Received: 1 September 2022 | Revised: 17 March 2023 | Accepted: 27 March 2023

DOI: 10.1002/eco.2547

RESEARCH ARTICLE

WILEY

Differences between stem and branch xylem water isotope composition in four tropical tree species

Md. Shawkat I. Sohel[1,2,3] | John Herbohn[1,3] | Magali F. Nehemy[4] | Jeffrey J. McDonnell[5,6]

[1]Tropical Forestry Group, School of Agriculture and Food Sciences, The University of Queensland, St. Lucia, Queensland 4072, Australia

[2]Department of Environmental Science and Management, North South University, Dhaka, Bangladesh

[3]Tropical Forests and People Research Centre, University of the Sunshine Coast, Maroochydore, Queensland 4556, Australia

[4]Trent School of the Environment, Trent University, Peterborough, ON K9L 0G2, Canada

[5]Global Institute for Water Security and School of Environment and Sustainability, University of Saskatchewan, Saskatoon, SK S7N 3H5, Canada

[6]School of Geosciences, University of Aberdeen, Aberdeen, Scotland AB24 3FX, UK

Correspondence
Md. Shawkat I. Sohel, Tropical Forests and People Research Centre, University of the Sunshine Coast, Maroochydore, Queensland 4556, Australia.
Email: shawkat.sohel@northsouth.edu

Funding information
IPRS

Abstract

Stable isotope studies (δ^2H and $\delta^{18}O$) of water within plants are providing new information on water sources, competitive interactions and water use patterns under natural conditions. This is based on the assumption that there is no fractionation at the time of water uptake or during its transport within trees. However, previous studies have found fractionation does occur in water taken up through the roots in halophytic and xerophytic plants. It is unclear how widespread such fractionation is in other species. In this study, we tested this fractionation by comparing stem and branch xylem water isotopes over the period of one day (from 8 am to 5 pm) in four wet tropical rainforest tree species (*Dendrocnide photinophylla*, *Aphananthe philippinensis*, *Daphnandra repandula* and *Mallotus polyadenos*). We found branch water isotope ratios (δ^2H and $\delta^{18}O$) were enriched compared with stem xylem water isotopes. *D. photinophylla* had a significantly different branch $\delta^{18}O$ ratio than *A. philippinensis*, *D. repandula* and *M. polyadenos*. In contrast, there were no significant differences in stem xylem $\delta^{18}O$ among the four observed tree species. We found clear differences in the stable isotope ratios of $\delta^{18}O$ for stem and branch xylem water for *D. photinophylla* and *D. repandula* of upto −0.85% and 0.50%, respectively. Remarkable δ^2H differences were also found between stem and branch xylem water isotope ratios for *A. philippinensis*, *D. repandula* and *M. polyadenos*, being upto −12.14%, −16.17% and −9.65%, respectively. A dual isotope (δ^2H–$\delta^{18}O$) plot showed branch water values were more enriched than stem xylem water values for *D. photinophylla* and *D. repandula*, indicating a clear difference between stem and branch xylem water. This study suggests that δ^2H and $\delta^{18}O$ fractionation could be a species-specific phenomenon in tropical trees, which has important implications for plant water source identification and evapotranspiration partitioning.

KEYWORDS
branch water, fractionation, hydrogen isotope, isotope enrichment, oxygen isotope, stem water, xylem water

1 | INTRODUCTION

Stable isotope measurements are widely employed in plant-water relations research to investigate physiological and hydrological processes from whole plant to ecosystem scales. There has been an increasing number of studies using hydrogen and oxygen isotope ratios to study water uptake by plants (Bertrand et al., 2014; Dawson, 1993; Dawson & Ehleringer, 1991; Flanagan et al., 1992; Kukowski et al., 2013; Liu et al., 2014; Penna et al., 2013; Schwendenmann et al., 2015; Snelgrove et al., 2021; Tetzlaff et al., 2021; Thorburn & Walker, 1994; White et al., 1985), to undertake palaeoclimatic reconstructions based upon the isotopic analysis of wood cellulose (Ballantyne et al., 2006; Burk & Stuiver, 1981; Masson-Delmotte et al., 2005; O'Reilly Sternberg, 2009; Ramesh et al., 1986; Rebetez et al., 2003; Robertson et al., 2001; Saurer et al., 1997; Treydte et al., 2006), and evapotranspiration partitioning (Liebhard et al., 2022). Many of these studies assume that there is no fractionation at the time of water uptake or during its transport within trees, and others have raised fractionation as a possible explanation for the mismatch between tree water and soil water (e.g., Snelgrove et al., 2020; Tetzlaff et al., 2021). However, a large fractionation of the water taken up through the roots in halophytic and xerophytic plants, and in plants associated with mycorrhizal fungi has also been observed (Adar et al., 1995; Barbeta et al., 2019; Ellsworth & Williams, 2007; Lin et al., 1993; Poca et al., 2019). Additionally, Zhao et al. (2016) reveal that large deuterium fractionation occurred between source water and plant tissue waters in *Populus euphratica*, which is the only deciduous tree species found in deserts. Isotopic fractionation phenomena have become more commonly reported in plant water investigations (Barbeta et al., 2019; Evaristo et al., 2017; Marshall et al., 2020; Nehemy et al., 2022; Oerter & Bowen, 2017, 2019; Vargas et al., 2017; Vega Grau et al., 2021). This has challenged the commonly accepted idea that the isotopic composition of xylem water will solely reflect source water. The mechanisms that drive observed xylem water offset and potential internal fractionation are not well known. A recent meta-analysis suggested that this observed xylem offset is larger in cold and wet sites, but smaller in hot climates (De la Casa et al., 2022).

Information on isotope fractionation and xylem water offset from soil water sources is critically important for studying plant water sources, palaeoclimatic reconstruction, and isotope-based evapotranspiration partitioning. Therefore, there is an urgent need to investigate this phenomenon across diverse plant species and bioclimatic regions. For instance, it is uncertain whether an isotopic fractionation in different tissue water can be detected in tropical rainforest tree species. Here, we investigate the internal variability in stem and branch xylem isotopic composition across different tree species growing in the same environment. We set the null hypothesis that xylem water isotopic composition is similar between stem and branches. To test this hypothesis, we investigated xylem water within daylight hours (i.e., from 8 am to 5 pm) among four wet tropical rainforest species. We asked the following research question:

How does tree stem xylem water compare to water samples from branches over a short period of time?

2 | MATERIALS AND METHODS

2.1 | Sampling location and studied species

The study site was located in a wet tropical rainforest in the Danbulla State Forest on the Atherton Tableland in northeastern Australia (Figure 1). The site is at an elevation of 760 m above sea level (Drake & Franks, 2003). Trees were located within a long-term experimental plot established by the Queensland Department of Forestry in 1948. The plot measures 200 m by 20 m (4,000 m^2 or 0.4 ha). Regular measurements of the growth, mortality and recruitment of trees within the plot that are greater than 10 cm diameter at breast height (DBH) have been undertaken since the plot's establishment. Soils within the plot are sandy clay loams to clay loams. The soils have developed from basalt set down in volcanic lava flows between the Pliocene to Holocene eras (Laffan, 1988). The annual average rainfall for the site is 1,680 mm, with over 1,000 mm falling between December and February (Drake & Franks, 2003). The area can be prone to seasonal droughts resulting from infrequent rainfall during the typically drier months of April to October (Drake & Franks, 2003). Most of the plant species in the study site are highly moisture-dependent (Drake & Franks, 2003; Tracey, 1982).

2.2 | Plant and soil sample collection

To investigate the isotopic differences between stem and branch xylem water, an opportunistic sampling of trees within the plot was conducted within a short period of time during daylight hours (i.e., from 8 am-5 pm) in a single campaign. Sampled trees were selected based on the ease of access to their branches using an extendable pruner. This resulted in trees of four species being selected for sampling: –*Dendrocnide photinophylla*, *Aphananthe philippinensis*, *Daphnandra repandula* and *Mallotus polyadenos*. For each species, four individual trees were sampled. Four branches and stem samples were considered for each species. Details of the sampled tree species, including their sample size, successional status and the DBH of individual trees, are given in Table 1. Xylem samples were collected from tree stems using a battery-operated drill with an 18 mm spade bit. A single xylem sample was collected from each tree. The xylem samples were collected from the northern side of the tree trunk at just above breast height (i.e., >1.37 m). Before collecting the samples, all the bark tissues were removed. To distinguish between sapwood and heartwood, a visual observation technique was applied. In most cases, there was a very clear colour distinction between heartwood and sapwood, which was also true for our sampled tree species. Branch samples were also collected from the same tree from where stem xylem samples were collected. All the branches were under shaded conditions. All the branch's diameters

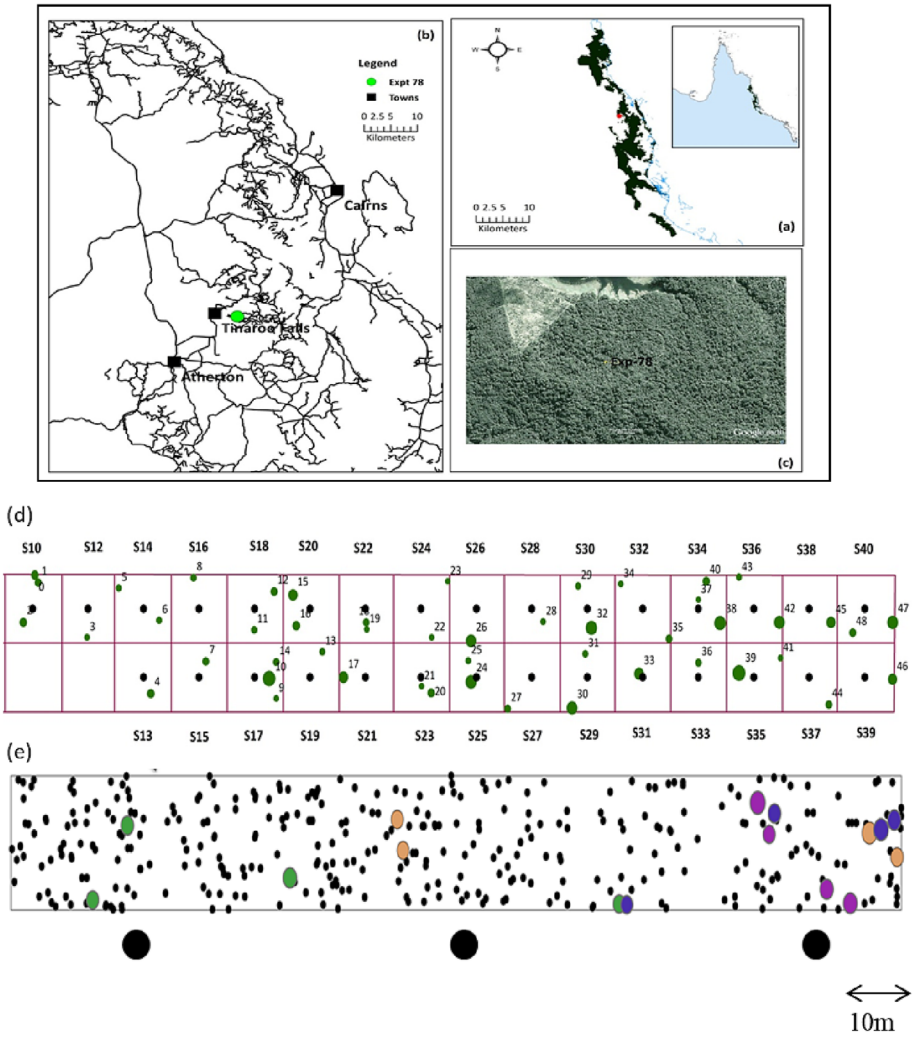

FIGURE 1 (a–c) Location of the study area. Green colour circle indicate the permanent plot where the study has been conducted. (d) The spatial distribution of all the studied tree species throughout the 20 × 160 m plot. Different sizes of the green circles indicate DBH differences among the trees. The whole plot was then divided into a 10 × 10 m grid. The codes outside of the grid indicate the grid ID. The small black dots in the figure show centre of the sub-plot. The numbers within the grid represent the tree species ID. Species details can be found in Sohel et al. (2021). (e) The coloured circles indicate the locations of the trees assessed for stem and branch xylem water isotope differences—green is *Mallotus polyadenos*, blue is *Dendrocnide photinophylla*, purple is *Daphnandra repandula* and brown is *Aphananthe philippinensis*. The different sizes of the coloured circles represent the diameter differences among the sampled trees (Table 1). The black circles outside of the plot (20 m away from the south edge of the plot) indicate the locations of the boreholes used for soil sample collection. Small black colour circle.

TABLE 1 Sampled tree species information.

Species	Common name	Family	Growth form	Successional status	Sample (branch + stem)	DBH (cm) of the sampled trees
A. philippinensis	Grey Handlewood	Ulmaceae	Moderate	Late secondary	4 + 4 = 8	122, 128, 138, 184
D. Repandula	Sassafras	Atherospermataceae	Slow	Mature phase	4 + 4 = 8	131, 160, 172, 208
M. Polyadenos	Kamala	Euphorbiaceae	Very fast	Pioneer	4 + 4 = 8	151,155, 167, 190
D. Photinophylla	Stinging tree	Urticaceae	Very fast	Pioneer	4 + 4 = 8	120, 135, 152, 181

N.B. Species successional status and growth form are based on Goosem and Tucker (2013).

were <5 cm. We did not record cardinal direction and the height from which the branch samples were collected. The extracted xylem tissues were placed into 24 ml glass vials, and the vials were immediately sealed and wrapped in parafilm. Samples were then placed into a refrigerator until laboratory analysis was undertaken. Soil samples were collected from three holes at six different depths (0 cm, 20 cm, 40 cm, 60 cm, 80 cm and 100 cm). Surface soil (0 cm) samples were considered because of the very shallow rooting pattern of the sampled trees. Here surface soil (0 cm) means scraping the surface until 3–5 cm. The upper 50 cm soil was mostly sandy, and the lower 50 cm soil was mainly composed of hard clay. All three soil boreholes (Figure 1) had similar soil textures. To define soil shallow and deeper soil depth, ecologist generally considers the rooting depth of vegetation. In this experimental plot, majority of the trees have shallow root systems (<50 cm). So, in that sense, we have considered <50 cm as shallow soil depth and > 50 cm as deep soil layer. The locations of the boreholes in relation to the trees are shown in Figure 1. All soil samples were placed in capped glass vials, wrapped with parafilm, placed into a refrigerator, and stored until laboratory analysis.

2.3 | Fractionation investigation through water stable isotope analysis

Water was extracted from the xylem (stem and branch) and soil samples using cryogenic vacuum distillation (Koeniger et al., 2011). The extracted water was then analysed for δ^2H and $\delta^{18}O$. In the case of δ^2H, an established method on a Delta V Advantage mass spectrometer and an HDevice peripheral were used. For $\delta^{18}O$, samples were run using an established method on a Delta V Advantage mass spectrometer and a GasBench II peripheral. For a description of this methodology, see Nelson (2000).

All δ^2H and $\delta^{18}O$ values were expressed as a percentage (%) relative to Vienna Standard Mean Ocean Water (VSMOW), using the following formula:

$$\delta^2H \text{ or } \delta^{18}O = \left(R_{sample}/R_{standard} - 1\right)1000$$

where R is the ratio of $^{18}O/^{16}O$ or $^{2}H/^{1}H$ in the sample or in Standard Mean Ocean Water (VSMOW).

The precision of analyses of δ^2H and $\delta^{18}O$ (including sampling, extraction and analytical errors) were estimated to be $+/-$ 1 permil and $+/-$ 0.2 permil, respectively. ANOVA was applied to know the differences in isotopic composition (Oxygen and Hydrogen water isotope) among soil, branch and stem xylem tissue among the sampled tree species since the data were normally distributed. Independent sample t-test was used to determine species-specific differences in branch and stem xylem water isotopic composition. In addition to that species-specific isotopic differences and their analytical precision was addressed by applying 95% bootstrapped confidence intervals (CIs) across different plant parts. Here, narrower CIs bar indicates lower uncertainty while wider CIs represent higher uncertainty in the samples to represent population parameters.

3 | RESULTS

3.1 | Differences in $\delta^{18}O$ and δ^2H isotope composition in stem and branch xylem water

Independent sample t-test showed statistically significant differences between branch and stem xylem hydrogen isotope ratios ($p < 0.05$), whereas oxygen isotope ratios showed no significant differences ($p > 0.05$) when species-specific variation was not considered (Figure 2). Statistically significant differences in branch $\delta^{18}O$ were found among the sampled tree species: *D. photinophylla* had significantly different branch $\delta^{18}O$ values than *A. philippinensis*, *D. repandula* and *M. polyadenos* (Figure 3). In contrast, there were no significant differences in stem xylem $\delta^{18}O$ among the four observed tree species (Figure 3). There were distinct differences between the branch and stem xylem $\delta^{18}O$ within the *A. philippinensis* and *D. photinophylla* trees (Table 2). The mean differences between stem and branch xylem water $\delta^{18}O$ were 0.50‰ and −0.85‰ for *A. philippinensis* and *D. photinophylla*, respectively (Table 2). There were no differences between branch and stem xylem $\delta^{18}O$ within the *D. repandula* and *M. polyadenos* trees (Table 2). There were also distinct patterns in the difference between branch and stem xylem δ^2H within the *A. philippinensis*, *D. repandula* and *M. polyadenos* trees (Table 2). All the species branch and stem show statistically significant differences in hydrogen isotope ($p < 0.05$). (Figure 4). The dual isotope plot shows both *A. philippinesis* and *M. polydenos* stem water seems to be more depleted in δ^2H than branch water. The stem water plots "straight" below the branch water indicates depleted in δ^2H. However, branch water were more enriched for both isotope than stem xylem water isotope values for the *D. photinophylla* and *D. repandula* trees (Figure 5), indicating a clear difference between the stem and branch xylem waters. It seems that for *D. photinophylla* branch water in more enriched in both heavy isotopes ($\delta^{18}O$ and δ^2H) than stem water, but in *D. repandula* this difference seems to be only in δ^2H. It seems that *D. repandula* shows the same pattern as the other two species. The only species not following the observed pattern (stem water being more depleted in δ^2H than branch water) is the *D. photinophylla*.

3.2 | Differences in $\delta^{18}O$ and δ^2H isotope composition between soil water and xylem (stem and branch) water

Soil water $\delta^{18}O$ and δ^2H showed a regular pattern of isotopic enrichment with samples near the soil surface (Figure 5), indicating that water in the upper soil layers was subjected to evaporative isotopic fractionation. For all of the observed tree species except *D. photinophylla* and *D. repandula*, the stem xylem water stable isotope values were consistent with water use from shallow soil depths (<60 cm deep; Figure 5). There were significant differences in δ^2H values between soil water and stem xylem water ($p > 0.05$) for all species except *D. repandula* (Figure 5 and Table 2). δ^2H and $\delta^{18}O$ showed significant differences in the values between soil water and stem water for all the species ($p > 0.05$). Branch water isotope values of *D. photinophylla* and *M. polyadenos* are more enriched and are significantly different than soil water isotope values (Figure 5 and Table 2).

4 | DISCUSSION

Plant functional trait divergence, such as different morphological traits, allows tree species to exploit soil water resources differently (Dawson & Ehleringer, 1991; Trogisch et al., 2016). Therefore, there is the possibility of isotopic composition variation among different tree species. However, less is known about this potential variation at spatial scales and within individual trees. A recent study in a humid temperate climate showed large soil water spatial heterogeneity but small variability in xylem isotopic composition within individual tree crowns and among crowns of distinct individuals of two species (*Fagus sylvatica* and *Picea abies*) (Goldsmith et al., 2019). A controlled

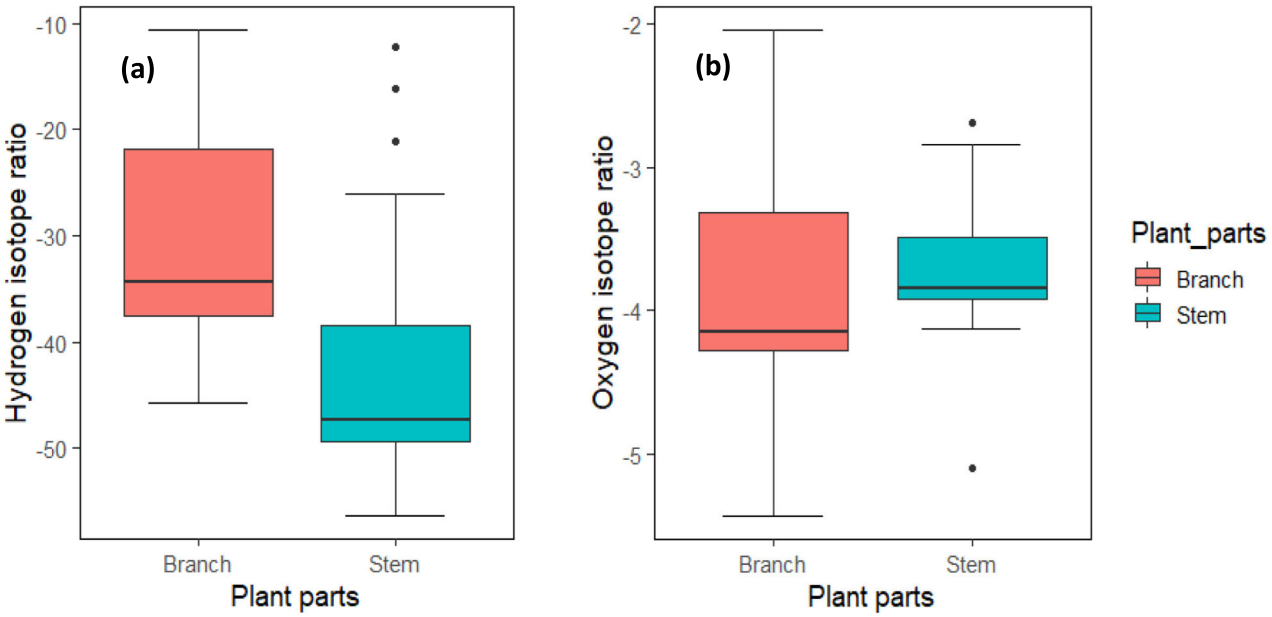

FIGURE 2 Branch and stem xylem hydrogen (a) and oxygen (b) isotope differences were displayed using boxplot. Line within the box plots shows the median of hydrogen and oxygen isotope ratios observed for the sampled trees. Edges of box show lower and upper quartiles, and whiskers show the maximum and minimum number of isotope ratios. Black dots show outlier observations. Independent sample t-test shows significant differences in branch and stems hydrogen isotope ratios ($p < 0.05$), whereas oxygen isotope ratios show no significant differences ($p > 0.05$). A total of 16 branch and 16 stem samples of four species were considered to show the differences.

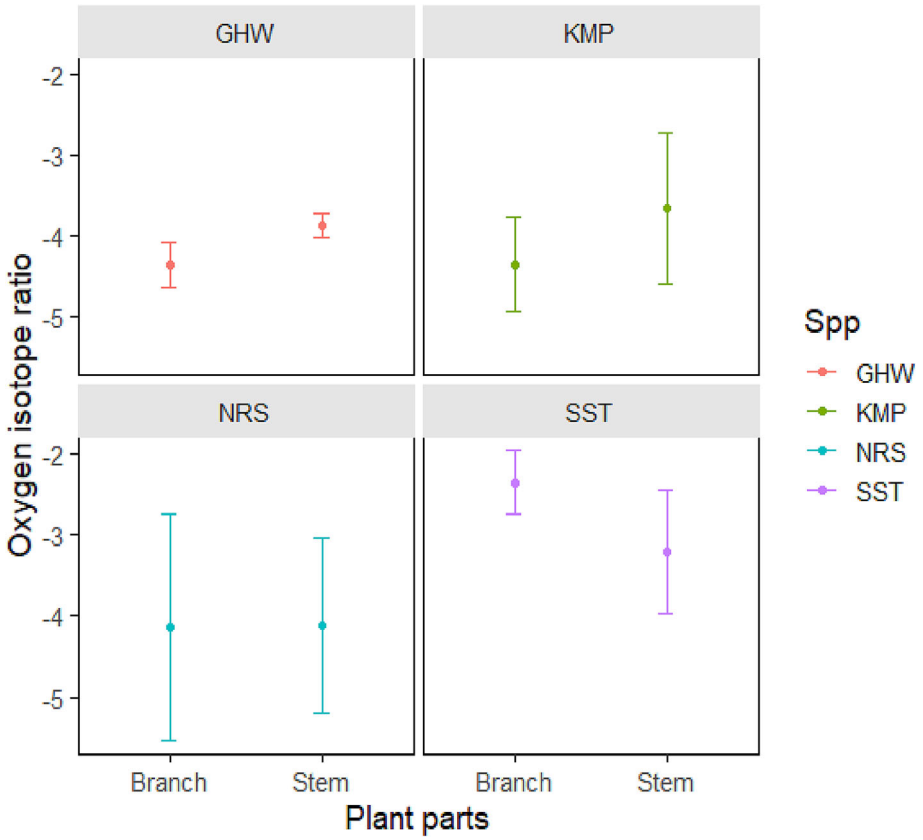

FIGURE 3 Branch and stem xylem oxygen isotope differences among the observed species. A point indicates mean values of the isotopic rations while bars around the means indicate their 95% bootstrapped confidence intervals (CIs) across different plant parts. Here, SST = *D. photinophylla*, KMP = *M. polyadenos*, GHW = *A. philippinensis*, NRS = *D. repandula*. Independent sample t-test shows species-specific significant differences in branch and stem oxygen isotope ratios ($p < 0.05$). Only NRS did not show any statistically significant difference ($p > 0.05$). A total of 16 branch and 16 stem samples of four species were considered to show the differences.

TABLE 2 The differences in isotopic composition (oxygen and hydrogen water isotope) among soil, branch and stem xylem tissue among the sampled tree species.

	Differences in hydrogen water isotope				Differences in oxygen water isotope			
	A. philippinensis:	*M. Polyadenos*:	*D. Repandula*:	*D. Photinophylla*:	*A. philippinensis*:	*M. Polyadenos*:	*D. Repandula*:	*D. Photinophylla*:
Stem-branch								
Mean	−**12.14****	−**9.65****	−**16.17****	−**6.53**^{ns}	**0.50****	**0.70**^{ns}	**0.02**^{ns}	−**0.85****
Max	−50.46	−50.05	−47.23	−26.10	−4.62	−4.90	−5.43	−3.83
Min	−34.29	−36.24	−23.51	−10.72	−3.72	−2.85	−3.55	−2.05
SD	6.71	6.19	9.28	5.61	0.30	0.59	0.72	0.58
Soil-stem								
Mean	**7.27****	**10.19****	**3.13**^{ns}	−**22.17****	−**1.46****	−**1.67****	−**1.20****	−**2.11****
Max	−50.46	−56.29	−56.29	−56.29	−6.84	−6.84	−6.84	−6.84
Min	−31.52	−36.15	−37.01	−12.22	−3.72	−2.85	−2.85	−2.69
SD	5.20	5.23	4.65	9.16	0.86	0.95	1.01	1.12

Note: The "**" ($p < 0.05$) indicates a significant difference between stem and branch xylem water and the "ns" ($p > 0.05$) indicates a non-significant difference. Branch sample/species = 4 and stem sample/species = 4.

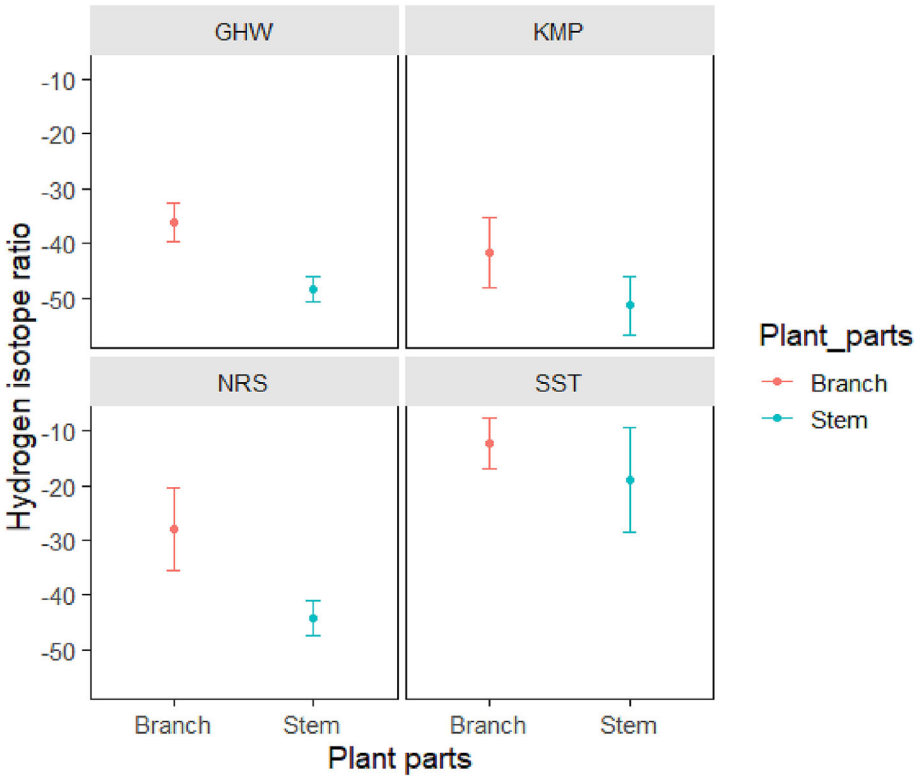

FIGURE 4 Branch and stem xylem hydrogen isotope differences among the observed species. A point indicates mean values of the isotopic rations while bars around the means indicate their 95% bootstrapped confidence intervals (CIs) across different plant parts. Here, SST = *D. photinophylla*, KMP = *M. polyadenos*, GHW = *A. philippinensis*, NRS = *D. repandula*. Independent sample t-test shows species specific significant differences in branch and stem hydrogen isotope ratios ($p < 0.05$). All the species branch and stem shows statistically significant differences in hydrogen isotope ($p < 0.05$). Total of 16 branch and 16 stem samples of four species were considered to show the differences.

experiment with two olive trees (*Olea europaea*; 1.9 m tall and 6 years old) showed no significant differences in isotopic composition between the xylem collected from the stem and branches (Amin et al., 2021). On the other hand, a recent study by Vega Grau et al. (2021) found evidence of xylem isotopic variability within trees, from roots to stems to branches in a tropical savanna. These differences were shown to affect the interpretation of water sources. We found that stem water was more depleted in δ^2H than branch xylem water in four wet tropical rainforest tree species (i.e., *Dendrocnide photinophylla*, *Aphananthe philippinensis*, *Daphnandra repandula* and *Mallotus polyadenos*).

We found large difference in δ^2H values between branch and stem xylem water for all the observed four species and a small difference in $\delta^{18}O$ for two species (Table 2; Figure 5). Despite the differences between stem and branch xylem water, both plotted within soil water isotopic distribution, with the exception of *D. photinophylla and D. repandula*. The evidence of distinct isotopic composition between

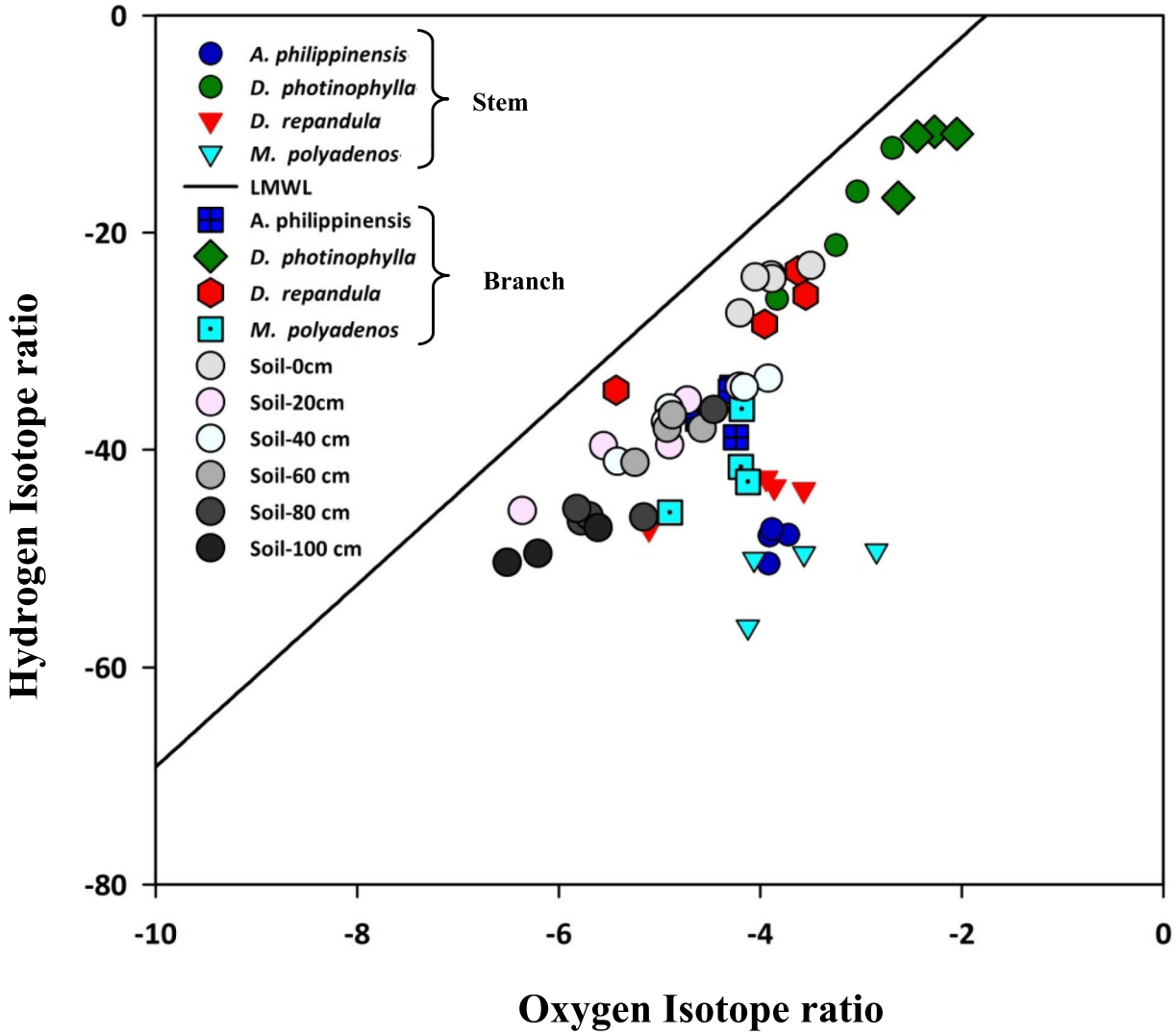

FIGURE 5 Oxygen and hydrogen dual isotope plot showing stem-branch-soil water isotope composition.

xylem water in the branch and stem could reflect different processes discussed below.

The observed difference between xylem isotopic composition in stem and branches could be explained by slow tree water transit times (i.e., days to weeks) (Nehemy et al., 2022; Seeger & Weiler, 2021). The stem and branch xylem isotopic composition represent distinct sampling locations along the water travel path length (i.e., sapwood) and could represent root water uptake from different days, as the water parcel slowly travels inside the tree because of the slow velocities. Another mechanism that could potentially explain the difference between stem and branch xylem water is the use of internally stored water outside sapwood (e.g., heartwood and phloem) that could impact stem and branch xylem water differently. A recent study showed evidence of functional exchange between heartwood and sapwood water in broadleaf species (Fabiani et al., 2022). Fabiani et al. (2022) showed that, overall, sapwood water in the stem is more depleted in heavy isotopes than heartwood in broadleaves. Assuming this same pattern in our broadleaves species, heartwood exchange could potentially explain the observed difference between stem and branch water. The sampled xylem water in the stem would show only sapwood water, whereas the water arriving in the branch (i.e., near the "outlet") could represent the overall contribution of storages (i.e., sapwood and more enriched heartwood water). Alternatively, another functional compartment that could result in direct exchange with xylem water and explain the observed difference between stem and branch water is phloem. Nehemy et al. (2022) showed that the isotopic difference between phloem and xylem water is larger in δ^2H than $\delta^{18}O$ and can increase with increased use of phloem water in periods of tree water deficit. Xylem water becomes more enriched in heavy isotopes with the increased use of phloem water, whereas phloem water becomes more depleted in heavy isotopes as result of this radial exchange (Nehemy et al., 2022). Larger stems generally have a lower proportion of phloem in relation to xylem (i.e., sapwood). Thus, the effect of phloem water exchange could be more easily observed in the branches, where proportionally a more significant volume of phloem water could contribute radially. This could result in

xylem water enrichment because of the preferential transport of lighter isotopes (i.e., specially ^{1}H) to phloem via the symplastic pathway (i.e., mediated by aquaporins) leaving behind heavier isotopologues during phloem recharge (Nehemy et al., 2022). However, more enriched phloem water in relation to xylem in trees have also been observed and explained by contribution enriched leaf water (Cernusak et al., 2005). The radial transfer of enriched phloem to xylem in the morning could potentially explain branch water enrichment in relation to stem water. Despite some plausible explanations, the direct measurement of heartwood and phloem water in both locations, stem and branch, would be necessary to clarify the dominant mechanism, and if those are important in the studied environment.

Previous research have found evaporative enrichment in suberized stems during leafless periods when trees are under water stress or reduced sap flow rates (Bertrand et al., 2014; Cernusak et al., 2005; del Castillo et al., 2016; Ellsworth & Sternberg, 2014; Martín-Gómez et al., 2016). We did not observe leafless periods, but reduced sap flow rates could be potentially observed in partially shaded branches and explains the observed difference between stem and branch water. Besides reduced transpiration, the exchange of xylem water with enriched water from the leaf could be another reason for branch water enrichment in relation to stem water (Brandes et al., 2007; Ellsworth & Williams, 2007) via back-diffusion of enriched leaf water (Dawson & Ehleringer, 1993).

Recent literature has also focused on seeking mechanisms to explain xylem water depletion in ^{2}H (Barbeta et al., 2022; Chen et al., 2020; Diao et al., 2022; Zhao et al., 2016). We observed this depletion pattern in some species. Stem xylem water was more depleted in $\delta^{2}H$ compared to branch and soil water (Figure 5). Some of these previous studies suggested that the observed depletion in hydrogen can be an artefact of the cryogenic extraction method. Chen et al. (2020) argued this is an artefact influenced by the proportional water content of the sample. In contrast, Diao et al. (2022) showed this might be instead related to an absolute extracted minimal water volume of each sample. Zhao et al. (2016) and Barbeta et al. (2022) suggest that this depletion is a result of the heterogeneity in tree water tissues. These studies indicate that intercellular water and water within xylem conduits are in equilibrium, whereas more compartmentalized water pools or pools with exchangeable H-atoms (i.e., cellulosic fibres, hydrophilic organic substances) and with more depleted $\delta^{2}H$ may exist, and does not participate in the transpiration stream but are extracted using the cryogenic method. This would explain our observations if such pools would be more commonly present in stem tissues rather than branch water, since same method was applied in both samples (i.e., cryogenic extraction). We do not have evidence that exclude or support this hypothesis. Allen and Kirchner (2022) emphasized the limitations of the existing methodology of water sources study and pointed that existing water extraction method and analysis techniques cannot fully explain water isotopic composition, water functioning and partitioning inside trees. Besides all this explanation, xylem sample collection procedure (battery operated drill) used in this study could also be a potential source of evaporative enrichment of xylem samples. The observed difference in xylem water variability within individual trees observed here and in other studies (Vega Grau et al., 2021), and the insignificant or lack of thereof in other studies (Amin et al., 2021; Goldsmith et al., 2019) highlights the importance of standardization in plant water source investigations (Millar et al., 2022).

5 | CONCLUSION

In this study, through extensive field-work utilizing four common wet tropical rainforest tree species, we have shown that there are significant species-specific differences in isotopic composition of branch and stem xylem water. Research into $\delta^{2}H$ and $\delta^{18}O$ fractionation has important implications for studying plant water sources, palaeoclimatic reconstruction and evapotranspiration partitioning. Commonly-held assumptions about isotopic fractionation could potentially lead to errors in water source calculations. Such miscalculations of water source could lead to misinterpretations of plant water source usage. Although our study did not resolve the mechanism responsible for species-specific differences in isotopic composition, it does provide strong evidence that species-specific fractionation of both $\delta^{2}H$ and $\delta^{18}O$ can occur.

ACKNOWLEDGEMENTS

We would like to thank Kim Janzen, Lab manager and isotope technician at the Global Institute for Water Security, University of Saskatchewan, Canada for her heartiest cooperation for extracting water from the samples and analysis. Thanks are also due to Kurt von Kleist for his great help during the field-work. This research was supported by a scholarship from the Australian Government through an IPRS (International Postgraduate Research Scholarship, currently named Research Training Program, RTP) to Md. Shawkat Islam Sohel. The research was also partially funded by the Wet Tropics Management Authority.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Adar, E. M., Gev, I., Li.p, J., Yakir, D., & Gat, J. R. (1995). Utilization of oxygen-18 and deuterium in stream flow for the identification of transpiration sources: Soil water versus groundwater in sand dune terrain. In E. Adar & C. Leibundgut (Eds.), *Application of tracers in arid zone hydrology* (Vol. 232) (pp. 329–338). International Association of Scientific Hydrology. Publication.

Allen, S. T., & Kirchner, J. W. (2022). Potential effects of cryogenic extraction biases on plant water source partitioning inferred from xylem-water isotope ratios. *Hydrological Processes*, *36*(2), e14483.

Amin, A., Zuecco, G., Marchina, C., Engel, M., Penna, D., McDonnell, J. J., & Borga, M. (2021). No evidence of isotopic fractionation in olive trees (*Olea europaea*): A stable isotope tracing experiment. *Hydrological Sciences Journal*, *66*(16), 2415–2430. https://doi.org/10.1080/02626667.2021.1987440

Ballantyne, A. P., Rybczynski, N., Baker, P. A., Harington, C. R., & White, D. (2006). Pliocene Arctic temperature constraints from the growth rings and isotopic composition of fossil larch. *Palaeogeography*

Palaeoclimatology Palaeoecology, *242*, 188–200. https://doi.org/10.1016/j.palaeo.2006.05.016

Barbeta, A., Burlett, R., Martín-Gómez, P., Fréjaville, B., Devert, N., Wingate, L., Domec, J. C., & Ogée, J. (2022). Evidence for distinct isotopic compositions of sap and tissue water in tree stems: Consequences for plant water source identification. *New Phytologist*, *233*(3), 1121–1132. https://doi.org/10.1111/nph.17857

Barbeta, A., Jones, S. P., Clavé, L., Wingate, L., Gimeno, T. E., Fréjaville, B., Wohl, S., & Ogée, J. (2019). Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrologyand Earth System Sciences*, *23*, 2129–2146.

Bertrand, G., Masini, J., Goldscheider, N., Meeks, J., Lavastre, V., Celle-Jeanton, H., Gobat, J. M., & Hunkeler, D. (2014). Determination of spatiotemporal variability of tree water uptake using stable isotopes ($\delta^{18}O$, $\delta^{2}H$) in an alluvial system supplied by a high-altitude watershed, Pfyn forest, Switzerland. *Ecohydrology*, *7*(2), 319–333. https://doi.org/10.1002/eco.1347

Brandes, E., Wenninger, J., Koeniger, P., Schindler, D., Rennenberg, H., Leibundgut, C., Mayer, H., & Gessler, A. (2007). Assessing environmental and physiological controls over water relations in a Scots pine (*Pinus sylvestris* L.) stand through analyses of stable isotope composition of water and organic matter. *Plant, Cell and Environment*, *30*, 113–127. https://doi.org/10.1111/j.1365-3040.2006.01609.x

Burk, R. L., & Stuiver, M. (1981). Oxygen isotope ratios in trees reflect mean annual temperature and humidity. *Science*, *211*, 1417–1419. https://doi.org/10.1126/science.211.4489.1417

Cernusak, L. A., Farquhar, G. D., & Pate, J. S. (2005). Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. *Tree Physiology*, *25*, 129–146. https://doi.org/10.1093/treephys/25.2.129

Chen, Y., Helliker, B. R., Tang, X., Li, F., Zhou, Y., & Song, X. (2020). Stem water cryogenic extraction biases estimation in deuterium isotope composition of plant source water. *Proceedings of the National Academy of Sciences, USA*, *117*(52), 33345–33350. https://doi.org/10.1073/pnas.2014422117

Dawson, T. E. (1993). Water sources of plants as determined from xylem-water isotopic composition: Perspectives on plant competition, distribution, and water relation. In J. R. Ehleringer, A. E. Hall, & G. D. Farquhar (Eds.), *Stable isotopes and plant carbon/water relations* (pp. 465–496). Academic Press. https://doi.org/10.1016/B978-0-08-091801-3.50040-4

Dawson, T. E., & Ehleringer, J. R. (1991). Streamside trees that do not use stream water. *Nature*, *350*, 335–337. https://doi.org/10.1038/350335a0

Dawson, T. E., & Ehleringer, J. R. (1993). Isotopic enrichment of water in the "woody" tissues of plants: Implications for plant water source, water uptake and other studies which use the stable isotopic composition of cellulose. *Geochimica et Cosmochimica Acta*, *57*, 3487–3492. https://doi.org/10.1016/0016-7037(93)90554-A

De la Casa, J., Barbeta, A., Rodríguez-Uña, A., Wingate, L., Ogée, J., & Gimeno, T. (2022). Isotopic offsets between bulk plant water and its sources are larger in cool and wet environments. *Hydrology and Earth System Sciences*, *26*(15), 4125–4146. https://doi.org/10.5194/hess-26-4125-2022

del Castillo, J., Comas, C., Voltas, J., & Ferrio, J. P. (2016). Dynamics of competition over water in a mixed oak-pine Mediterranean forest: Spatio-temporal and physiological components. *Forest Ecology and Management*, *382*, 214–224. https://doi.org/10.1016/j.foreco.2016.10.025

Diao, H., Schuler, P., Goldsmith, G. R., Siegwolf, R. T. W., Saurer, M., & Lehmann, M. M. (2022). Technical note: On uncertainties in plant water isotopic composition following extraction by cryogenic vacuum distillation. *Hydrology and Earth System Sciences*, *26*, 5835–5847.

Drake, P. L., & Franks, P. J. (2003). Water resource partitioning, stem xylem hydraulic properties, and plant water use strategies in a seasonally dry riparian tropical rainforest. *Oecologia*, *137*, 321–329. https://doi.org/10.1007/s00442-003-1352-y

Ellsworth, P., & Williams, D. (2007). Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant and Soil*, *291*(1–2), 93–107. https://doi.org/10.1007/s11104-006-9177-1

Ellsworth, P. Z., & Sternberg, L. S. L. (2014). Seasonal water use by deciduous and evergreen woody species in a scrub community is based on water availability and root distribution. *Ecohydrology*, *8*(4), 538–551. https://doi.org/10.1002/eco.1523

Evaristo, J., McDonnell, J. J., & Clemens, J. (2017). Plant source water apportionment using stable isotopes: A comparison of simple linear, two-compartment mixing model approaches. *Hydrological Processes*, *31*, 3750–3758.

Fabiani, G., Penna, D., Barbeta, A., & Klaus, J. (2022). Sapwood and heartwood are not isolated compartments: Consequences for isotope ecohydrology. *Ecohydrology*, *15*(8), e2478. https://doi.org/10.1002/eco.2478

Flanagan, L. B., Ehleringer, J. R., & Marshall, J. D. (1992). Differential uptake of summer precipitation and groundwater among co-occurring trees and shrubs in the southwestern United States. *Plant, Cell and Environment*, *15*(7), 831–836. https://doi.org/10.1111/j.1365-3040.1992.tb02150.x

Goldsmith, G. R., Allen, S. T., Braun, S., Engbersen, N., González-Quijano, C. R., Kirchner, J. W., & Siegwolf, R. T. (2019). Spatial variation in throughfall, soil, and plant water isotopes in a temperate forest. *Ecohydrology*, *12*(7), e2059.

Goosem, S., & Tucker, N. I. J. (2013). *Repairing the rainforest* (second edition). Wet Tropics Management Authority and Biotropica Australia Pty. Ltd.

Koeniger, P., Marshall, J. D., Link, T., & Mulch, A. (2011). An inexpensive, fast, and reliable method for vacuum extraction of soil and plant water for stable isotope analyses by mass spectrometry. *Rapid Communications in Mass Spectrometry*, *25*, 3041–3048. https://doi.org/10.1002/rcm.5198

Kukowski, K. R., Schwinning, S., & Schwartz, B. F. (2013). Hydraulic responses to extreme drought conditions in three co-dominant tree species in shallow soil over bedrock. *Oecologia*, *171*, 819–830. https://doi.org/10.1007/s00442-012-2466-x

Laffan, M. D. (1988). *Soils and land use on the Atherton tableland, North Queensland. Report no 61* (pp. 55–57). CSIRO Division of Soils.

Liebhard, G., Klik, A., Stumpp, C., & Nolz, R. (2022). Partitioning evapotranspiration using water stable isotopes and information from lysimeter experiments. *Hydrological Sciences Journal*, *67*, 646–661. https://doi.org/10.1080/02626667.2022.2030866

Lin, G., Sternberg, L., Ehleringer, J., Hall, A., & Farquhar, G. (1993). Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In *Stable isotopes and plant carbon-water relations* (pp. 497–510). Academic Press Inc. https://doi.org/10.1016/B978-0-08-091801-3.50041-6

Liu, W., Li, J., Lu, H., Wang, P., Luo, Q., Liu, W., & Li, H. (2014). Vertical patterns of soil water acquisition by non-native rubber trees (*Hevea brasiliensis*) in Xishuangbanna, Southwest China. *Ecohydrology*, *7*, 1234–1244.

Marshall, J. D., Cuntz, M., Beyer, M., Dubbert, M., & Kuehnhammer, K. (2020). Borehole equilibration: Testing a new method to monitor the isotopic composition of tree xylem water in situ. *Frontiers in Plant Science*, *11*, 358.

Martín-Gómez, P., Serrano, L., & Ferrio, J. P. (2016). Short-term dynamics of evaporative enrichment of xylem water in woody stems: Implications for ecohydrology. *Tree Physiology*, *37*, 511–522. https://doi.org/10.1093/treephys/tpw115

Masson-Delmotte, V., Raffalli-Delerce, G., Danis, P. A., Yiou, P., Stievenard, M., Guibal, F., Mestre, O., Bernard, V., Goosse, H.,

Hoffmann, G., & Jouzel, J. (2005). Changes in European precipitation seasonality and in drought frequencies revealed by a four-century-long tree-ring isotopic record from Brittany, western France. *Climate Dynamics*, *24*, 57–69. https://doi.org/10.1007/s00382-004-0458-1

Millar, C., Janzen, K., Nehemy, M. F., Koehler, G., Hervé-Fernández, P., Wang, N., Orlowski, A., Barbeta, J. J., & McDonnell, J. J. (2022). On the urgent need for standardization in isotope-based ecohydrological investigations. *Hydrological Procesess*, *36*(10), e14698.

Nehemy, M. F., Benettin, P., Allen, S. T., Steppe, K., Rinaldo, A., Lehmann, M. M., & McDonnell, J. J. (2022). Phloem water isotopically different to xylem water: Potential causes and implications for ecohydrological tracing. *Ecohydrology*, *15*(10), e2417.

Nelson, S. T. (2000). A simple, practical methodology for routine VSMOW/SLAP normalization of water samples analyzed by continuous flow methods. *Rapid Communications in Mass Spectrometry*, *14*(12), 1044–1046. https://doi.org/10.1002/1097-0231(20000630)14:12<1044::AID-RCM987>3.0.CO;2-3

Oerter, E. J., & Bowen, G. (2017). In situ monitoring of H and O stable isotopes in soil water reveals ecohydrologic dynamics in managed soil systems. *Ecohydrology*, *10*, e1841.

Oerter, E. J., & Bowen, G. (2019). Spatio-temporal heterogeneity in soil water stable isotopic composition and its ecohydrologic implications in semiarid ecosystems. *Ecohydrology*, *33*, 1724–1738.

Penna, D., Oliviero, O., Assendelft, R., Zuecco, G., van Meerveld, I., Anfodillo, T., Carraro, V., Borga, M., & Fontana, G. D. (2013). Tracing the water sources of trees and streams: Isotopic analysis in a small pre-alpine catchment. *Procedia Environmental Sciences*, *19*, 106–112. https://doi.org/10.1016/j.proenv.2013.06.012

Poca, M., Coomans, O., Urcelay, C., Zeballos, S. R., Bodé, S., & Boeckx, P. (2019). Isotope fractionation during root water uptake by *Acacia caven* is enhanced by arbuscular mycorrhizas. *Plant and Soil*, *441*, 485–497. https://doi.org/10.1007/s11104-019-04139-1

Ramesh, R., Bhattacharya, S. K., & Gopalan, K. (1986). Climatic correlations in the stable isotope records of silver fir (*Abies pindrow*) trees from Kashmir, India. *Earth and Planetary Science Letters*, *79*(1-2), 66–74. https://doi.org/10.1016/0012-821X(86)90041-5

Rebetez, M., Saurer, M., & Cherubini, P. (2003). To what extent can oxygen isotopes in tree rings and precipitation be used to reconstruct past atmospheric temperature? A case study. *Climatic Change*, *61*, 237–248. https://doi.org/10.1023/A:1026369000246

Robertson, I., Waterhouse, J. S., Barker, A. C., Carter, A. H. C., & Switsur, V. R. (2001). Oxygen isotope ratios of oak in East England: Implications for reconstructing the isotopic composition of precipitation. *Earth and Planetary Science Letters*, *191*, 21–31. https://doi.org/10.1016/S0012-821X(01)00399-5

Saurer, M., Borella, S., & Leuenberger, M. (1997). $\delta^{18}O$ of tree rings of beech (*Fagus silvatica*) as a record of $\delta^{18}O$ of the growing season precipitation. *Tellus Series B – Chemical and Physical Meteorology*, *49*(1), 80–92. https://doi.org/10.3402/tellusb.v49i1.15952

Schwendenmann, L., Pendall, E., Sanchez-Bragado, R., Kunert, N., & Hölscher, D. (2015). Tree water uptake in a tropical plantation varying in tree diversity: Interspecific differences, seasonal shifts and complementarity. *Ecohydrology*, *8*(1), 1–12. https://doi.org/10.1002/eco.1479

Seeger, S., & Weiler, M. (2021). Temporal dynamics of tree xylem water isotopes: In situ monitoring and modeling. *Biogeosciences*, *18*(15), 4603–4627. https://doi.org/10.5194/bg-18-4603-2021

Snelgrove, J. R., Buttle, J. M., Kohn, M. J., & Tetzlaff, D. (2020). Assessing ecohydrological separation in a northern mixed forest biome using stable isotopes. *Hydrology and Earth System Sciences Discussions*, *2020*(1), 1–30.

Snelgrove, J. R., Buttle, J. M., Kohn, M. J., & Tetzlaff, D. (2021). Co-evolution of xylem water and soil water stable isotopic composition in a northern mixed forest biome. *Hydrology and Earth System Sciences*, *25*(4), 2169–2186. https://doi.org/10.5194/hess-25-2169-2021

Sohel, M. S. I., Grau, A. V., McDonnell, J. J., & Herbohn, J. (2021). Tropical forest water source patterns revealed by stable isotopes: A preliminary analysis of 46 neighboring species. *Forest Ecology and Management*, *494*, 119355.

Sternberg, L. (2009). Oxygen stable isotope ratios of tree-ring cellulose: The next phase of understanding. *New Phytologist*, *181*(3), 553–562. https://doi.org/10.1111/j.1469-8137.2008.02661.x

Tetzlaff, D., Buttle, J., Carey, S. K., Kohn, M. J., Laudon, H., McNamara, J. P., Smith, A., Sprenger, M., & Soulsby, C. (2021). Stable isotopes of water reveal differences in plant–soil water relationships across northern environments. *Hydrological Processes*, *35*(1), e14023. https://doi.org/10.1002/hyp.14023

Thorburn, P. J., & Walker, G. R. (1994). Variations in stream water uptake by *Eucalyptus camaldulensis* with differing access to stream water. *Oecologia*, *100*, 293–301.

Tracey, J. G. (1982). *Vegetation of the humid tropical region of North Queensland*. CSIRO.

Treydte, K. S., Schleser, G. H., Helle, G., Frank, D. C., Winiger, M., Haug, G. H., & Esper, J. (2006). The twentieth century was the wettest period in northern Pakistan over the past millennium. *Nature*, *440*, 1179–1182. https://doi.org/10.1038/nature04743

Trogisch, S., Salmon, Y., He, J. S., Hector, A., & Scherer-Lorenzen, M. (2016). Spatio-temporal water uptake patterns of tree saplings are not altered by interspecific interaction in the early stage of a subtropical forest. *Forest Ecology and Management*, *367*, 52–61. https://doi.org/10.1016/j.foreco.2016.02.018

Vargas, A. I., Schaffer, B., Yuhong, L., & Sternberg, L. D. S. L. (2017). Testing plant use of mobile vs immobile soil water sources using stable isotope experiments. *New Phytologist*, *215*, 582–594.

Vega Grau, A. M., McDonnell, J., Schmidt, S., Annandale, M., & Herbohn, J. (2021). Isotopic fractionation from deep roots to tall shoots: a forensic analysis of xylem water isotope composition in mature tropical savanna trees. *Science of the Total Environment*, *795*, 148675. https://doi.org/10.1016/j.scitotenv.2021.148675

White, J. W. C., Cook, E. R., Lawrence, J. R., & Broecker, W. S. (1985). The D/H ratios of sap in trees: Implications for water sources and tree ring D/H ratios. *Geochimica et Cosmochimica Acta*, *49*, 237–246. https://doi.org/10.1016/0016-7037(85)90207-8

Zhao, L., Wang, L., Cernusak, L. A., Liu, X., Xiao, H., Zhou, M., & Zhang, S. (2016). Significant difference in hydrogen isotope composition between xylem and tissue water in *Populus euphratica*. *Plant, Cell & Environment*, *39*, 1848–1857. https://doi.org/10.1111/pce.12753

How to cite this article: Sohel, M. S. I., Herbohn, J., Nehemy, M. F., & McDonnell, J. J. (2023). Differences between stem and branch xylem water isotope composition in four tropical tree species. *Ecohydrology*, e2547. https://doi.org/10.1002/eco.2547