

Plant source water apportionment using stable isotopes: A comparison of simple linear, two-compartment mixing model approaches

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Abstract

Plant source water identification using stable isotopes is now a common practice in ecohydrological process investigations. Notwithstanding, little critical evaluation of the approaches for source apportionment have been conducted. Here, we present a critical evaluation of the main methods used for source apportionment between vadose and saturated zone water: simple mass balance and Bayesian mixing models. We leverage new isotope stem water samples from a diverse set of tree species in a strikingly uniform terrain and soil conditions at the Christchurch Botanic Garden, New Zealand. Our results show that using $\delta^2\text{H}$ alone in a simple, two-source mass balance approach leads to erroneous results, particularly an apparent overestimation of groundwater contribution to xylem. Alternatively, using both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in a Bayesian inference framework improves the source water estimates and is more useful than the simple mass balance approach, particularly when soil and groundwater contributions are relatively disproportionate. We suggest that plant source water quantification methods should take into consideration the possible effects of $^2\text{H}/^1\text{H}$ fractionation. The Bayesian inference approach used here may be less sensitive to $^2\text{H}/^1\text{H}$ fractionation effects than simple mass balance methods.

KEYWORDS

Bayesian inference, hydrogen isotope fractionation, mixing model, plant source water, water stable isotopes

1 | INTRODUCTION

Stable isotope ratios of hydrogen ($^2\text{H}/^1\text{H}$) and oxygen ($^{18}\text{O}/^{16}\text{O}$) in water within plants provide information on water sources, competitive interactions, and water-use patterns (Ehleringer & Dawson, 1992). Because root water uptake is generally considered a nonfractionating process (Dawson & Ehleringer, 1991; Zimmermann et al., 1966), the isotopic composition of xylem (i.e., plant stem) water represents an integrated signal of its sources in the subsurface. Thus, end-member mixing models have become a popular method in stable isotope plant-water studies. Nevertheless, the effects of different mixing models on the interpretation of source apportionment results are poorly understood. But quantifying the contributions from various sources in the subsurface (i.e., source proportions) is needed given the growing interest in the role of groundwater as a water source for vegetation in the Critical Zone (Evaristo & McDonnell, 2017; Fan, 2015). While recent work

(Evaristo, Jasechko, & McDonnell, 2015; Good, Noone, & Bowen, 2015; Zhang, Evaristo, Li, Si, & McDonnell, 2016) has provided evidence for ecohydrological separation (Brooks, Barnard, Coulombe, & McDonnell, 2010; McDonnell, 2014) whereby plants appear to use water of a character different to mobile water found in soils, groundwater, and streams, methods used for quantifying the contribution of groundwater to xylem water have yet to be critically examined.

Here, we examine the stable isotope composition ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) of 35 tree species (10 angiosperms, 25 gymnosperms), bulk soil water, and groundwater from two plots at the Christchurch Botanic Garden, New Zealand. We leverage a large number of different tree species together with the exceptionally homogenous soil water isotope depth profiles of two plots—one irrigated with known isotopic composition and one nonirrigated—to answer the following question regarding plant source water identification using stable isotope mixing models:

- How do two-source (vadose and saturated zone) mixing models compare using simple mass balance (SMB) and Bayesian mixing models (BMM)?

This paper seeks to demonstrate the pros and cons of the two main approaches by leveraging a diverse set of tree species isotope data in isotopically homogeneous soils and a well-characterized site.

1.1 | Mixing model theory

In the simplest case where xylem water may represent an integrated signal of two sources, the proportional contribution of each source may be resolved using a single isotope in a two-source system of mass balance equation (Dawson, 1993; Phillips & Ehleringer, 1995):

$$\delta_{xyl} = f_A \delta_A + f_B \delta_B \quad (1)$$

where δ_{xyl} is plant xylem water (either $\delta^2\text{H}$ or $\delta^{18}\text{O}$) and the proportions (f_A , f_B) of sources with isotopic signatures (δ_A , δ_B), respectively.

In underdetermined systems where the number of sources is greater than the number of isotopes plus one, the most widely used approach to date is a simple linear and iterative algorithm, providing the user with a range of feasible solutions (i.e., IsoSource; Phillips and Gregg, 2003). There are numerous reviews in the literature on the use of IsoSource and other approaches from simple linear to more complex process-based models (e.g., Ogle, Tucker, & Cable, 2014). Their advantages over simple two-source mixing models range from having the ability to assign feasibility estimates in multiple-source systems (e.g., IsoSource, IsoError), or better yet quantify the likelihood of sources via a more robust statistical approach (e.g., stable-isotope analysis in R [SIAR], Parnell, Inger, Bearhop, & Jackson, 2010), to having the ability to predict outcomes by incorporating biophysical parameters in a Bayesian framework (e.g., Ogle, Wolpert, & Reynolds, 2004). Ultimately, the utility of an isotope mixing model depends on two practical, nonmutually exclusive drivers: modelling goals of the research question and availability of pertinent model information. However, in many if not most cases, the latter driver precedes the former.

In early, mostly pre-2000s era studies when the cost of isotope analysis was prohibitive, the use of either $\delta^2\text{H}$ or $\delta^{18}\text{O}$ ("single isotope ratio") in most of the aforementioned approaches was deemed sufficient. As the cost of isotope analysis decreased, analysing for isotopes of both H and O ("dual isotope ratio") rapidly became the norm (see Evaristo & McDonnell, 2017). Consequently, the insights from H and O information, in the context of plant source water research, did not always reinforce each other. A single isotope ratio approach in a two-source model may lead to erroneous interpretations when root water uptake takes place simultaneously from several zones (Thorburn & Ehleringer, 1995), when soil water potentials dominate water uptake more than root distributions (Schwinning, Davis, Richardson, & Ehleringer, 2002), or in the case of saline and xeric environments where hydrogen isotope fractionation may be a confounding issue (Ellsworth & Williams, 2007; Lin & da Sternberg, 1993). Moreover, single isotope ratio approach in a multiple-source model (e.g., IsoSource) may yield "overlapping" feasibility solutions, with poorly constrained accounting of uncertainties (see Parnell et al., 2010).

The dual isotope ratio approach may provide information that is not apparent in the single isotope ratio method. Evaristo et al. (2015); Evaristo, McDonnell, Scholl, Bruijnzeel, and Chun (2016), for example, have shown that the single isotope analysis is problematic because the physical process of evaporation has a disproportionately greater effect on $\delta^{18}\text{O}$ than on $\delta^2\text{H}$ (Craig, 1961). This means that either isotope ratio may provide different information on potential water sources of plant water uptake (McDonnell, 2014). Conversely, the dual isotope ratio approach in a Bayesian framework (e.g., SIAR) may or may not be sensitive to processes such as H-fractionation, which has a disproportionately greater effect on $\delta^2\text{H}$ than on $\delta^{18}\text{O}$ (Ellsworth & Williams, 2007). These interpretation caveats notwithstanding a comparative assessment of outputs between models are lacking. One reason for this knowledge gap is in the nature of available datasets. Where variability in more than two sources may maximize the utility of a Bayesian approach (Leng et al., 2013), the same could not be resolved from first principles using mass balance (e.g., Equation 1). Conversely, where two-source mass balance may be invoked (Brunel, Walker, & Kennetsmith, 1995), a Bayesian approach may not be able to arrive at likely solutions if the two sources are in close proximity to each other. Altogether, examining the possible nuances of two-compartment mixing model approaches vis-à-vis processes such as H-fractionation ($^2\text{H}/^1\text{H}$ fractionation) could prove useful given the widespread and growing interest in source water partitioning approaches (Evaristo & McDonnell, 2017).

1.2 | Study site

Our study was conducted in the Christchurch Botanic Gardens, Christchurch, New Zealand (43°31'49"S 172°37'16"E). Christchurch has a temperate maritime climate with mean daily maximum/minimum temperatures of 22.5/12.2 °C and 11.3/1.7 °C in midsummer and mid-winter months, respectively. Mean annual rainfall is 625 mm and largely evenly distributed throughout the year.

The study site is generally flat with a maximum height above sea level of 6.7 m. The Avon River runs through the site dividing the botanic garden into a larger area (21 ha) within a bend of the river, and a smaller area (12 ha) outside the bend. The groundwater system underlying the Christchurch Botanic Gardens is at a point of transition from a semiconfined to confined aquifer with the occurrence of an approximately 3-m confining layer that overlies the aquifer system near the coast (Stewart, 2012; Taylor et al., 1989 and references therein). We identify and delineate the aquifers underlying the study site (m below ground level) following Blackstock (2011) and after the classification scheme of Weir (2007): Aquifer 1 (0–30 m), Aquifer 2 (30–80 m), Aquifer 3 (80–130 m), Aquifer 4 (130–190 m), and Aquifer 5 (>190 m). One of our two sampling locations (the Archery Lawn, "irrigated site") is located within the bend where plantings are irrigated as required using bore water drawn from an aquifer at a depth of 175 m (i.e., Aquifer 4). The soil of the Archery Lawn is a well-drained Waimakariri fine sandy loam. The second sampling location (the Pinetum, "nonirrigated") is located outside the river bend where plantings are not irrigated. The Kaiapoi soil at this site is a moderately well-drained fine sandy loam (Christchurch City Council 2007).

2 | MATERIALS AND METHODS

2.1 | Field samples

We performed our soil sampling and tree sampling on January 13 and 14, 2016. We sampled soil water with an auger. Samples were collected and stored in glass scintillation vials until they underwent cryogenic extraction. We followed Orłowski et al. (2016) procedures for extraction of soil and water. The trees were of diverse ages and dimensions as documented by field survey and Botanic Gardens documented records (Table 1). Tree samples were obtained from suberized branches for all tree species—wood chips were obtained in the field and placed immediately into glass scintillation vials until extraction—

to minimize the effect of evaporative enrichment by water loss through unsuberized stems (following Dawson, 1993). Soil water samples recovered were analysed on a Los Gatos laser spectrometer. All plant samples were run on an Isotope Ratio Mass Spectrometry (IRMS) mass spectrometer, due to possible spectral contamination of plant water. We also sampled stream water in the Avon River that flows through the gardens, Christchurch tap water and groundwater from the 175-m bore.

Historical mean monthly rainfall amount at Christchurch was 47 mm, ranging between 36 and 60 mm, with relatively even distribution throughout the year; the wettest months were July and August (60 mm), and the driest month was March (36 mm; Christchurch Botanic Gardens meteorological station). Monthly total rainfalls in the 3 months leading up to January 2016 were 12.1, 15.5, and

TABLE 1 Trees sampled at the irrigated Archery Lawn and the unirrigated Pinetum with age since planting (when known), diameter at breast height (DBH), overall tree height, and canopy spread

Species	Common name	Age (years)	DBH (m)	Height (m)	Spread (m)
Archery Lawn (irrigated)					
<i>Agathis australis</i>	Kauri	96	0.69	20	8
<i>Betula pendula</i>	Birch	96	0.77	20	20
<i>Cedrus atlantica</i>	Atlas cedar	126	1.57	25	23
<i>Chamaecyparis pisifera</i>	Sawara cypress	126	0.92	22	12
<i>Cryptomeria japonica</i>	Japanese cedar	59	0.33	14	7
<i>Eugenia uniflora</i>	Brush cherry	30	0.23	10	7
<i>Fagus sylvatica</i>	Beech	51	1.03	20	20
<i>Fraxinus excelsior</i>	Ash	126	1.15	26	24
<i>Larix kaempferi</i>	Japanese larch	126	0.68	19	18
<i>Liriodendron tulipifera</i>	Tulip tree	38	0.28	12	7
<i>Quercus coccinea</i>	Scarlet oak	96	1.08	26	30
<i>Quercus ilex</i>	Holm oak	126	1.51	25	26
<i>Quercus oblongata</i>	Himalayan oak	126	1.00	23	16
<i>Sequoia sempervirens</i>	Coast redwood	126	1.26	31	11
<i>Sequoiadendron giganteum</i>	Wellingtonia	126	2.01	33	14
<i>Taxus baccata</i>	Irish yew	126	1.00	12	10
<i>Tilia cordata</i>	Lime	126	1.36	28	26
<i>Ulmus glabra</i>	Elm	126	0.70	10	14
Pinetum (unirrigated)					
<i>Abies religiosa</i>	Sacred fir	36	0.7	18	10
<i>Cedrus deodara</i>	Deodar cedar	38	0.5	12	8
<i>Cryptomeria japonica</i>	Japanese cedar	32	0.35	15	6
<i>Cunninghamia lanceolata</i>	Chinese fir		0.57	20	8
<i>Cupressus duclouxiana</i>	Yunnan cypress	22	0.1	7	3
<i>Juniperus phoenicea</i>	Phoenician juniper	26	0.2	6	10
<i>Picea omorika</i>	Serbian spruce	24	0.11	6	4
<i>Pinus sabineana</i>	Digger pine	22	0.1	6	4
<i>Pinus brutia</i>	Turkish red pine		0.92	16	16
<i>Pinus montezumae</i>	Montezuma pine	21	0.26	12	6
<i>Pinus mugo</i>	Mountain pine	26	0.2	6	10
<i>Pinus muricata</i>	Bishop pine	26	1.34	25	15
<i>Pinus nigra 1</i>	Crimean pine	55	0.74	21	21
<i>Pinus nigra 2</i>	Black pine		0.13	6	4
<i>Pinus radiata</i>	Radiata pine	24	0.35	8	5
<i>Pinus sp.</i>	Pine		0.45	12	8
<i>Pinus walllichiana</i>	Bhutan pine	28	0.99	25	20

50.9 mm, respectively. In January 2016, 25.2 mm fell before January 13–14 sampling dates; 19.1 mm of this amount fell on January 3, a daily average of 2.1 mm.

2.2 | Source partitioning models

To answer our research question, we sought to compare model results from two approaches: simple mass balance (SMB) and Bayesian mixing model (BMM).

We represented the subsurface as two compartments: a vadose zone (“soil”) compartment and a saturated zone (“groundwater”) compartment. Examination of two-compartment models in ecohydrological studies is timely given the growing interest in the role of groundwater as a water source for vegetation at catchment (Evaristo et al., 2016; McDonald et al., 2015), continental (Maxwell, Condon, & Kollet, 2015), and global scales (Clark et al., 2015; Evaristo & McDonnell, 2017; Fan, 2015). Given that our groundwater samples were from a bore at a depth of ~175 m, we made the simplifying assumption that the isotopic composition of water from all underlying aquifers were not isotopically different from each other; future work at the site should systematically sample the underlying aquifers and explore possible differences in the isotopic composition of water in space and time. At the Archery Lawn (“irrigated”) site, therefore, the two-component mixing is between a soil water component and an irrigation plus shallow aquifer component. At the Pinetum (“nonirrigated”) site, the two-component mixing is between a soil water component and a shallow aquifer component (i.e., Aquifer 1). We define soil water component, sampled as bulk soil water and extracted via cryogenic vacuum distillation, as “less mobile” soil water following Evaristo et al. (2016). We refrain from identifying this soil water component as “tightly bound water” (*sensu* Brooks et al., 2010), given that we did not have soil lysimetry water isotopic measurements in this study, which might have represented the “more mobile” soil water component. Nonetheless, we make the distinction between the more mobile subsurface water expressed in the shallow aquifer and the less mobile subsurface water represented by our bulk soil water samples. Based on the assumption that xylem water represents an integrated signal of sources in the subsurface, we performed a single isotope ratio (i.e., either $\delta^2\text{H}$ or $\delta^{18}\text{O}$) two-source mass balance approach and calculated the fraction of groundwater f_{gw} contribution to xylem:

$$f_{\text{gw}} = \frac{\delta_{\text{xyl}} - \delta_{\text{soil}}}{\delta_{\text{gw}} - \delta_{\text{soil}}} \quad (2)$$

To be able to compare any discrepancies in the results, we solved Equation 2 using $\delta^2\text{H}$ and $\delta^{18}\text{O}$ independently. Rarely considered in plant source water identification studies, it has been shown that some trees in certain environments (Ellsworth & Williams, 2007; Lin & da Sternberg, 1993) do fractionate hydrogen isotopes (“ $^2\text{H}/^1\text{H}$ fractionation”) during uptake (Note: For consistency with terminology across multiple disciplines, here we use “ $^2\text{H}/^1\text{H}$ fractionation” to refer to fractionation between the deuterium and protium isotopes of hydrogen; Horita, 2005; Le Losq, Mysen, & Cody, 2016; Sachs & Kawka, 2015; Wang, Sessions, Nielsen, & Goddard, 2009). When the soil as a source can be modelled as a single compartment (i.e., minimal to nil isotopic variability with depth), the possible effect of $^2\text{H}/^1\text{H}$ fractionation can

be calculated as the magnitude of isotopic separation (Ellsworth & Williams, 2007):

$$\Delta^2\text{H} = \delta^2\text{H}_{\text{soil}} - \delta^2\text{H}_{\text{xyl}} \quad (3)$$

Positive $\Delta^2\text{H}$ is indicative of $^2\text{H}/^1\text{H}$ fractionation, while zero or negative $\Delta^2\text{H}$ indicates that there is no $^2\text{H}/^1\text{H}$ fractionation. Because $^2\text{H}/^1\text{H}$ fractionation violates the general assumption that root water uptake is a nonfractionating process (Dawson & Ehleringer, 1991), we used $\delta^{18}\text{O}$ to solve for Equation 2 instead of $\delta^2\text{H}$ if $\Delta^2\text{H}$ is positive. Nevertheless, we will show model results from using either $\delta^2\text{H}$ or $\delta^{18}\text{O}$ to demonstrate any discrepancies in source contribution estimates.

Uncertainties W in the SMB approach were quantified following Genereux (1998):

$$W_{f_{\text{soil}}} = \left\{ \left[\frac{\delta_{\text{gw}} - \delta_{\text{xyl}}}{(\delta_{\text{gw}} - \delta_{\text{soil}})^2} W_{\delta_{\text{soil}}} \right]^2 + \left[\frac{\delta_{\text{xyl}} - \delta_{\text{soil}}}{(\delta_{\text{gw}} - \delta_{\text{soil}})^2} W_{\delta_{\text{gw}}} \right]^2 + \left[\frac{-1}{(\delta_{\text{gw}} - \delta_{\text{soil}})} W_{\delta_{\text{xyl}}} \right]^2 \right\}^{1/2} \quad (4)$$

We then employed a simple linear mixing model—SIAR BMM statistical package (Parnell et al., 2010)—implemented in a Bayesian inference framework to determine the sources of water uptake of trees at both sites. The Bayesian framework presents a relatively new avenue of probabilistic modelling, particularly in biogeosciences, where datasets may exhibit high variance (Eddy, 2004). As with the SMB approach, SIAR was used here to determine the relative importance of the two compartments (soil water and groundwater) to xylem water using Markov Chain Monte Carlo (MCMC) methods. The trophic enrichment factor and concentration dependence of the original model were set to 0. The model was run with 500,000 iterations (discarding the first 50,000), and a source water's most likely contribution (i.e., the mean of the posterior distribution of the MCMC simulation) to xylem water was obtained. The justification for the choice of a uniform (i.e., noninformative prior) distribution was complemented by comparing the insights drawn from MCMC method (in proportion p -space) with the interpretation drawn from isotope biplot approach (in δ -space; Newsome, del Rio, Bearhop, & Phillips, 2007).

The BMM assumes that the variability and uncertainty associated with the sources are normally distributed. These sources of uncertainty (including measurement errors) are explicitly propagated into the posterior probability distributions.

3 | RESULTS

3.1 | How do potential subsurface water sources vary with depth?

Figure 1 shows the soil water isotope depth profiles at both sites. Soil $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values (mean \pm 1 SD) at the irrigated site (Figure 1a) were $-52 \pm 7.4\text{‰}$ and $-6.5 \pm 1.2\text{‰}$, respectively. While soil $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values at the nonirrigated site (Figure 1b) were $-53.5 \pm 7.8\text{‰}$ and $-6.6 \pm 1.8\text{‰}$, respectively, groundwater values were $-62 \pm 0.8\text{‰}$ and $-9.1 \pm 0.6\text{‰}$. Group means of $\delta^2\text{H}$, $\delta^{18}\text{O}$ and d -excess were not statistically different as determined by one-way ANOVA ($.25 < p < .92$ and $.08 < p < .20$ at irrigated and nonirrigated sites,

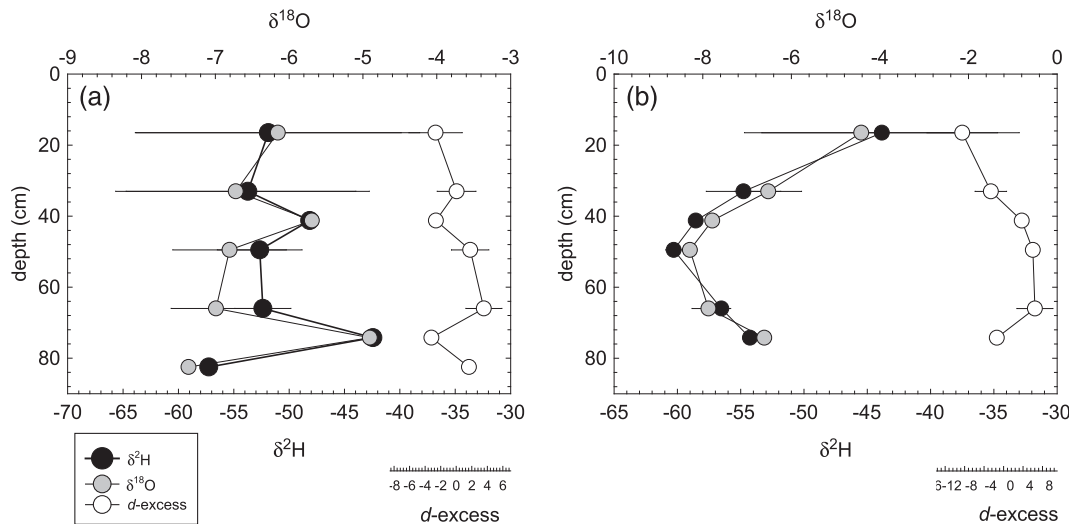


FIGURE 1 Soil water isotope depth profiles at irrigated (a) and nonirrigated (b) sites. Means of $\delta^2\text{H}$, $\delta^{18}\text{O}$ and $d\text{-excess}$ are not statistically different ($p > .10$, Tukey's honest significant difference (HSD) post hoc test) across depths at both sites. Error bars are 1 SD

respectively). The difference between soil water and groundwater were on average -8.8‰ and -2.6‰ $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively. The homogeneity (i.e., statistically insignificant differences) of soil water isotopes provides justification for treating the soil as the first compartment. The marked contrast between water in the unsaturated zone and deep groundwater (sampled from bore holes) sets the rationale for treating the latter as the second compartment.

3.2 | Where do the 35 tree species plot in relation to these sources and what are the differences between irrigated and nonirrigated?

Figure 2 shows all samples in $\delta^{18}\text{O}$ - $\delta^2\text{H}$ dual isotope space. The local meteoric water line ($\delta^2\text{H} = 7.7\delta^{18}\text{O} + 7.4$) and 95% confidence intervals are also shown (Blackstock, 2011). All soil and xylem samples plot to the right of the local meteoric water line, a consequence of evaporative processes as a water parcel infiltrates the unsaturated zone (Brunel et al., 1995). The fate and transport of unsaturated zone water—subject to cycles of evaporation, drainage, mixing, and redistribution (Barnes & Allison, 1988; Soderberg, Good, Wang, & Caylor, 2012)—therefore, results in a composite “mixing space” of evaporatively enriched waters. If or when root water uptake takes place from within the unsaturated zone, such space may be referred to as soil-xylem mixing space (Figure 2b inset). Nevertheless, it is apparent that many of the xylem samples plot outside the soil-xylem mixing space. Calculating the magnitude of isotopic separation (Equation 3), we found that 12 of 18 trees at the irrigated and 15 of 17 trees at the nonirrigated site showed signs of $^2\text{H}/^1\text{H}$ fractionation. In dual isotope space, $^2\text{H}/^1\text{H}$ fractionation results in a xylem sample plotting outside the soil-mixing space along the $\delta^2\text{H}$ axis (Figure 2b inset).

The calculated magnitude of isotopic separation was $4.7 \pm 5.6\text{‰}$ and $5.6 \pm 3.8\text{‰}$ at the irrigated and nonirrigated sites, respectively (one-way ANOVA, $p = .6$). Isotopic separation was also not significantly different between angiosperms and gymnosperms ($p = .90$).

3.3 | How do the source mixing models compare?

Figure 3 shows results from the two-compartment model using SMB (Equations 1 and 2). Using $\delta^2\text{H}$ only (left panel), without considering $^2\text{H}/^1\text{H}$ fractionation (i.e., “ $^2\text{H}/^1\text{H}$ fractionation insensitive”), shows that groundwater and soil water contributions to xylem were $52 \pm 44\%$ and $48 \pm 44\%$, respectively, at the irrigated site. A contribution from groundwater was found in 67% (12 of 18) of trees at this site. Moreover, groundwater and soil water contributions to xylem were $68 \pm 35\%$ and $32 \pm 35\%$, respectively, at the nonirrigated site. Contributions from groundwater were found in 88% (15 of 17) of trees at this site.

Using $\delta^{18}\text{O}$ only (middle panel, i.e., “ $^2\text{H}/^1\text{H}$ fractionation insensitive”) shows that groundwater and soil contribution to xylem was $1 \pm 11\%$ and $92 \pm 11\%$, respectively, at the irrigated site. Contribution from groundwater was found in 50% (9 of 18) of trees at this site. Moreover, groundwater and soil water contributions to xylem were $28 \pm 32\%$ and $72 \pm 32\%$, respectively, at the nonirrigated site. Contribution from groundwater was found in 65% (11 of 17) of trees at this site. Right panel shows results from the BMM. Groundwater and soil water contributions to xylem were $29 \pm 10\%$ and $71 \pm 10\%$, respectively, at the irrigated site. At the nonirrigated site, groundwater and soil water contributions to xylem were $42 \pm 10\%$ and $58 \pm 10\%$, respectively.

In Figure 4, grouping all trees into angiosperms and gymnosperms shows that contribution from groundwater was greater in gymnosperms ($60 \pm 4\%$) than in angiosperms ($52 \pm 5\%$). Soil water and groundwater contributions were significantly different between the two groups (homoscedastic, two-tailed t test, $p < .0001$). Soil water and groundwater contributions were significantly different in gymnosperms (two-tailed t test, $p < .0001$).

3.4 | Uncertainty analysis

Figure 5 shows the uncertainty estimates in the fractions of groundwater and soil water contributions to xylem. As the difference in the isotopic composition between groundwater and soil water sources (ΔC) increases, uncertainty in mixing fractions decreases. ΔC for this study

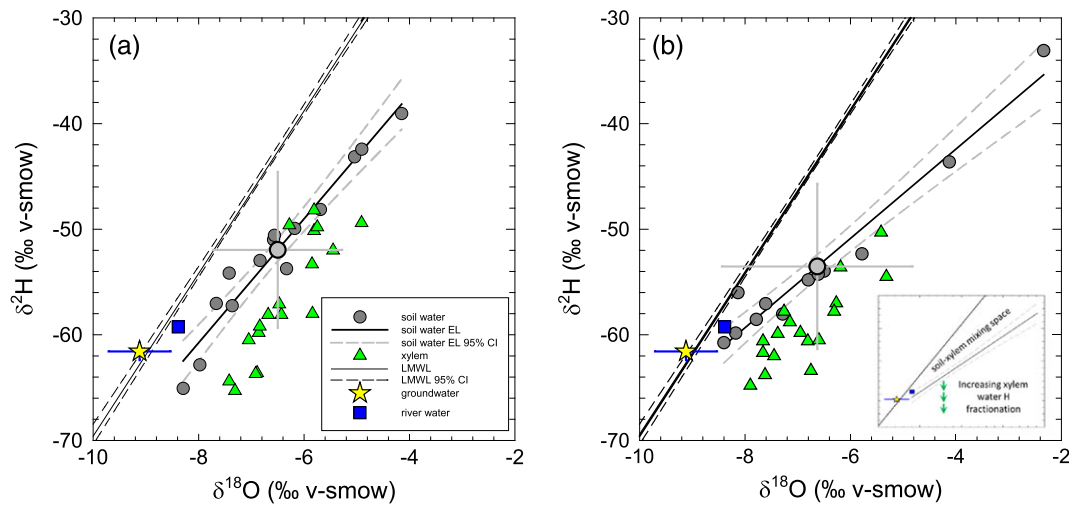


FIGURE 2 Crossplot between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ at irrigated (a) and nonirrigated (b) sites. Inset in (b) is a schematic of the soil-xylem mixing space; xylem samples that fall outside this space may be indicative of $^2\text{H}/^1\text{H}$ fractionation. Error bars are 1 SD

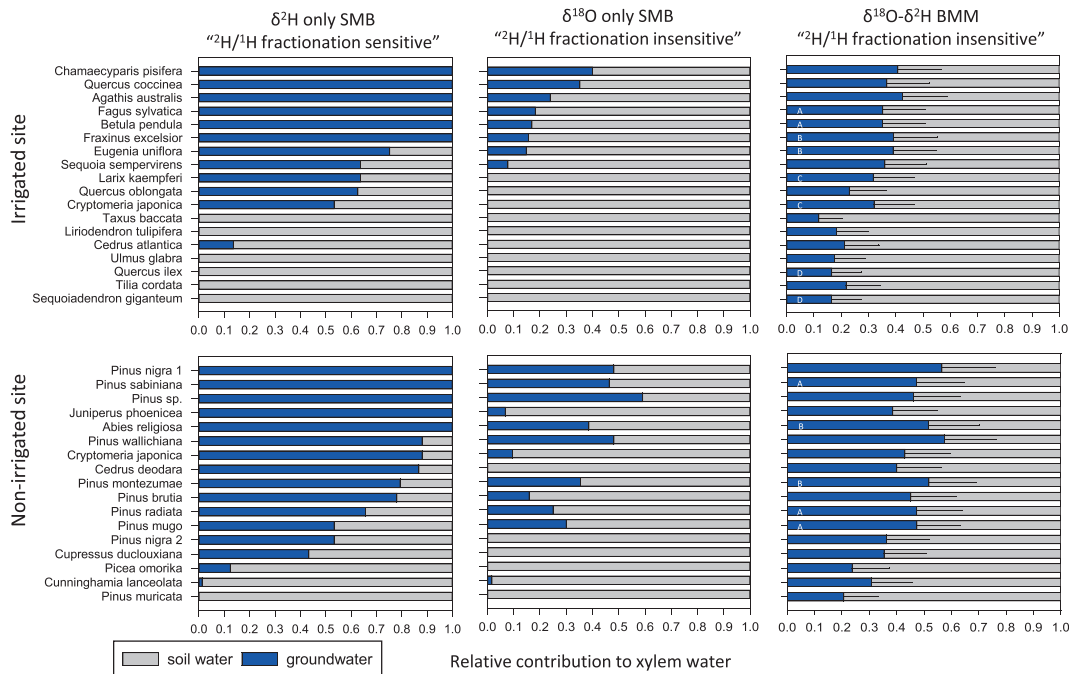


FIGURE 3 Simple mass balance (SMB) methods: $\delta^2\text{H}$ only without accounting for $^2\text{H}/^1\text{H}$ fractionation (left panel, “ $^2\text{H}/^1\text{H}$ fractionation sensitive”), $\delta^{18}\text{O}$ only (middle panel, “ $^2\text{H}/^1\text{H}$ fractionation insensitive”). Bayesian mixing model (BMM) method (right panel, “ $^2\text{H}/^1\text{H}$ fractionation insensitive”); pairwise comparison of groundwater proportions (post hoc tests $p < .001$) in BMM; groundwater proportions connected by the same letters are not statistically different from each other; error bars are 1 SD

was 2.5, with an average uncertainty of 11%, 95% CI (Craig, 1961; Dawson & Ehleringer, 1991) for the SMB approach (Figure 5a). Meanwhile, uncertainty in the BMM was 14%, 95% CI (Dawson & Ehleringer, 1991; Ellsworth & Williams, 2007; Figure 5b). Doubling ΔC from 2.5 to 5.0 resulted in much lower uncertainty for the SMB approach at 5%, 95% CI (Brooks et al., 2010; Brooks et al., 2010). Uncertainty in the BMM at this ΔC value was 14%, 95% CI (Ehleringer & Dawson, 1992; Ehleringer & Dawson, 1992). The concave up shape of the curves in the SMB approach shows that the lowest uncertainties are associated with increasingly equal proportions of groundwater and soil water contributions to xylem. The opposite is true for the BMM

approach. That is, the concave down shape of the curves demonstrates that greater certainty results as the proportions of two sources become increasingly dissimilar.

4 | DISCUSSION

4.1 | Two-compartment model: $\delta^2\text{H}$ versus $\delta^{18}\text{O}$

One of the main objectives of this study was to compare the results of a two-source SMB mixing model using either $\delta^2\text{H}$ or $\delta^{18}\text{O}$. We were

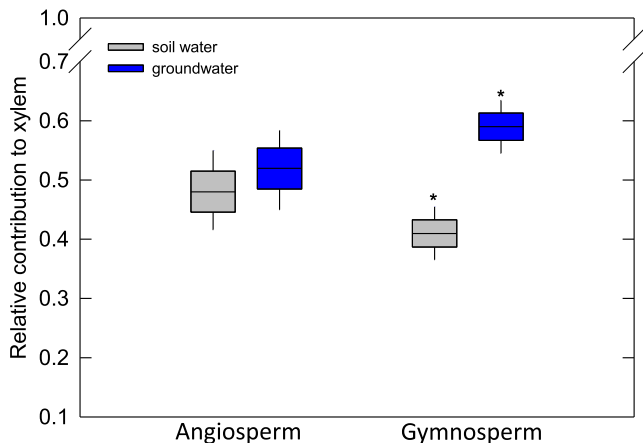


FIGURE 4 Bayesian mixing model derived groundwater and soil water contributions to xylem grouped by angiosperms and gymnosperms. Error bars are 1 SD. Asterisks indicate statistically significant difference (two-tailed *t* test, $p < .0001$)

able to demonstrate that using $\delta^2\text{H}$ resulted in groundwater contribution to xylem 1.2 times greater than when using $\delta^{18}\text{O}$. Sensitivity analysis showed that better agreement between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ would result if the magnitude of isotopic separation $\Delta^2\text{H}$ was nonpositive, highlighting the nontrivial effect of $^2\text{H}/^1\text{H}$ fractionation in using $\delta^2\text{H}$ in a two-source mass balance framework.

4.2 | Two-compartment model: $^2\text{H}/^1\text{H}$ fractionation

$^2\text{H}/^1\text{H}$ fractionation related to root water uptake was reported in earlier studies (e.g., Ellsworth & Williams, 2007; Lin & da Sternberg, 1993). Lin and da Sternberg (1993) demonstrated that the magnitude of $^2\text{H}/^1\text{H}$ fractionation in mangroves was positively correlated with growth and transpiration rates. Ellsworth and Williams (2007) provided evidence supporting the hypothesis that the movement of water into roots via the symplastic pathway (through cytoplasmic continuities and plasmodesmata) resulted in $^2\text{H}/^1\text{H}$ fractionation.

Water movement via the apoplastic path, resulting mainly from the hydrostatic gradient created by transpiration (Barzana et al., 2012), allows for flow of water as “aggregates” as opposed to flow of

dissociated water molecules that characterizes movement via the symplastic route. Movement of water via the apoplastic route (within the cell wall continuum) is believed to not result in $^2\text{H}/^1\text{H}$ fractionation. The relative importance of these pathways to overall movement of water into roots, however, is dependent on species, environmental conditions, and developmental stages (Chaumont & Tyerman, 2014). Whereas earlier papers by Lin and da Sternberg (1993) and Ellsworth and Williams (2007) reported $^2\text{H}/^1\text{H}$ fractionation amongst species in “extreme” environments—mangroves and woody xerophytes, respectively—our finding that $^2\text{H}/^1\text{H}$ fractionation is relatively common amongst 35 tree species in a “nonextreme” environment is possibly novel. If generalizable, we suggest that future source water partitioning studies should check for indications of $^2\text{H}/^1\text{H}$ fractionation. Its implications may be profound especially in understanding and quantifying plant source water in natural and cultivated ecosystems (Giambelluca et al., 2016). As we demonstrated here, disregarding its potential effect when using $\delta^2\text{H}$ in a two-source SMB approach could lead to erroneous results. We propose that the utility of the MCMC algorithm used here, particularly in cases where fractionation associated with water uptake is suspect, is fundamentally valid following the niche concept for isotopic ecology (Newsome et al., 2007), particularly on the biogenic dimension. For more information, the interested reader is referred to Newsome et al. (2007).

4.3 | Two-compartment model: Bayesian mixing model

As with the BMM approach, we found no effect between species and other tree-specific attributes and f_{gw} . Nevertheless, we found that f_{gw} was significantly higher than f_{soil} in gymnosperms. The same comparison was not statistically significant in angiosperms. This finding supports the interpretation that gymnosperms have access to deeper subsurface water sources than angiosperms, which may be related to key anatomical differences between the two groups (Anderegg, 2015). We note, however, that this site-level finding is in contrast to the aggregated result of a recent global-in-scale stable isotope metaanalysis by Evaristo and McDonnell (2017), possibly reflecting site-specific differences.

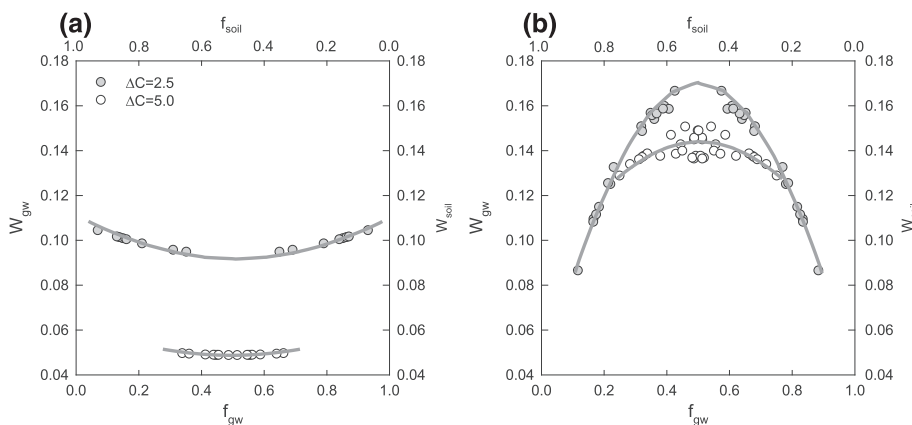


FIGURE 5 Uncertainty W in fractions of groundwater (f_{gw}) and soil water (f_{soil}) contributions to xylem based on the mass balance approach (a) using $\delta^{18}\text{O}$ (following Genereux, 1998), and Bayesian mixing model (b). ΔC is the absolute difference between groundwater and soil water isotopic composition. $\Delta C = 2.5$ is for this study

4.4 | Uncertainties in source water identification

The utility of mixing models is contingent on the distinct isotopic composition of sources (Gannes, del Rio, & Koch, 1998). The rationale behind modelling the approximately 1-m-deep soil as one compartment is the relatively homogeneous isotopic composition of the soil profile (Phillips, Newsome, & Gregg, 2005; Phillips et al., 2014). And because the soil compartment is isotopically different from groundwater, the SMB and BMM approaches used here may prove useful in settings similar to our two sites. Moreover, the same approaches may be suitable when the research question is about quantifying source water apportionment between the vadose and saturated zones. Notwithstanding, as with any model approaches, there are benefits and tradeoffs. For example, SMB approaches such as the one used here could only provide point estimates. Uncertainties, however, may be quantified and improved (i.e., higher certainty) if the difference between sources (ΔC) is large. The extent to which ΔC is maximized will depend on the nature of the data. SMB approach showed that doubling the ΔC could lead to improved certainty by about the same magnitude (Figure 5a). In some cases, however, the difference between sources may not be large and the researcher may be more interested in quantifying the magnitude of separation between sources than in maximizing certainty. Figure 5b shows that the BMM may be more useful in achieving such objective albeit at a relatively higher uncertainty than the SMB approach. Increasing the ΔC may not improve the average uncertainty but may simply narrow the credible interval (*sensu* Bayesian inference). It is noteworthy that the uncertainties in the BMM approach become more variable as the proportion between the two sources becomes more or less equal.

5 | CONCLUSIONS

A total of 35 trees were sampled for stable isotopic compositions of water in bulk soil and xylem at two sites in Christchurch, New Zealand. We compared and evaluated critically an SMB and BMM in quantifying the magnitude of contribution from various sources in the subsurface. When using a single isotope ratio system in a simple, two-source mass balance approach, our study highlights the importance of checking for indications of $^2\text{H}/^1\text{H}$ fractionation via root water uptake. Using $\delta^2\text{H}$ over $\delta^{18}\text{O}$ when $^2\text{H}/^1\text{H}$ fractionation is present can lead to erroneous results, particularly an apparent overestimation of groundwater contribution to xylem. When using two isotopes together, the BMM approach may prove more useful than the SMB approach, particularly when soil and groundwater contributions are relatively disproportionate. The Bayesian approach is also insensitive to possible effects of $^2\text{H}/^1\text{H}$ fractionation. When full accounting of uncertainties in sources and mixtures ("xylem") are desired, and possible violation of the supposed nonfractionating nature of root water uptake is suspected, our results show that the BMM approach used here may prove most appropriate.

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AUTHOR CONTRIBUTIONS

J. J. M. designed the research. J. J. M. and J. C. collected the data. J. E. performed the data analysis, modelling, and interpretation. J. E. led the writing of the manuscript with contributions from J. J. M. and J. C.

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