifugation technique to extract xylem water and compare its isotopic signature to that of CVD-extracted stem water as well as source water. Conducted under field conditions in tropical northern Australia, our study spans seven tree species naturally experiencing a range of water stress levels. Our findings reveal a significant deuterium bias in CVD-extracted bulk stem water when compared to xylem water (median bias -14.9‰), whereas xylem water closely aligned with source water (median offset -1.9‰). We find substantial variations in deuterium bias among the seven tree species (bias ranging from -19.3 to -9.1‰), but intriguingly, CVD-induced biases were unrelated to environmental factors such as relative stem water content and pre-dawn leaf water potential. These results imply that inter-specific differences may be driven by anatomical traits rather than tree hydraulic functioning. Additionally, our data highlight the potential to use a site-specific deuterium offset, based on the isotopic signature of local source water, for correcting CVD-induced biases.

Introduction

Understanding plant rooting depth and the sources of plant water is critical for effective water resource management (Fan et al., 2017; Miguez-Macho & Fan, 2021; Penna et al., 2018). For over four decades, scientists have used stable isotope analyses of plant water and potential source water (such as soil water from different depths and groundwater) to infer rooting depth and characterise plant water sources (e.g. Ehleringer & Dawson, 1992; Goldsmith et al., 2012; Thorburn et al., 1993; White et al., 1985; Zencich et al., 2002). Central to this isotope-enabled approach is the extraction of water from plant stems.

One widely adopted technique for this purpose is cryogenic vacuum distillation (CVD). In CVD, water is extracted through sublimation under vacuum conditions. While relatively easy to implement in the laboratory, a limitation of CVD is that it provides a bulk measurement of total stem water (hereafter 'bulk stem water'), rather than a more targeted measurement of the water that transits from root uptake through xylem conduits and contributes to transpiration (hereafter 'xylem water'). Less destructive and more selective *in-situ* alternatives have recently emerged (Kübert et al., 2023; Kühnhammer et al., 2022; Marshall et al., 2020; Volkmann et al., 2016), but CVD remains the most commonly used method among the scientific community (de la Casa et al., 2022; Millar et al., 2022; Millar et al., 2018).

Recent studies have raised concerns about the use of CVD for plant water sourcing investigations (Barbeta et al., 2022; Chen et al., 2020; Wen et al., 2022). These concerns have resulted from increasing evidence of systematic deuterium offsets in plant water, reaching up to -40‰ relative to source water (e.g. Barbeta et al., 2019; de la Casa et al., 2022; Duvert et al., 2022; Ellsworth & Williams, 2007; Lin & Sternberg, 1993; Poca et al., 2019; Tetzlaff et al., 2021). The underlying reasons for these CVD-induced deuterium offsets are now becoming clearer, and may be related to a combination of (1) hydrogen exchange with organic tissues during the CVD extraction process (Chen et al., 2020; Diao et al., 2022; Wen et al., 2022) and (2) isotopic heterogeneity between xylem water and the water stored in non-conductive tissues, as CVD extracts both water pools (Barbeta et al., 2022; Bowers & Williams, 2022; Wen et al., 2022).

Much of the evidence regarding these CVD-induced biases has been derived from controlled laboratory and potted experiments, and few studies have examined isotopic biases under field conditions. Several authors have conducted rehydration experiments with labelled water (Chen et al., 2020; Diao et al., 2022; Wen et al., 2022), but such experiments may lead to artificially high deuterium biases (Diao et al., 2022). Conversely, field studies often lack source water isotopic data, making bias inferences uncertain (Bowers & Williams, 2022; Zuecco et al., 2022). To our knowledge, Barbeta et al. (2022) and He et al. (2023) are the only authors who have investigated the differences between xylem water, bulk stem water and source water under natural field conditions. However, both studies focused on a limited number of tree species, warranting further investigation into how CVD-induced biases may vary across different species. Additionally, questions remain regarding the influence of environmental factors on deuterium biases, such as water availability and resulting tree water stress levels. A meta-analysis by de la Casa et al. (2022) suggests that environmental conditions may influence deuterium biases, but this hypothesis requires more detailed testing at specific locations.

As an alternative to CVD approaches, the Cavitron, a custom-made rotor fitted to a centrifuge (Cochard, 2002; Cochard et al., 2005), is now emerging as a powerful tool for non-destructive extraction of xylem water and subsequent evaluation of CVD-induced isotopic biases (Barbeta et al., 2022; He et al., 2023; Wen et al., 2022). In this study, we use Cavitron-extracted xylem water as a reference to assess the extent and magnitude of CVD-induced biases across several tree species under natural field conditions in the Australian tropics. We deliberately selected tree species spanning a range of water stress levels, as indicated by pre-dawn leaf water potentials. We also measured the isotopic composition of groundwater and soil water to characterise source water. Our work addresses the following questions:

Does the isotopic composition of Cavitron-extracted xylem water align with source water, and does CVD-extracted bulk stem water exhibit a deuterium offset?

Does the CVD-induced deuterium bias occur consistently across tree species, regardless of water stress conditions and xylem water isotopic composition?

We provide a detailed operating procedure for our Cavitron xylem water extraction (see Supplementary Information). Following the recommendations of Millar et al. (2022), we hope that this procedure can contribute to the establishment of a unified protocol for use by other research groups.

Methods

Site description

Stem, soil and groundwater samples were collected at Elsey National Park, a site located in northern Australia, about 400km southeast of Darwin (15.00°S, 133.19°E). This region experiences a wet-dry tropical climate (*Aw* according to the Köppen-Geiger classification), with an average annual rainfall of 1017 mm for the period 2004–2022 (Bureau of Meteorology's Cave Creek Station #14650), >95% of which falls between November and April. Elsey National Park is an area of regional groundwater discharge from an extensive limestone aquifer (Jolly et al., 2004; Karp, 2008; Lamontagne et al., 2021). The park supports a diverse array of vegetation types along a groundwater depth gradient, ranging from vine forests in areas with shallow

groundwater (i.e. ≈1 m below ground level) to woodland savannas in areas where the water table is deeper (i.e. >10 m below ground level).

We conducted two sampling campaigns in October 2021 and October 2022, coinciding with the later part of the dry season. During that period, water availability is at a minimum and we expected some trees to rely entirely on groundwater, whereas others without access to groundwater would likely experience water stress as soil moisture stores become depleted (Canham et al., 2021; Duvert et al., 2022). Because our aim was to capture a range of tree species and water stress conditions, we sampled trees across several sites covering varying habitats and depths to water table. We sampled common tree species from seasonally dry woodlands (*E. tetrodonta*, *E. chlorostachys*), to riparian species (*M. argentea*, *M. dealbata*), as well as species tolerant of both dry conditions and short-term seasonal inundation (*T. arostrata*, *H. arborescens*). For the purposes of this study, we sampled a total of seven tree species and 17 individual trees (Table 1).

Stem sampling and leaf water potential measurements

Originally, our plan was to sample a total of 40 stems from nine tree species, including two replicate trees per species and two replicate stems per individual tree – except for *Hakea arborescens*, for which we aimed to increase the number of replicate trees to eight. This specific focus on *H. arborescens* was motivated by preliminary leaf water potential data, which showed a considerable variability among trees of this species.

We encountered various challenges associated with the Cavitron extraction procedure, including difficulties in sampling stem segments that were straight enough to be secured in the rotor, and an inability for some stems to extract sufficient volumes of xylem water for isotope analysis. As a result, we were only able to analyse a total of 18 stems from seven tree species. This still permitted the sampling of one to two replicate trees per species and one to two replicate stem segments per individual tree – except for *H. arborescens*, for which we sampled eight stems from seven individual trees (Table 1).

Species	# of stems (# of individual trees)
Erythrophleum chlorostachys	1 (1)
Terminalia arostrata	1 (1)
Corymbia bella	2 (2)
Eucalyptus tetrodonta	2 (2)
Melaleuca argentea	2 (2)
Melaleuca dealbata	2 (2)
Hakea arborescens	8 (7)
Total	18 (17)

Table 1: List of tree species sampled as part of this work. Number of stem samples for which we obtained the isotopic composition of both CVD-extracted bulk water and Cavitron-extracted xylem water.

All stem samples were collected before dawn. For each tree, a large branch (≈0.8 to ≈2 m length; ≈10 to ≈25 mm base diameter) was removed from the canopy using a telescopic pruner. The cut edge of each branch was immediately sealed with parafilm and electric tape, and sealed branches were transported to our base (an air-conditioned, darkened room) within 30 minutes of cutting. Upon arrival, leaves were cut with a sharp razor blade for pre-dawn leaf water potential (LWP) measurements (see description below). Two separate stems from the same branch were then cut, sealed at both ends with parafilm, wrapped in cling wrap, and double Ziploc bagged. Stems were subsequently stored at 4°C for later water extraction through CVD and Cavitron centrifugation methods. The sampled stems typically ranged from 210 to 260 mm in length and 6 to 13 mm in diameter.

We used a Model-1000 pressure chamber (PMS Instrument Company, United States) with nitrogen gas and a mounted eye lens for the LWP measurements. Two replicate measurements were made for each branch sample, plus a third measurement when the difference between the first two measurements was > 10%. All

LWP measurements were finalised within two hours of sampling. For each sample we report the average of the two to three measurements.

Source water sampling

To characterise the spectrum of isotopic compositions of potential source waters, we sampled both groundwater and soil water at various locations and depths within and around Elsey National Park. We obtained groundwater from five observation bores and soil water from six soil cores. For groundwater, we used a submersible pump (Tornado, Proactive, USA) except at one site where we used a pre-installed solar-powered pump, and collected samples once three bore volumes had been purged and/or once pH and conductivity measurements had stabilised. For soil samples, we used a hand auger at five sites to extract shallow soil (maximum depth of 2.0 m), while at one site, we used a small drill rig to extract deeper soil horizons (maximum depth of 5.4 m). We collected a total of 36 soil samples at depths ranging from 0.1 to 5.4 m. Each sample comprised approximately 100 to 300g of soil material, was sealed in double Ziplock bags with minimised headspace, and kept at 4°C until further analysis. Additionally, a local meteoric water line was obtained from 18 rainfall samples collected on site between 2019 and 2021 (Lamontagne et al., in prep.).

Cavitron extractions

To extract xylem water from stem samples, we used a standard 270-mm diameter Cavitron manufactured by DG-Meca (France) and fitted to an ultracentrifuge (Avanti J-E, Beckman Coulter, United States) at Charles Darwin University. We broadly followed the method outlined in Barbeta et al. (2022). Briefly, small plastic containers were inserted into each end of the stem and sealed with parafilm to collect xylem water. Samples were spun for two minutes at speeds ranging from 3,000 to 9,000 rpm, corresponding to xylem pressures of –0.57 to –6.40 MPa based on stem length (Alder et al., 1997; Cochard, 2002). Extracted water was then collected from the containers using a micropipette, filtered through 0.45 μm and stored in 2 mL glass vials fitted with 0.1 mL micro-inserts. Samples were then preserved at 4°C until analysis. Stem samples were weighed before and after centrifugation, oven-dried at 105°C for 24h after centrifugation and reweighed to determine the relative stem water content (RSWC). A more detailed description of our operating procedure is provided in the Supplementary Information.

Cryogenic extractions

Bulk stem water and soil water were extracted at the West Australian Biogeochemistry Centre (WABC), University of Western Australia, following the CVD procedure outlined in West et al. (2006). Samples were fully frozen using liquid nitrogen, after which they were subjected to a vacuum with pressure < 10 Pa. Frozen samples were then heated under vacuum conditions, causing water vapour to be collected in a liquid nitrogen cold trap. Extraction times were set at 60 and 90 min for soil and stem samples, respectively, aligning with the recommendations of West et al. (2006). To ensure the quality and accuracy of the extraction process, water was also extracted from four different standards using the same procedure.

Isotopic analyses

All extracted water samples were analysed for oxygen and hydrogen isotopic ratios ($\delta^{18}O$ and δD) at the WABC using a cavity ring-down spectrometer (Picarro Inc., model L2130-I) fitted with a micro-combustion module to remove organic compounds that may be present in extracted water. The raw isotopic values are expressed relative to VSMOW and are reported in per mil (‰). According to analyses on replicate stem samples, overall precision for the CVD extraction and measurement procedure was ±0.5‰ and ±3.0‰ for $\delta^{18}O$ and δD, respectively. Cavitron-extracted xylem water and groundwater samples had a precision of ±0.1‰ and ±0.5‰ for $\delta^{18}O$ and δD, respectively.

Data analyses

Statistical analyses and plotting were conducted using MATLAB R2022a. We define *deuterium bias* as the difference between δD of CVD-derived bulk stem water and that of Cavitron-derived xylem water of the same branch:

$\delta D_{bias} = \delta D_{bulk} - \delta D_{xylem}$ (1)

We define *deuterium offset* as the difference between δD of xylem or bulk stem water and their expected δD based on the source water line:

$\delta D_{offset} = \delta D_{xylem\ or\ bulk} - \delta D_{source}$ (2)

with $\delta D_{source} = \alpha_{swl} * \delta^{18}O_{xylem\ or\ bulk} + \beta_{swl}$ (3)

where α_{swl} and β_{swl} are the slope and intercept of the source water line, respectively.

To test whether the means of the δD offsets of xylem and bulk stem water were significantly different from zero, we used a one-sample *t*-test (MATLAB function *ttest*). To test whether the difference between the δD offsets of xylem and bulk stem water was significant, we used the non-parametric two-sample Kolmogorov-Smirnov test (MATLAB function *kstest2*). Unlike other tests (e.g. paired *t*-test) that only compare the means of each group, this test evaluates the entire distribution of each group. To test cross-species differences in mean δD bias, we used a Kruskal-Wallis test (MATLAB function *kruskalwallis*) followed by a Dunn-Sidák post-hoc test for pairwise comparison (MATLAB function *multcompare*).

To test the potential effect of tree species, RSWC, pre-dawn LWP and xylem water isotopic composition on the CVD-induced δD bias, we used linear mixed-effects models (MATLAB function *fitlme*). We used species, pre-dawn LWP, δD of xylem water and RSWC as predictor variables and δD bias as the response variable. In addition, we included sampling site as a random effect in the mixed-effects model.

Results

Isotopic biases between xylem water and bulk stem water

The δD of CVD-derived bulk stem water was significantly and consistently more depleted than that of Cavitron-derived xylem water (Figure 1), with a median δD bias of –14.9‰ and values ranging between –20.4 and –8.6‰ (Kolmogorov-Smirnov; $p<0.01$). In contrast, there was no significant $\delta^{18}O$ bias between CVD and Cavitron data, with $\delta^{18}O$ biases centred around 0.3‰ and ranging from –1.7 to 0.9‰ (Kolmogorov-Smirnov; $p=0.945$).

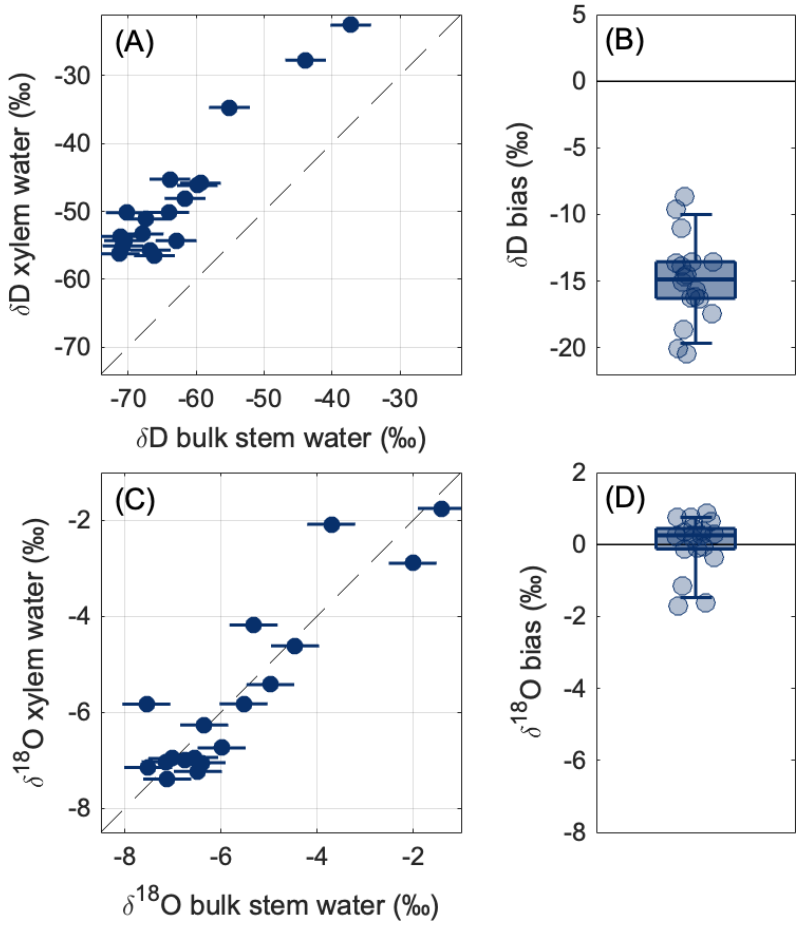

Figure 1. δD (A,B) and δ^{18}O (C,D) biases between Cavitron-extracted xylem water and CVD-extracted bulk stem water across all samples. Boxplots in (B) and (D) show the 10^{th}, 25^{th}, 50^{th}, $75^{th}$and 90^{th} percentiles.

Deuterium offsets between stem water and source water

We found large differences in the relationships between xylem water, bulk stem water and source water (Figure 2). While Cavitron-extracted xylem water aligned well with the source water line (Kolmogorov-Smirnov; p=0.945), CVD-extracted water plotted well below the source water line (Kolmogorov-Smirnov; p<0.01). The δD offsets of Cavitron-extracted water ranged from –6.5 to 5.5‰ (median –1.9‰) and their mean was not significantly different from zero (t-test; p=0.458), while the δD offsets of CVD-extracted water ranged from –22.8 to –8.8‰ (median –15.5‰) and their mean was significantly different from zero (t-test; p<0.01) (Figure 2b). Some stem water samples (both CVD- and Cavitron-extracted) were more enriched than any of the source water samples, which is likely due to our inability to extract soil water from shallow (i.e. evaporated) horizons. The similarity in slopes between all three trend lines (5.02, 5.06 and 4.86 for the source water, xylem water and bulk stem water lines, respectively) is a first indication that the CVD-induced δD offset may be independent of the δD composition of tree water.

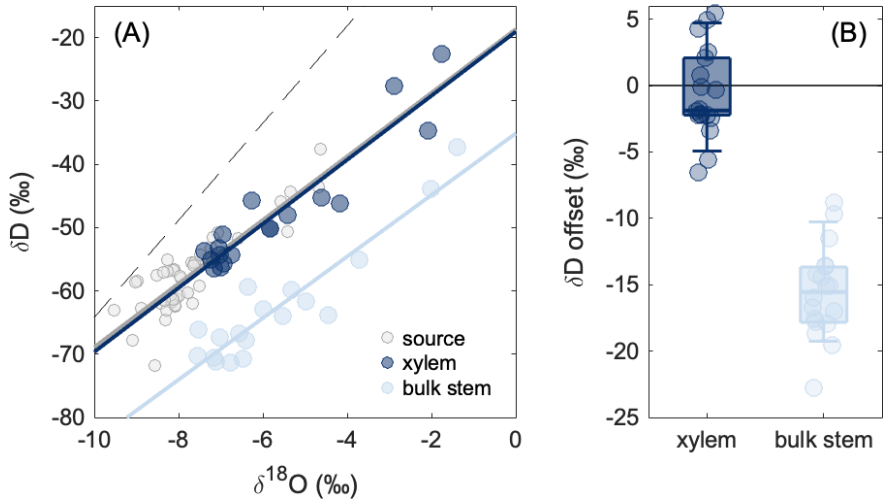

Figure 2. (A) Dual isotope plot showing source water, xylem water (Cavitron) and bulk stem water (CVD) and their trend lines. (B) Distribution of the δD offset relative to source water. In (A), the black dashed line reflects the local meteoric water line (Lamontagne et al., in prep.), while the grey, dark blue and light blue lines reflect the source water, xylem water and bulk stem water lines, respectively. The boxplot shows the 10th, 25th, 50th, 75th and 90th percentiles of offset values.

Deuterium bias across tree species

We found significant differences in the CVD-induced δD bias among different tree species (Kruskal-Wallis; $p<0.05$) (Figure 3). Mean biases spanned a relatively broad range, from –19.3‰ (*C. bella*) to –9.1‰ (*M. argentea*). Pairwise comparisons indicated that most species had non-significant differences in mean δD bias, except for *M. argentea* which had significantly smaller δD biases than *H. arborescens* and *C. bella* (Dunn-Sidák; $p<0.05$) (Figure 3).

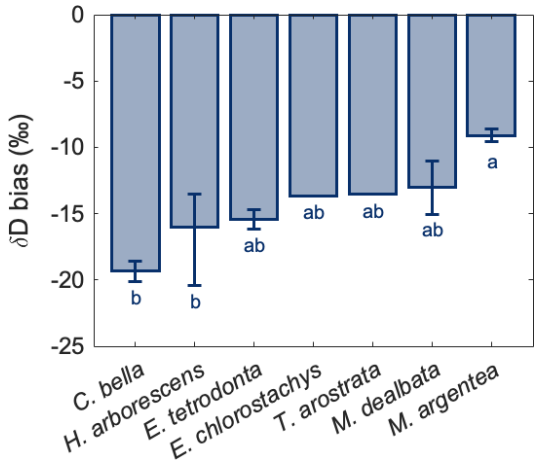

Figure 3. Distribution of mean δD biases per tree species. The error bars correspond to minima and maxima. Letters indicate significant differences between species based on a Dunn-Sidák test ($p < 0.05$).

Deuterium biases and offsets against environmental variables

Both pre-dawn LWPs (–1.97 to –0.18 MPa), RSWC (0.60 to 1.64 g/g) and xylem water δD (–56.5 to –22.5‰) varied over broad ranges, making it possible to test the ability of these variables to predict variations in δD biases and offsets (Figure 4). We found that neither δD biases nor δD offsets were correlated with isotopic composition (Spearman; p=0.17 and p=0.78, respectively) (Figure 4a, 4d), pre-dawn LWP (Spearman; p=0.73 and p=0.22, respectively) (Figure 4b, 4e), or RSWC (Spearman; p=0.38 and p=0.42, respectively) (Figure 4c, 4f). The linear mixed-effect modelling confirmed that xylem water δD, pre-dawn LWP and RSWC where not significantly related to the δD bias. The model also found significant differences between *C. bella* and all other species (Table 2). The 'site' random effect had very low ($<10^{-5}$) variance, implying that sites had a minimal effect on the δD bias. These modelling results suggest that while CVD-induced δD biases were independent of water content and water stress levels, inter-specific differences cannot be ignored.

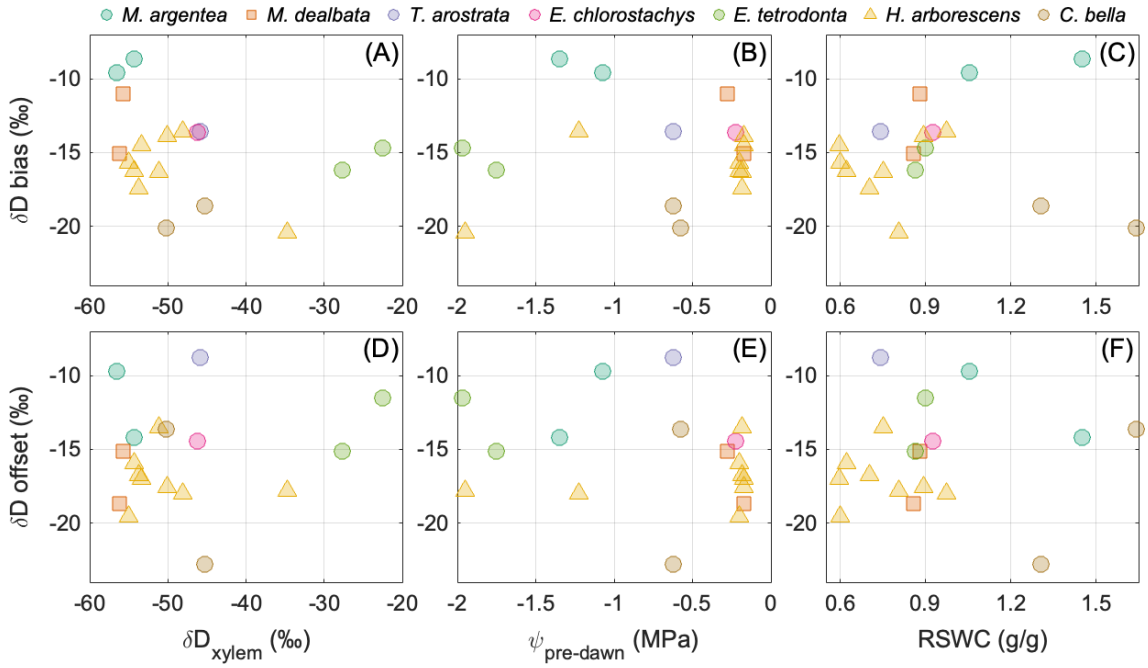

Figure 4. Relationship between δD biases (i.e. Cavitron-derived xylem water versus CVD-derived bulk stem water) and δD offsets (i.e. source water versus CVD-derived bulk stem water) with δD_{xylem}(A,D), $\Psi_{pre\text{-}dawn}$ (B,E) and RSWC (C,F). δD_{xylem} is the δD of xylem water as measured via Cavitron extraction, $\Psi_{pre\text{-}dawn}$ is the pre-dawn leaf water potential, and RSWC is the relative stem water content.

Parameter	Estimate	SE	t-stat	dff	p-value	95% CI (lower)	95% CI (upper)
Intercept	-30.48	9.66	-3.16	8	0.01	-52.76	-8.21
E. chlorostachys	7.81	2.3	3.4	8	0.01	2.51	13.12
E. tetrodonta	10.11	3.12	3.24	8	0.01	2.91	17.31
H. arborescens	6.04	2.43	2.48	8	0.04	0.42	11.65
M. argentea	10.78	3.03	3.56	8	0.01	3.8	17.77
M. dealbata	7.53	2.41	3.12	8	0.01	1.96	13.09
T. arostrata	9.08	2.68	3.38	8	0.01	2.89	15.27
‗D xylem water	-0.12	0.18	-0.66	8	0.53	-0.53	0.29
pre-dawn LWP	0.88	1.89	0.47	8	0.65	-3.48	5.24
RSWC	4.13	2.91	1.42	8	0.19	-2.58	10.84

Table 2: Fixed effects coefficients in the linear mixed-effect model. P-values in bold are significant at a 95% confidence level. 'SE' stands for standard error. 'CI' stands for confidence interval. 'dff' stand for degrees of freedom. Here the reference species is C. bella. None of the other inter-species differences were significant at a 95% confidence level.

Discussion

Xylem water matches source water, but bulk stem water doesn't

Our data show that the Cavitron-extracted xylem water had δD signatures that closely aligned with source water (mean of δD offsets not significantly different from zero), with virtually identical trend lines between these two groups. This result corroborates the findings of recent studies that used a Cavitron for xylem water extraction (Barbeta et al., 2022; He et al., 2023; Wen et al., 2023). Similar centrifugation methods relying on smaller stem segments have also resulted in relatively good alignments with source water (Sánchez-Murillo et al., 2023). While not directly tested against source water measurements, other techniques targeting mobile xylem water, such as direct vapour equilibration (Millar et al., 2018) and pressure chamber extraction (Bowers & Williams, 2022; Zuecco et al., 2022), have also produced δD signatures likely reflective of source water.

We found that unlike xylem water, bulk CVD-extracted water was strongly depleted in δD relative to both source water (median δD offset –15.5‰) and Cavitron-extracted water (median δD bias –14.9‰). The tendency of CVD extraction to introduce systematic isotopic biases is now well-known and has been thoroughly described through recent experimental work by Wen et al. (2022). These authors have shown that CVD-induced bias is caused by both within-stem isotopic differences between xylem and tissue (as per Barbeta et al., 2022) and hydrogen exchange with organics (as per Chen et al., 2020), with hydrogen exchange being the dominant process. Diao et al. (2022) propose an alternative perspective, suggesting that while hydrogen exchange does occur, CVD-induced biases are mostly related to isotopic fractionation during CVD extraction, and that extracting larger volumes of plant water through CVD can reduce these biases. In our case, the exact CVD-extracted volumes were not recorded, but were on average 0.25 mL. According to Diao et al. (2022), this small sample volume size could lead to a δD bias roughly ranging from –10 to –35‰, consistent with our observations – although we note that the volume effect would be specific to the CVD setup used.

Overall, our results add to the growing empirical evidence that sampling xylem water yields more reliable estimates of plant water sources, compared to methods analysing bulk stem water.

Cryogenic bias is species-specific, but independent of stem water content and status

Our data indicate that while the CVD-induced δD bias was apparent across seven tree species, it affected each species differently, with mean biases ranging from –19.3‰ (*C. bella*) to –9.1‰ (*M. argentea*). These results support the findings of Chen et al. (2020), who showed via rehydration experiments that CVD-induced biases differed in the range –5 to –11‰ across nine species, and those of Barbeta et al. (2022) who observed significant inter-specific differences in the range –12.7 to –22.3‰ across three species. More broadly, the global synthesis by de la Casa et al. (2022) suggests considerable variations in δD biases across different species – although this study inferred biases based on δD offset calculations using source water, rather than through direct measurements.

Variability in δD biases within single species was relatively low, although the small number of replicate trees was a limitation of our study – except for *H. arborescens* for which we have data for seven individual trees. For this species, the measured δD biases varied between –20.4 and –13.6‰. Limited within-species variation was also reported by Barbeta et al. (2022) for *Fagus sylvatica* and by Bowers and Williams (2022) for a range of conifer species. Wen et al. (2022) found large variations in the δD of xylem water between apple trees of the same species (and even within single individuals), but their δD biases were less variable, similar to our findings.

CVD-induced δD biases were not correlated with RSWC or stem water isotopic composition. This is at odds with recent laboratory studies where stem water content emerged as a key driver of the CVD δD bias. Chen et al. (2020) and Wen et al. (2022) showed that a higher stem content resulted in a lower bias, and Wen et al. (2022) showed that more δD-depleted stem water resulted in lower biases. However, these two studies were rehydration experiments so may not be reflective of natural conditions. Our results are more in line with those of Barbeta et al. (2022), Bowers and Williams (2022) and He et al. (2023) who found, under field conditions, non-significant or weakly significant relationships between RSWC and δD bias.

CVD-induced δD biases were not correlated with pre-dawn LWP either. This lack of a relationship between δD biases and water availability suggests that the extent of tree water stress may not affect the CVD bias. Bowers and Williams (2022) observed a negative correlation between species-specific xylem vulnerability to cavitation and δD bias, and hypothesised that less vulnerable species might have less well-mixed xylem conduits, potentially leading to higher δD biases. While our data do not allow us to test this hypothesis, it is plausible that the observed inter-specific differences may be related to anatomical differences between species, rather than to point-in-time water stress conditions (e.g. differences in connectivity of xylem conduits, variable xylem residence times; Bowers and Williams (2022)). The wide range of observed LWPs (–1.97 to –0.18 MPa) suggests that the sampled species may represent a spectrum of anatomical adaptations to aridity, given this is a strong driver shaping stem hydraulic traits in Australian trees (Peters et al., 2021). Overall, there is a clear need for research that further untangle the respective roles of anatomical and functional tree properties in CVD-induced δD biases.

Concluding remarks

Our dataset provides robust evidence of (1) a strong δD bias in CVD-extracted bulk stem water relative to xylem water and source water, (2) significant differences in the magnitude of these biases among tree species, and (3) the limited influence of RSWC and LWP in explaining variations in δD bias. However, our inability to extract sufficient water from some stem samples resulted in a low number of replicates per species. These low numbers might have hindered the detection of any species-specific patterns in the data, suggesting that our third conclusion might not hold for individual species. To improve water extraction yields, particularly for trees in seasonally dry environments, we recommend using a larger version of the Cavitron (500-mm diameter), which can host longer stems hence yield higher water volumes.

In the context of future plant water sourcing studies, we recommend that non-destructive extraction techniques that target xylem water, such as Cavitron centrifugation (e.g. Barbeta et al., 2022; He et al., 2023),

pressure chamber (e.g. Wen et al., 2023; Zuecco et al., 2022) or *in-situ* techniques (e.g. Kübert et al., 2023; Kühnhammer et al., 2022), be preferred over CVD extraction. Should no alternative be available, one should ensure that large volumes (specific to each experimental setup) are extracted via CVD (Diao et al., 2022). In any case, the similarities we found between average δD biases and average δD offsets lead us to the conclusion that CVD-derived bulk stem water isotopic signatures can potentially be corrected to provide a reasonable approximation of xylem water isotopic signatures. Yet this adjustment can only be done using a site-specific δD offset, a step that requires the local source water line to be known.

Unlike Chen et al. (2020), we discourage the indiscriminate use of a uniform offset correction for all sites, because δD offsets may be highly site- and method-dependent (Diao et al., 2022; Millar et al., 2018). We also recommend using an average, site-specific δD offset for the correction of CVD data (Duvert et al., 2022) rather than individual offsets (Barbeta et al., 2019; He et al., 2023), as using individual offsets for each sample eliminates the natural variability in δD among samples. In turn, this can introduce additional uncertainties to plant water source identification. Until a complete understanding of the mechanisms generating δD offsets is achieved, corrections of CVD data should be made with a high degree of caution, and researchers should consider assessing plant water sources based on $\delta^{18}O$ data alone.

Our work emphasises how considering an appropriate methodology when seeking to characterise plant water uptake is key to advancing our understanding of the role of vegetation in partitioning rainfall into evapotranspiration and recharge. From a water resource management perspective, this field of research is also becoming increasingly relevant, particularly in areas where exploited groundwater systems support groundwater-dependent ecosystems of ecological and cultural significance.

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