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Citation for final published version:

Sargeant, Christopher I, Singer, Michael Bliss and Vallet-Coulomb, Christine 2019. Identification of source-water oxygen isotopes in trees Toolkit (ISO-Tool) for deciphering historical water use by forest trees. *Water Resources Research* 55 (12) , pp. 10954-10975. 10.1029/2018WR024519 file

Publishers page: <https://doi.org/10.1029/2018WR024519> <<https://doi.org/10.1029/2018WR024519>>

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1 **Identification of Source-water Oxygen isotopes in trees Toolkit (ISO-Tool) for deciphering**
2 **historical water use by forest trees**

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16
17 **Key Points:**

- 18 • A toolkit for determining the oxygen isotopic signature of historical source water to trees is
19 presented.
- 20 • The parameterization of ISO-Tool is subdivided based on data availability and output resolution.
- 21 • The tool is designed to retrospectively assess water source usage by plants at annual and sub-
22 annual timescales.

23
24 **ABSTRACT**

25 Hydrological regimes are being perturbed under climate change due to the regional expression
26 of the water cycle across the globe, leading to alterations in the spatial and temporal distribution of
27 water near the Earth's surface. Water is a critical resource for forest ecosystems and hydrological
28 limitations on vegetative health are particularly complex. To anticipate how subsurface water availability
29 may evolve in the future and affect the dynamics of plant water-source usage, as well as the health and
30 functioning of vegetation in various biomes, we need a robust, quantitative framework for linking water
31 availability to past plant water-use, which is constrained by historical data. Here, we outline the
32 Identification of Source-water Oxygen isotopes in trees Toolkit (ISO-Tool), designed to retrospectively

33 investigate the dynamics of tree-water uptake. ISO-Tool utilizes tree-ring isotopes ($\delta^{18}\text{O}$) combined with
34 a biomechanistic fractionation model to predict the $\delta^{18}\text{O}$ of water utilized during any period of growth.
35 Through comparisons with measured $\delta^{18}\text{O}$ in local water sources, climatic and hydrological variables,
36 ISO-Tool can reconstruct and inform on past ecohydrological interactions. We provide an overview of
37 the modeling components and data requirements necessary to constrain the retrodictions of source-
38 water $\delta^{18}\text{O}$. We demonstrate the utility and efficacy of ISO-Tool for three riparian field sites
39 characterized by differences in climatic, geomorphic and hydrologic complexity. However, ISO-Tool can
40 be applied to a range of vegetated environments where distinct isotopic endmembers exist. We present
41 a set of tool groups, which can be applied adaptively, ensuring that scientific progress in understanding
42 retrospective ecohydrology can be made, even under varying degrees of data availability.

43

44 **1 INTRODUCTION**

45 **1.1 Background**

46 Forests worldwide are becoming increasingly vulnerable to variations in water availability as
47 hydrological regimes respond to climate change (Allen et al., 2010, 2015; Choat et al., 2012; Clark et al.,
48 2011; Hartmann et al., 2013). Yet, despite the fundamental role water plays in the health, productivity
49 and distribution of tree species (Currie & Paquin, 1987; Hsiao, 1973; Schulze et al., 1987; Stephenson,
50 1990), there remain considerable uncertainties in how terrestrial water availability to forests will evolve
51 under future climate (Allen & Ingram, 2002; Donat et al., 2016; IPCC, 2014; Sippel et al., 2017; Trenberth
52 et al., 2014). Such shortcomings result from the incomplete characterization of moisture sources over a
53 range of timescales, which is further complicated by the contribution of several potential sources to
54 tree-available water. These sources include infiltrated precipitation in the vadose zone and shallow
55 groundwater in the phreatic zone, where the latter can be derived from hyporheic streamflow
56 contributions to shallow water tables (Busch et al., 1992; Evans et al., 2018; Singer et al., 2014; White &
57 Smith, 2013). Therefore, for any interval of time, the particular water source used by a tree is a function
58 of specific tree rooting depths as well as by the time-varying availability of root-zone water, which varies
59 in response to climatic trends and fluctuations (Dawson & Pate, 1996; Snyder & Williams, 2000). The
60 details surrounding these dynamic ecohydrological relationships are poorly understood, a knowledge
61 gap which restricts our ability to anticipate how forests will respond to alterations in hydrological
62 regimes that may affect one or both soil hydrological reservoirs (vadose and phreatic), particularly in
63 regions where water is expected to become a limiting resource to tree growth (Bréda et al., 2006;
64 García-Ruiz et al., 2011; Lindner et al., 2010).

65 To address this shortcoming, we take a retrospective approach and identify two fundamental
66 questions: 1) What water source was used by a tree during a particular period of growth? 2) Does this
67 water source utilization correspond to the dynamics of root-zone water availability? If these questions
68 can be answered satisfactorily for a particular site, we believe this information on plant-water
69 relationships could dramatically improve studies of ecohydrology, paleoclimate, and land surface
70 dynamics within forest ecosystems.

71 Severe moisture deficits can reduce tree growth and biomass production (Berner et al., 2017;
72 Charru et al., 2010; Sarris et al., 2011; Silva et al., 2010), increase the susceptibility to pathogen
73 infestations and eventually lead to mortality from hydraulic failure and/or carbon starvation (Allen et al.,
74 2010; Breshears et al., 2005; McDowell et al., 2011; Schlesinger et al., 2016). Through differential
75 responses to moisture stress, individual tree and species' mortality rates can lead to altered stand
76 demographics and composition, and thus ecosystem functioning (Clark et al., 2016; Hansen et al., 2001;
77 Milad et al., 2011), whilst moisture limitations in the rooting zone may also determine whether
78 particular tree species can get recruited and established at riparian sites (Mahoney & Rood, 1998; Singer
79 & Dunne, 2004). These are key concerns, given the significant role trees play globally in carbon and
80 water cycling (Bonan et al., 2008; Ciais et al., 2005; Ellison et al., 2017; Jasechko et al., 2013; Kurz et al.,
81 2008; Settele et al., 2014) and in the provision of ecological services (Anderegg et al., 2013). Critically,
82 forest vulnerability to drought conditions is not restricted to environments typically considered as
83 moisture limited. Amazonian forest mortality was linked to drought conditions experienced in 2005
84 (Phillips et al., 2009) and 2010 (Lewis et al., 2011), with the later drought spanning 3.2 million km² (25 %
85 greater than that of 2005). Furthermore, Chen et al., (2017) attributed trembling aspen die back in
86 western Canadian boreal forests to insufficient water availability.

87 Retrospective insights into patterns of tree source water use (at seasonal and annual resolution)
88 would be particularly useful for riparian zones, where phreatophytic forest species are highly dependent
89 on the hydrological connectivity between the river and floodplain phreatic aquifer (Singer et al., 2014).
90 Phreatophytes are sensitive to changes in shallow alluvial groundwater availability which can manifest
91 as declines in healthy forest stands, reduced ecological services (Amoros & Bornette, 2002; Steiger et al.,
92 2005; Stromberg et al., 2007) and shifts in the successional states of forest communities (e.g. Stromberg
93 et al., 1996; Shafroth et al., 2000). Information about the timing and origin of tree source waters would
94 also allow for accurate determinations of the ecohydrological impacts arising from river flow regulation
95 practices (i.e. minimum flow requirements), river bed-level changes (e.g. gravel extraction / downstream

96 of dam construction) and to identify critical thresholds for groundwater abstraction. Such information
97 would promote more sustainable water management strategies in forests where water resources are
98 also under increasing population pressures (Jackson et al., 2001).

99 We believe that a simple, yet effective, methodology for determining historical sources of water
100 used by trees, would be highly valuable in improving scientific enquiry in isotopic ecohydrologic
101 investigations. Such an approach would need to be well defined, easily accessible and adaptable.

102 In this paper, we build on existing research by combining and enhancing a set of techniques to
103 identify historical water-sources ($\delta^{18}O_{sw}$) used by trees, based on information from tree-ring cellulose
104 ($\delta^{18}O_{cell}$), plant physiology, hydroclimate and a biomechanistic fractionation model (Roden et al., 2000;
105 Barbour et al., 2004). This is presented in the form of a methodological 'toolkit' called the Identification
106 of Source water Oxygen isotope Toolkit (ISO-Tool), for which we identify and explain the necessary data
107 requirements and provide examples of its application. We distill these requirements and data sources
108 and present them in the form of a tiered 'Tool Group' scheme, to provide a means to straightforwardly
109 reconstruct the historical source water signatures of trees at annual or sub-annual scales. Our goal here
110 is not to necessary develop a method for highly accurate characterization of past water usage by plants,
111 but rather to enable researchers to better ascertain likely water sources to plants for particular periods
112 of time, to distinguish between plant water sources within sites for different species, and to identify
113 how plant water sources may vary along hydroclimatic gradients.

114

115 **1.2 Isotope echydrology**

116 Stable isotope ratios of oxygen and hydrogen in water ($\delta^{18}O$ and δ^2H) have been used to trace
117 the sources of waters used by plants through the comparison of the isotopic signatures of potential
118 endmember sources (e.g. soil, groundwater, surface waters) with those of waters extracted from the
119 xylem (e.g. Bertrand et al., 2014; Dawson & Ehleringer, 1991; Dawson & Pate, 1996; Horton et al., 2003;
120 Jackson et al., 1999; Schulze et al., 1996; White et al., 1985). This is based on broad evidence that no
121 isotopic fractionation occurs between the soil water pool and the plant during root uptake (Allison et al.,
122 1983; White et al., 1985), although δ^2H fractionation has been reported in some halophytes and woody
123 xerophytes (Ellsworth & Williams, 2007; Lin & Sternberg, 1993). This means that the water taken up by a
124 tree retains isotopic information specific to its origin and history within the hydrological cycle at the
125 point of uptake. Characterizing plant source waters via isotope analysis is particularly powerful if
126 potential endmember water sources, e.g. precipitation (*P*) or groundwater (*GW*) are isotopically distinct

127 and locally defined. This a fundamental principle when considering the use of $\delta^{18}\text{O}/\delta^2\text{H}$ for
128 ecohydrological studies (Barbeta et al., 2018).

129 If combined measurements are made of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in xylem and potential endmember water
130 sources, information relating to the evaporative history of the water (relative to local precipitation
131 input) can be obtained, although such information is not necessary for identifying plant water sources
132 (Sprenger et al., 2016).

133 Soil composition, texture and water status (e.g. Chen et al., 2017; Oshun et al., 2016; Vargas et
134 al., 2017) have all been proposed causes of the fractionation of oxygen and hydrogen isotopes in plant-
135 available water, prior to uptake, highlighting the need for careful consideration of these factors during
136 investigations, as well as further research (for an overview, see Barbeta et al., 2018). Nevertheless, if
137 such results are interpreted carefully, then stable isotope analyses remain a powerful method for
138 understanding plant-water interactions. Herein, only $\delta^{18}\text{O}$ ecohydrology is discussed, since this isotope
139 ratio functions as a better conservative tracer of water in ecosystems than $\delta^2\text{H}$ due to its higher mass
140 and binding energy.

141 Commonly, the distinct hydrological reservoirs i.e. vadose (unsaturated) and phreatic
142 (saturated) zones are characterized by differences in their water $\delta^{18}\text{O}$ content and water in these zones
143 is preferentially used by different species (Singer et al., 2014). However, where potential source waters
144 are isotopically similar, disentangling the water source use can be challenging (Drake & Franks, 2003).

145 Water in the vadose zone typically inherits an isotopic signature reflective of precipitation
146 ($\delta^{18}\text{O}_{ppt}$) inputs (Robertson & Gazis, 2006) which varies seasonally as a function of temperature, air mass
147 origin and history (Dansgaard, 1964; Gat, 1996). For temperate environments, this is exemplified by
148 depleted and enriched $\delta^{18}\text{O}$ of precipitation in the winter and summer, respectively. The isotopic
149 composition of soil moisture can also represent a time-varying mixture of isotopically separate
150 precipitation events, which may also include the effects of near-surface evaporative enrichment, leading
151 to the development of isotopic profiles in soil water with depth (Allison et al., 1983; Gazis & Feng, 2004;
152 Hsieh et al., 1998; Sprenger et al., 2016).

153 In contrast, the $\delta^{18}\text{O}$ signature of phreatic water typically has low temporal variability, and it is
154 often isotopically depleted in relation to vadose zone moisture, especially if GW is sourced from regional
155 snowmelt runoff (colder percolated precipitation) (Gat, 1996) and/or is mixed with streamflow (Q)
156 (Dawson & Ehleringer, 1991). However, there may be situations where isotopically distinct

157 groundwaters contribute to a rooting zone. Sargeant and Singer (2016) suggested that isotopically
158 dissimilar waters, derived from different climatic regimes, may interact within a shallow alluvial aquifer
159 receiving both a regionally derived *GW* and water derived from locally infiltrated precipitation. A
160 pragmatic approach would be to isotopically characterize the potential water sources within a field site,
161 ensuring that isotopic separation of plant available waters is possible (Barbareta et al., 2018).

162

163 **1.3 Source water availability**

164 In environments where both vadose and shallow phreatic waters are available, tree species can
165 employ different physiological strategies for obtaining water. Deeply rooted phreatophytic species
166 prefer phreatic water sources derived from shallow aquifers (Busch et al., 1992) and capillary rise, yet
167 may also exhibit opportunistic behavior, switching to shallow soil moisture (or using combination of
168 both) under periods of water deficit (Singer et al. 2013, 2014; Snyder & Williams, 2000; Sun et al., 2016).
169 Shallowly rooted species are unable to access deeper phreatic water, so they rely on vadose moisture
170 which is sensitive to balance between precipitation inputs and near-surface evaporation, although it is
171 possible that capillary rise during a period of elevated water table can supply phreatic water to the
172 unsaturated zone (Sánchez-Pérez et al., 2008). Additionally, plant roots can redistribute water across a
173 soil-water potential gradient (hydraulic redistribution), both vertically and laterally (Brooks et al., 2002;
174 Caldwell et al., 1998; Richards & Caldwell, 1987), allowing water from different depths and distances to
175 be utilized by neighboring plants (Dawson, 1993), and complicating source-water identification. Studies
176 which provide detailed ecohydrological information are typically based on comparisons between the
177 contemporaneous measurements of tree xylem waters with those of local water sources (e.g.
178 Plamboeck et al., 1999; Sánchez-Pérez et al., 2008). Whilst direct analysis of xylem $\delta^{18}\text{O}$ circumvents the
179 leaf fractionation and exchange mechanisms which mask the source water $\delta^{18}\text{O}$ information stored in
180 $\delta^{18}\text{O}_{\text{cell}}$ (McCarroll & Loader, 2004), studies of this sort are limited by the temporal domain of field work
181 (typically 2-3 years), restricting the development of broader conclusions about ecohydrological
182 interactions (Pettit & Froend, 2018). Long-term, seasonal reconstructions of tree source-waters $\delta^{18}\text{O}$
183 could yield potentially powerful new information about water availability to trees, and thus hydrological
184 processes, whilst also providing historical context for real-time investigations.

185

186 **1.4 Tree-ring oxygen isotopes**

187 In order to gain retrospective insights into plant-water interactions over longer timescales, tree-
188 ring isotopes represent an under-utilized excellent library of accurately dated isotopic information on
189 tree water usage. The oxygen isotope ratio ($^{18}\text{O}/^{16}\text{O}$) contained within tree-ring cellulose ($\delta^{18}\text{O}_{\text{cell}}$) can be
190 used to characterize the isotopic signature of the source water(s) ($\delta^{18}\text{O}_{\text{sw}}$) used during the time of ring
191 formation, thereby providing an extended record of ecohydrological processes (McCarroll & Loader,
192 2004). Tree-ring $\delta^{18}\text{O}$ fractionation theory is largely well established (McCarroll & Loader, 2004),
193 although some uncertainties remain regarding the timing and transfer of photosynthates from source to
194 sink tissues, which is relevant for finer scale studies (Gessler et al., 2009; Offerman et al., 2011; Ogée et
195 al., 2009). Whilst there is the possibility for the remobilization of stored carbohydrates, formed at an
196 early period, this more evident in $\delta^{13}\text{C}$ measurements than those of $\delta^{18}\text{O}$ in tree rings (Hill et al., 1995)

197 The $\delta^{18}\text{O}_{\text{cell}}$ records an integrated isotopic signal of three components: (a) the signatures of
198 trees' source water taken up from the rooting zone; (b) leaf-water enrichment as a function of
199 evaporation (Dongmann et al., 1974; Flanagan et al., 1991); and (c) the biochemical fractionation that
200 occurs during photosynthesis (Sternberg et al., 1986; Yakir & DeNiro, 1990). A final mechanism during
201 cellulose synthesis allows for a proportion of oxygen atoms in sucrose to exchange with $\delta^{18}\text{O}_{\text{sw}}$, damping
202 the leaf-level fractionation effects (Farquhar et al., 1998; Hill et al., 1995; Roden et al., 2000). While
203 there is a consistent enrichment ($27 \pm 4 \text{‰}$) of photosynthate $\delta^{18}\text{O}$, compared with leaf water ($\delta^{18}\text{O}_{\text{lw}}$),
204 leaf water enrichment is much more variable responding to atmospheric conditions and leaf level gas
205 exchange relating to species' physiology (McCarroll & Loader, 2004; Roden et al., 2000).

206 Studies utilizing tree-ring $\delta^{18}\text{O}_{\text{cell}}$ have been able to provide insights into historical water
207 availability to trees, although use of the 'raw' $\delta^{18}\text{O}_{\text{cell}}$ to infer water source assumes that climatic
208 variables and tree-physiological responses are uniform across a study site or between sites, and the
209 relative differences measured in $\delta^{18}\text{O}_{\text{cell}}$ are therefore solely a function of $\delta^{18}\text{O}_{\text{sw}}$ differences. For
210 example, Marshall and Monserud (2006) suggested that differences observed in tree-ring $\delta^{18}\text{O}_{\text{cell}}$ of
211 three co-located species over eight decades could be attributed to shifts in tree water source use, whilst
212 Singer et al. (2013) suggested that fluctuations in floodplain hydrology strongly controlled the source
213 water availability and utilization for two tree species with contrasting rooting profiles. However, in order
214 to provide direct comparisons with endmember water sources, it is necessary to determine the $\delta^{18}\text{O}_{\text{sw}}$
215 from the $\delta^{18}\text{O}_{\text{cell}}$ value. Using detailed measurements of the primary controlling variables of $\delta^{18}\text{O}$
216 fractionation in leaves (E - transpiration, g_s - stomatal conductance, $\delta^{18}\text{O}_{\text{wv}}$ - atmospheric water vapor
217 $\delta^{18}\text{O}$, RH - relative humidity and T - air temperature), it is possible to model the relationship between
218 xylem water ($\delta^{18}\text{O}_{\text{xyl}}$) and $\delta^{18}\text{O}_{\text{cell}}$, to varying degrees, based on the quality of input data (Barbour et al.,

219 2004; Roden et al., 2000). This relationship can be used to inversely hindcast (model) the $\delta^{18}\text{O}$ of source
220 waters used for the formation of each tree ring ($\delta^{18}\text{O}_{msw}$) (Bose et al., 2016; Sargeant & Singer, 2016;
221 Singer et al., 2014). Earlier work by Anderson et al., (2002) demonstrated the potential for
222 reconstructing historical $\delta^{18}\text{O}_{ppt}$ from $\delta^{18}\text{O}_{cell}$ by calibrating the model (incorporating climate variables
223 and tree growth) against records of $\delta^{18}\text{O}_{ppt}$.

224 The model calculations of $\delta^{18}\text{O}_{msw}$ can then be verified against field measurements of the
225 evolving isotopic composition of contributing water sources (*P*, *GW*). Using this approach, direct
226 comparisons between $\delta^{18}\text{O}_{msw}$ with local endmember water source $\delta^{18}\text{O}$ have suggested that co-
227 occurring tree species with distinct rooting depths (e.g. *Fraxinus* spp. and *Populus* spp.) often access and
228 utilize different mixes of water sources at annual and sub-annual timescales, as a function of fluctuating
229 hydro-climate and relative depths to local GW (Sargeant & Singer, 2016; Singer et al., 2014).

230 Identifying tree source water(s) requires information on how the potential local endmembers
231 vary in their $\delta^{18}\text{O}$ signature on both annual and sub-annual timescales, which is particularly complex in
232 situations where there may exist more than one potential water source available for tree growth
233 (Sargeant & Singer, 2016; Singer et al., 2014). Furthermore, fluctuations in source water mixtures modify
234 the isotopic signature of root-available water, thus challenging interpretations of water sources
235 consistently available to trees (Busch et al., 1992; Dawson & Ehleringer, 1991; Sánchez-Pérez et al.,
236 2008; Snyder & Williams, 2000). Despite these challenges, the identification of $\delta^{18}\text{O}_{sw}$ is critically
237 important, especially considering that changes in soil water content may outpace the ability for new
238 root growth to track such shifts (Plamboeck et al., 1999).

239 We demonstrate our methodology for three Mediterranean, riparian forest sites, at both annual
240 and sub-annual resolutions, highlighting the ability of the ISO-Tool to discern valuable ecohydrological
241 information at different timescales. Whilst our examples are based on riparian forests, ISO-Tool is also
242 suitable for other forested environments where a distinct isotopic separation exists between potential
243 water sources used for tree growth over different time periods. Our aim is to provide a new suite of
244 methods to enable a greater understanding of forest ecohydrology in response to climatic fluctuations
245 and trends in subsurface hydrology.

246 **2 METHODS**

247 **2.1 Identification of Source water Oxygen isotope ratios in trees Toolkit (ISO-Toolkit)**

248 The core principle of this research is that $\delta^{18}O_{cell}$ can be deconvolved to the $\delta^{18}O_{sw}$ taken up from
249 the rooting zone during a particular growing season. The method utilizes an iterative modeling approach
250 to model the source water $\delta^{18}O_{sw}$ signature ($\delta^{18}O_{msw}$), which is then directly comparable to the $\delta^{18}O$ of
251 potential endmember water sources. In this section, we explore the calculations of $\delta^{18}O_{msw}$ and how the
252 user defined input variables are used. We then analyze the sensitivity of the mechanistic model to these
253 different input variables. Subsequently, we describe how key variables can be constrained in order to
254 improve the accuracy of the $\delta^{18}O_{msw}$ calculations and we outline the data and methods that can be used
255 to help interpret the model output. Combined, these form a methodological toolkit, which can be
256 utilized for determining historical plant water use in a variety of forest ecosystems.

257 An overview of ISO-Tool is shown schematically in Figure 1. ISO-Tool, an open-source code
258 available in two forms, enables the back-calculation of $\delta^{18}O$ of water sources used by plants based on
259 measured $\delta^{18}O_{cell}$, climate and plant physiological variables. This code is based on the Barbour et al.
260 (2004) model for plant $\delta^{18}O$ fractionation but modified to invert the problem.

261 The required user inputs of the toolkit are predefined in the following sections, but the manner
262 in which these are constrained is governed by specific Tool Group selections. These data are added to
263 the input file and must comprise of an average value with an estimate of standard deviation (SD) . From
264 these, the model generates normal distributions that are repeatedly sampled by Monte-Carlo
265 simulation, which produces an error margin of ± 2 SD. From the mean input values, the model also
266 calculates the mean $\delta^{18}O_{msw}$ value. The mean and standard deviation error bounds are produced as a
267 plot and an associated output file is generated. The modeling script is editable to enable a 'bespoke'
268 analysis through the modification of individual parameters (detailed below), but which are not currently
269 included as requirements within the input file. Presently, the model uses an optimization routine to
270 predict the $\delta^{18}O$ value of source water, $\delta^{18}O_{msw}$. The model results ($\delta^{18}O_{msw}$) can then be interpreted by
271 the user from a selection of possible techniques contained within the Tool Groups necessary to
272 characterize source water availability to trees.

273 The $\delta^{18}O_{msw}$ modeling script is provided as a Matlab code (doi: 10.5281/zenodo/1161221) with
274 the input and output files in .csv (.txt) format. The model is provided at:
275 <https://github.com/blissville71/InverseBarbourModel>. Here, the user will find downloadable files of the
276 model as a Matlab code (includes Monte-Carlo simulations) and a Microsoft Excel version (excludes
277 Monte-Carlo simulations), plus an example input file and accompanying instructions /overview for the
278 model's rationale and use.

279 **2.2 Modeling source water $\delta^{18}O$ ($\delta^{18}O_{msw}$)**

280 The $\delta^{18}O_{msw}$ is predicted from a known $\delta^{18}O_{cell}$ by accounting for the tree-level fractionations and
281 exchange processes which alter the $\delta^{18}O_{sw}$ signature until cellulose formation. In Figure 2a-d, the key
282 components of the model are shown, as well as how required user input data is used in these equations,
283 with an accompanying conceptualization in Figure 2e.

284 The extent to which transpiration enriches $\delta^{18}O_{sw}$ at the sites of evaporation (Δ_e) in the leaf, is
285 fundamental in determining the recorded variability in $\delta^{18}O_{cell}$ values. The Δ_e model is based on the work
286 by Craig and Gordon (1965) and later modified for the leaf environment (Dongmann et al., 1974;
287 Farquhar & Lloyd, 1993; Flannagan et al., 1991):

$$\Delta_e = (1 + \varepsilon^*) \left[(1 + \varepsilon_k) \left(1 - \frac{e_a}{e_i} \right) + \frac{e_a}{e_i} (1 + \Delta_v) \right] - 1 \quad (1)$$

288

289 which can be approximated as:

$$\Delta_e \approx \varepsilon^* + \varepsilon_k + (\Delta_v - \varepsilon_k) \frac{e_a}{e_i} \quad (2)$$

290

291 The evaporative enrichment of source water within the leaf is given in Figure 2a & e, and
292 assumes an isotopic steady state, which is appropriate for our purposes since we rely on
293 photosynthetically derived substrates ($\delta^{18}O_{cell}$), reflecting daytime leaf conditions during which an
294 isotopic steady state is approached (Cernusak et al., 2016). Within the model, T_l is assigned as $T+1$ ($^{\circ}C$),
295 as a simplifying assumption. An energy balance equation could be incorporated to compute a realistic T_l ,
296 but it would require additional data inputs (e.g. wind speed, leaf width, photosynthetically active
297 radiation) that are typically unavailable for historical reconstruction (Barbour et al., 2000; Lorrey et al.,
298 2016).

299 Although boundary layer conductance is an important component of ε_k , difficulties in
300 determining time-varying values (Brenner & Jarvis, 1995) may necessitate the use of a fixed value of 1
301 $\text{mol m}^{-2}\text{s}^{-1}$ (Barbour et al., 2004). In reality, boundary layer conductance varies as a function of leaf shape,
302 size and thickness, stomatal density, wind velocities and energy balance (Buhay et al., 1996; Jarvis &
303 McNaughton, 1986; Schuepp, 1993; Stokes et al., 2006). Constraining all of these parameters requires a
304 much more detailed investigation beyond the scope of this study. However, both leaf temperature

305 (offset from air temperature) and boundary layer conductance values can be assigned at the user's
306 discretion.

307 The magnitude of leaf water evaporative enrichment has been shown to be over-predicted by
308 the equation in Figure 2a (Cernusak et al., 2016 and references therein). Farquhar and Lloyd, (1993)
309 proposed that such discrepancies resulted from a Péclet effect, whereby the advection of unenriched
310 source water via transpiration is opposed by the diffusion of enriched water to and from the sites of
311 evaporation, respectively, operating over a given distance divided by the molar density of water and
312 diffusivity of H_2^{18}O in water (Figure 2b, c & e). As such, under high transpiration rates, the level of leaf
313 water enrichment declines. Values of L (mm) can be calculated as a function of E based on the study of
314 Song et al., (2013). They developed a regression between L and E for several different tree species ($L =$
315 $2.36 \times 10^{-5} E^{1.20}$, $R^2 = 0.813$), where the regression function is expressed in meters (m). We included this
316 relationship within our code so L is computed only based on E (obviating direct measurements of L). We
317 also utilized the R-squared value to provide an error around these estimates. Barbour et al. (2004)
318 showed that the inclusion of the Péclet effect improved their model estimates of the isotopic signature
319 in cellulose (relative to source water), accounting for 89 % of the variability in their study.

320 During photosynthesis, sucrose molecules are enriched $+27 \pm 4$ ‰ above leaf water $\delta^{18}\text{O}$ as the
321 result of carbonyl-bound oxygen atom exchange between triose phosphates and the medium water
322 (Sternberg et al., 1986; Yakir & DeNiro, 1990). It is this sucrose which is utilized to form cellulose, a
323 process by which a proportion of intermediary molecules are able to exchange with unenriched xylem
324 water ($\delta^{18}\text{O}_{sw}$) (Farquhar et al., 1998; Hill et al., 1995) (Figure 2d & e). The fraction of oxygen atoms
325 undergoing this exchange is controlled by the proportion of unenriched source water within the cell
326 during cellulose synthesis. The fraction of exchanged oxygen atoms is reported as 0.42 for many tree
327 species (Roden et al., 2000), whilst the source water content is assumed to be at unity for large, mature
328 trees where the distance between leaf and sink tissue is considerable (Barbour et al., 2002). The overall
329 effect of this process is to dampen evaporative enrichment signature transferred to the cellulose
330 molecule at the leaf level.

331 **2.3 Sensitivity analyses**

332 We conducted a series of sensitivity analyses on the calculation of $\delta^{18}\text{O}_{msw}$ in response to
333 variations in T , RH , $\delta^{18}\text{O}_{ww}$, as well as to paired values of g_s and E . We began by varying each of these
334 input variables independently and then advanced the analysis through a covariance of two variables

335 (Figure 3). During each sensitivity test, variables not under analysis were kept constant as indicated in
336 Figure 3 'Default values'. Leaf gas exchange parameters used in all model runs are those from Singer et
337 al., (2014) for *Fraxinus excelsior*.

338 **2.3.1 $\delta^{18}O_{cell}$, $\delta^{18}O_{wv}$ and RH**

339 The $\delta^{18}O_{msw}$ predictions respond primarily to changes in RH , $\delta^{18}O_{wv}$ and $\delta^{18}O_{cell}$ when each
340 respective input is varied independently (Figure 3a). It should be noted that these results are only
341 representative of the interactions occurring between changing variable and the default values of the
342 remaining inputs indicated in Figure 3a.

343 We included $\delta^{18}O_{cell}$ to illustrate the positive response we expect on $\delta^{18}O_{msw}$ if all other variables
344 are constant, thus, Δ_e is static so with increasing $\delta^{18}O_{cell}$ there must be a corresponding enrichment in
345 $\delta^{18}O_{msw}$ (+1.5‰/‰). For $\delta^{18}O_{wv}$, a negative relationship exists with $\delta^{18}O_{msw}$ (-0.6‰/‰) (Figure 3a). The
346 dependence of Δ_e on $\delta^{18}O_{wv}$ can be shown using equation (2). If $e_a/e_i = 1$ (saturated conditions), then Δ_e
347 = $\epsilon^* + \Delta_v$ (Cernusak et al., 2016), because enrichment in $\delta^{18}O_{wv}$ causes depletion in $\delta^{18}O_{msw}$ in
348 compensation. For RH there is a strong, positive, curvilinear relationship with $\delta^{18}O_{msw}$, so as RH
349 increases, Δ_e decreases. This arises because $\delta^{18}O_{msw}$ must undergo enrichment to maintain a given
350 $\delta^{18}O_{cell}$.

351 **2.3.2 T , g_s and E**

352 When T (i.e. T_l) was varied from 2-40 °C, $\delta^{18}O_{msw}$ was shown to enrich by 3.3‰, a relatively weak effect
353 on Δ_e over this large temperature range, compared to that of $\delta^{18}O_{wv}$ and RH . As T_l increases, the
354 equilibrium fractionation factor, ϵ^* , is reduced causing a decline in Δ_e . This produces an enrichment of
355 $\delta^{18}O_{msw}$ to maintain the static input $\delta^{18}O_{cell}$ value.

356 In order to test the sensitivity of g_s (mol m⁻²s⁻¹) and E (mmol m⁻²s⁻¹), whilst also accounting for
357 the physiological coupling between the two processes, these two variables were paired based on a
358 simple expression derived from Fick's law of diffusion where Δw is the leaf-to-air vapour pressure
359 gradient (Dawson, 1996; Pearcy et al., 1989):

$$E = g_s \Delta w \quad (3)$$

360 Calculations were conducted using the Roden model of evaporative enrichment (Roden &
361 Ehleringer, 2000) in an Excel file format available at ftp://ecophys.biology.utah.edu/tree_ring/ (Barbour

362 et al., 2004). Input values of g_s ranged from 0.05 – 1.00 mol m⁻²s⁻¹ at 101.3 kPa to generate paired
363 estimates of E with a subsequent range of 0.40 – 4.20 mmol m⁻²s⁻¹. We recommend caution in
364 interpreting the results on the g_s/E pairing sensitivity test because we have forced the dependence
365 between the two. In reality, these variables might be expected to behave distinctly with variations in
366 climate, subsurface water availability and between species. The effect of g_s and E in the model is shown
367 in Figure 3a. It indicates a negative relationship between g_s / E and $\delta^{18}O_{msw}$ leaf water. The level of leaf
368 water enrichment declines (at a constant e_a) with increasing g_s due to a reduction in leaf temperature
369 and intercellular vapour pressure caused by increasing E (Barbour et al., 2007).

370 **2.3.3 Model sensitivity to covarying parameters**

371 We covaried input parameters to identify those which most strongly affect predictions
372 (retrodictions) of $\delta^{18}O_{msw}$ (Figure 3b-g). The $\delta^{18}O_{cell}$ was kept constant at 30 ‰. The $\delta^{18}O_{cell}$ represents
373 the starting point for the model calculations, and all other variables interact independently of it. The
374 variance of air T was shown to have very little interaction with other variables, as shifts in $\delta^{18}O_{msw}$ are
375 predominantly controlled RH , $\delta^{18}O_{wv}$ and g_s/E (Figure 3b, d & g, respectively). RH had the greatest
376 interaction with $\delta^{18}O_{wv}$ (Figure 3c) and g_s/E (Figure 3f), producing a range in $\delta^{18}O_{msw}$ of 29.1 ‰ and 21.5
377 ‰, respectively for the range of plotted values. The interaction between $\delta^{18}O_{wv}$ and g_s/E (Figure 3e)
378 induced a 10.7 ‰ change in $\delta^{18}O_{msw}$ over the range values for each respective variable. Since the range
379 in co-variables presented are unlikely to represent field conditions, we varied the input values (T , RH ,
380 $\delta^{18}O_{wv}$) using the range of variance observed for a study site in SE France for the growing seasons (May-
381 Sept) of 2000-2010. The results are shown as grey shading in Figure 3b-d and indicate that under the
382 growing season climatic conditions, RH and $\delta^{18}O_{wv}$ remain the primary interacting controls of $\delta^{18}O_{msw}$,
383 producing variations up to 6.1 ‰ over their respective ranges (Figure 3c). The effects were smaller for T -
384 RH and T - $\delta^{18}O_{wv}$, which produced $\delta^{18}O_{msw}$ ranges of 4.5 ‰ and 3.3 ‰, respectively (Figure 3b and d).

385 The sensitivity analyses allow for straightforward identification of how the input variables to the
386 biomechanistic model affect the predictions of $\delta^{18}O_{msw}$. By constraining the relevant variables and
387 increasing the accuracy of the input data, the reliability of model output obviously also increases, and
388 thus the characterization of the source water utilized by a tree during a particular time period is
389 improved. It is evident from the sensitivity analyses that $\delta^{18}O_{wv}$, RH and g_s/E require the greatest
390 constraints since their variability can introduce significant shifts in back-calculated values of $\delta^{18}O_{msw}$.

391 Through sensitivity analyses, we identified the required environmental (T , RH , $\delta^{18}O_{wv}$) and
392 physiological (g_s , E) input variables responsible for modulating leaf level $\delta^{18}O$ enrichment. By utilizing the
393 model in inverse mode, for a known $\delta^{18}O_{cell}$, and driving it with time-dependent input variables, it is
394 possible to calculate $\delta^{18}O_{msw}$ taken up by the tree.

395

396 **2.4 Tool Groups – constraining input variables and interpretative techniques**

397 In this section we outline how the necessary model input variables can be obtained and
398 summarize the interpretative techniques required to explain the resulting $\delta^{18}O_{msw}$ values within a
399 hydrological context. We begin by explaining the sampling and extraction procedures of tree-ring
400 cellulose at annual and sub-annual resolutions, the essential input of the $\delta^{18}O_{msw}$ model. Following this,
401 we present three different tiers of data or ‘Tool Groups’ within ISO-Tool (Figure 4), which highlight the
402 various approaches that can be drawn on to obtain values of T , RH , $\delta^{18}O_{wv}$, g_s/E required for model
403 calculations and the various ways in which these results can be interpreted. The calculated $\delta^{18}O_{msw}$
404 values are most useful if they can be compared to the potential water source endmembers which, in
405 turn, require knowledge of the hydrological processes which control the availability of a particular water
406 source to the tree’s rooting zone.

407 The idea is that Tool Group A represents the optimal suite of techniques and its data inputs
408 should be used whenever and wherever possible. However, when data limitations prevent the use of
409 Tool Group A for particular variable, the second Tool Group (B) should be employed, where
410 possible...and so on. Thus, in a real application, due to universal constraints on data, a researcher is
411 likely to draw relevant data from all three tool groups. In the worst-case scenario, one could use data
412 entirely from Tool Group C and still make progress in water source characterization from tree ring
413 cellulose. The rationale for this classification is to highlight the potential to undertake research into
414 historical ecohydrology even when the most desirable information is unavailable. Of course, the
415 research question also governs which tool group is utilized. A critical point is that prior to conducting
416 isotopic source water investigations, the location of study must exhibit isotopically distinct, potential
417 endmember water sources (isotopic separation), which can be confirmed through exploratory $\delta^{18}O$
418 analyses or from wider literature/ database searches. We encourage researchers to make additions and
419 refinements to the ISO-Tool.

420 For an overview of the techniques listed in Figure 4, please see the supporting information. A more
421 detailed discussion of the methodologies for Tool Group A can be found in supporting text S1 (Barbour
422 & Farquhar, 2000; Beyer et al., 2016; Böttcher et al., 2014; Busch et al., 1992; Cocozza et al., 2016; Cuny
423 et al., 2015; David et al., 2013; Dawson et al., 2002; Delattre et al., 2015; Gröning et al., 2012; Hao et al.,
424 2013; IAEA/GNIP precipitation sampling guide V2.02, 2014; Jarvis, 1995; Lambs et al., 2002; Meinzer et
425 al., 1999; Parnell et al., 2008, 2010; Pearcy et al., 1989; Phillips & Gregg 2003; Robock et al., 2000; Rossi
426 et al., 2006; Rundel & Jarrell, 1989; S.U. et al., 2014; Scott et al., 2004; Snyder & Williams, 2000; Song et
427 al., 2013; Soudant et al., 2016; Sprenger et al., 2016; Stokes, 2004; Volkmann & Weiler, 2014; West et
428 al., 2006; White & Smith, 2013; Zencich et al., 2002; Zweifel et al., 2001), Tool Group B in supporting text
429 S2 (Berkelhammer & Stott, 2009; Bowen & Revenaugh, 2003; Bowen & Wilkinson, 2002; Bowen et al.,
430 2005; Bowen, 2017; Delattre et al., 2015; Entekhabi et al., 2010; GNIP, IAEA/WMO, 2017; Granier et al.,
431 1999; GSOD – ‘Global Surface Summary of the Day’, 2018; Hsieh et al., 1998; Jonard et al., 2011; Jones,
432 1998; McNaughton & Jarvis, 1991; Oren et al., 1999; Richardson et al., 2007; Schwartz et al., 2002;
433 Sprenger et al., 2016; Srivastava, 2017; Vicente-Serrano et al., 2010; West et al., 2006; Yale University,
434 2017) and for Tool Group C, in supporting text S3 (Bowen et al., 2005; Bowen, 2017; Celle-Jeanton et al.,
435 2001; Delattre et al., 2015; Horita & Wesolowski, 1994; GNIP/GNIR, IAEA/WMO, 2017; Jacob & Sonntag,
436 1991; Majoube, 1971; NOAA – ‘National Oceanic Atmospheric Administration’, 2018; Poyatos et al.,
437 2016; Sargeant & Singer, 2016; Singer et al., 2014; Suni et al., 2003; Wen et al., 2010). This is not
438 designed to be an exhaustive review of all possible techniques for the interpretation of $\delta^{18}O_{msw}$, but to
439 broadly identify the tools which could be utilized in source-water characterization.

440

441 **2.4.1 Tree-ring $\delta^{18}O_{cell}$**

442 The tree(s) chosen for isotopic analysis generally reflect particular research questions, but they
443 would ideally be visually healthy individual specimens with distinguishable, annual growth rings in order
444 to determine the associated climatic variables needed for modeling. Otherwise, it is challenging to
445 constrain the timing of water use.

446 After tree selection, tree-rings can be collected the form of tree-cores, obtained by an
447 increment borer (e.g. at least 5 mm diameter) at breast height. Extracted tree-cores are ‘bladed’ to
448 expose a uniform surface to maximize ring visibility. Cores should not be mounted with adhesive to
449 prevent contamination of wood material and ethanol should be used to clean equipment. The tree-rings

450 corresponding to the years of interest can be identified through visual cross-dating or standard
451 dendrochronological methods whereby ring widths are measured under a microscope using a tree-ring
452 measurement program such as 'Measure J2X Tree-Ring Measuring Programme'. Validation of cross-
453 dating can be achieved using a cross-correlation software such as COFECHA (Holmes, 1983).
454 Alternatively, the cores may be scanned combined and ring-widths measured digitally with the
455 coordinate measuring and cross dating software programs CooRecorder and CDendro, respectively
456 (Larsson, 2014). Individual rings of interest are dissected with a scalpel and homogenized in preparation
457 α -cellulose extraction.

458 Sub-annual isotopic analysis of tree rings has been shown to contain additional information that
459 is masked by annual (homogenized) whole-ring analyses and which may be important for understanding
460 the seasonal dynamics of water availability (e.g. Roden et al., 2009; Sargeant & Singer, 2016). Tree-cores
461 can be collected and processed as above but with each whole ring divided into sub-annual segments
462 using a scalpel or microtome (e.g. Gärtner et al., 2014; Helle & Schleser, 2004). The number of divisions
463 is determined by the researcher and we recommend conducting trials on surplus rings to ensure that
464 each slice yields enough material cellulose mass for isotopic analysis.

465 Tree-ring α -cellulose is the preferred material for isotopic analysis since it retains the $\delta^{18}\text{O}$
466 information from the time of formation without undergoing any subsequent isotopic exchange (Wright,
467 2008). In comparison, other components of whole wood material (e.g. hemicellulose, lignin, lipids and
468 waxes) can exchange oxygen and may also represent different periods of synthesis (Battipaglia et al.,
469 2008; Gray & Thompson, 1977). The modified Brendel (MBrendel) method (Gaudinski et al., 2005),
470 allows for batch processing with minimal laboratory equipment. McCarroll and Loader (2004) suggest
471 that for softwoods, the high quantity of resins and extractions can be removed by a Soxhlet apparatus
472 and a toluene-ethanol solution.

473 The α -cellulose can be analyzed for $^{18}\text{O}/^{16}\text{O}$ using an online, continuous flow system of a TC/EA
474 (Temperature Conversion Elemental Analyzer) connected to an Isotope Ratio Mass Spectrometer
475 (IRMS). Results are reported in per mil (‰) deviation from Vienna Standard Mean Ocean Water
476 (VSMOW $\delta^{18}\text{O} = 2.0052 \times 10^{-3}$ ‰) (equation [3], where R_s is the isotopic ratio of the sample and R_{std} is
477 that of the standard). This follows correction to the IAEA-CH3 (cellulose [Hunsinger et al., 2010])
478 standard, co-run with an internal lab standard and the effects of drift and linearity are accounted for to
479 obtain $\delta^{18}\text{O}_{cell}$:

$$\delta^{18}O_{cell} (\text{‰}) = \left(\frac{R_s - R_{std}}{R_{std}} \right) \cdot 10^3 \quad (3)$$

480

481 **3 ISO-TOOL APPLICATIONS**

482 In this section, we present three example applications of ISO-Tool to demonstrate its
483 broad utility. Our examples illustrate water source characterizations drawing on components from each
484 Tool Group for constraining model inputs and the interpretation of results (Figure 4). The sites are all
485 located along the Rhône River, SE France (Table S1), and although we illustrate how ISO-Tool usage in
486 riparian environments, we emphasize that it can be employed in any site where isotopically distinct
487 water sources are present and where tree rings record annual growth. The $\delta^{18}O_{cell}$ results from which
488 $\delta^{18}O_{msw}$ were calculated are in Figure S1a-c. We refer the reader to Figure 4 for each of the following
489 case studies.

490 Statistical analyses were conducted using Minitab (version 17) and are reported relative to the
491 95% significance level. All statistical tests were conducted following the determination of sample set
492 normality with the Anderson-Darling (A-D) test and analysis of equal variance using the Levene test.
493 For the comparison of 2-sample means we used the T-test (T) or Mann-Whitney (Wilcoxon rank) test
494 (U), whilst for >2 datasets an ANOVA (F -test) and post-hoc test (Tukey-Kramer/Games-Howell) was used
495 to determine statistically similar groups.

496

497 **3.1 Example 1: Minimum flow restoration, Pierre-Bénite (PB)**

498 Riparian forests are often the focus of restoration schemes which aim to improve the level of
499 hydrological connectivity through the raising of minimum discharge levels below dams to benefit aquatic
500 ecology as well as riparian forests, but this has never been shown convincingly. This example is based on
501 data from the Pierre-Bénite (PB) site where water managers increased the minimum dry-season river
502 flow from 10 m/s to 100 m/s in the year 2000 to benefit aquatic ecology. This produced a mean water
503 table rise within the floodplain of ~0.5 m (Amoros et al., 2005; Singer et al., 2014). In Singer et al., (2014),
504 analysis of annual tree-ring $\delta^{18}O_{cell}$ records from two cohorts of *Populus nigra* situated at relatively 'high'
505 and 'low' floodplain elevations suggested that the flow restoration enabled phreatic water to become
506 available to *Populus* rooted at low floodplain elevations. Since $\delta^{18}O_{cell}$ cannot be directly compared with
507 local water source $\delta^{18}O$ signatures, we applied ISO-Tool to evaluate the conclusions of Singer et al.,
508 (2014).

509 Whole ring (annually resolved) $\delta^{18}O_{cell}$ was used in this example (Figure S1a), with climate
510 records of T and RH , corresponding to the observed growing season of May-September, obtained from a
511 local climate station (meteofrance.com, 2017). The $\delta^{18}O_{wv}$ values were calculated using an equilibrium
512 fraction factor (Majoube, 1971) using records of T and GNIP (IAEA/WMO, 2017)-OIPC (Online Isotopes in
513 Precipitation Calculator) (Bowen, 2017; Bowen et al., 2005) estimates of $\delta^{18}O_{ppt}$, assuming that $\delta^{18}O_{wv}$
514 was in isotopic equilibrium with $\delta^{18}O_{ppt}$. Monthly estimates of $\delta^{18}O_{ppt}$ were obtained from the records of
515 two equidistant GNIP (IAEA/WMO, 2017) monitoring stations (Figure S2a-b), averaged and correlated to
516 monthly $\delta^{18}O$ values for the site, obtained from the 'Online Isotopes in Precipitation Calculator' (Bowen,
517 2017; Bowen et al., 2005) (Figure S2c). Literature-derived values of g_s/E , were used. In situ
518 measurements of $\delta^{18}O_{RW}$ were unavailable, thus necessitating the use of $\delta^{18}O_{RW}$ from 34 km
519 downstream. Seasonal $\delta^{18}O_{ppt}$ for each year were computed by weighting the monthly $\delta^{18}O_{ppt}$ values by
520 the corresponding P totals recorded at the climate station. Piezometric measurements of water table
521 elevation were available for the study period (Figure S3).

522 In Figure 5a, the $\delta^{18}O_{msw}$ time series of each cohort is shown. Although there is a separation in
523 cohort water use from 2000 onwards, with the 'high' cohort utilizing more enriched $\delta^{18}O_{msw}$ than the
524 'low' cohort (+0.9 ‰), it is not statistically significant ($T_{18} = 1.68$, $p = 0.11$). Neither the 'high' or 'low'
525 poplar trees displayed a shift in $\delta^{18}O_{msw}$ following the flow restoration, drawing on an isotopically similar
526 water source during both periods ('high': $T_{19} = -1.20$, $p = 0.244$; 'low': $T_{19} = -0.65$, $p = 0.542$). The
527 implementation of a minimum river flow was seemingly insufficient to provide sustained phreatic water
528 access to either cohort of trees. Instead, both cohorts seemingly relied on different mixtures of seasonal
529 precipitation. It is possible that the water table elevation did make some portion of phreatic water
530 available to *Populus* at low elevations, e.g. by capillary rise (Sánchez-Pérez et al., 2008), but its signature
531 is masked by mixing with precipitation-sourced vadose moisture (Figure 5a). The lack of clear evidence
532 of a phreatic water signature in $\delta^{18}O_{msw}$ of either cohort after the flow restoration may be due to a
533 combination of insufficient river flow to raise the water table relative to the fixed rooting architecture
534 for mature trees of this species. It is possible that these *Populus* trees previously developed extensive
535 vadose zone roots at the expense of deeper, vertical roots during the period when phreatic water was
536 unavailable (i.e. pre-2000). This dimorphic rooting characteristic is advantageous in environments where
537 phreatic and vadose zone moisture are seasonally variable (Singer et al., 2014).

538

539 **3.2 Example 2: Sub-annual water source use, Donzère-Mondragon (DM)**

540 Seasonal variability in water sources may exert important controls on growing season water
541 availability and corresponding tree health, which could be masked by whole tree-ring isotopic analysis
542 (Sargeant & Singer, 2016). At the Donzère-Mondragon (DM) site we employed ISO-Tool to determine
543 the sub-annual progression of $\delta^{18}O_{msw}$ of two co-located (<5 m apart), streamside individuals of *Fraxinus*
544 *excelsior* (roots restricted to the vadose zone by a coarse gravel layer) and *Populus nigra* (roots capable
545 of phreatic zone access). The two-species approach can be used to investigate the seasonal dynamics of
546 water partitioning within different hydrological reservoirs.

547 We utilized the $\delta^{18}O_{cell}$ from Sargeant and Singer (2016) (Figure S1b), but employ a longer
548 growing season (MJJAS vs. MJJA) and the use of update to date GNIP (IAEA/WMO, 2017) records and the
549 latest version of the OIPC (Figure S4). Sub-annual patterns and inter-species differences in $\delta^{18}O_{msw}$ are
550 evident wherein *Fraxinus* $\delta^{18}O_{msw}$ is enriched by +2.4 ‰ compared to *Populus* ($U = 17211$, $p < 0.001$)
551 (Figure 5b) but a $\delta^{18}O_{msw}$ convergence for both species from 2007-2010 ($T_{58} = 0.59$, $p = 0.557$).

552 Prior to 2007, *Fraxinus*' $\delta^{18}O_{msw}$ generally follows a seasonal pattern, shifting from depleted
553 earlywood (EW) to enriched latewood (LW) (+1.5‰). This suggests the use of non-growing season (NGS)
554 $\delta^{18}O_{ppt}$ for EW ($U = 1083$, $p = 0.716$) and growing season (GS) $\delta^{18}O_{ppt}$ LW ($U = 1318$, $p = 0.053$) growth,
555 since phreatic water is typically unavailable to *Fraxinus* roots due to the presence of a local gravel layer
556 in floodplain soils (Singer et al., 2013, 2014). These water sources correspond to the evolving isotopic
557 signature of vadose zone water during the growing season. However, in 2001 and 2003 the $\delta^{18}O_{msw}$ and
558 sub-annual pattern for *Fraxinus* is depleted compared with $\delta^{18}O_{ppt}$. This may result from overbank
559 flooding and/or a very elevated water table in these years of high streamflow (Sargeant & Singer, 2016),
560 which delivers isotopically depleted water to the vadose zone. For this same period (2000-2006), the
561 *Populus* tree exhibits much smaller swings in seasonal water use and it appears to have used a mix of
562 NGS $\delta^{18}O_{ppt}$ and phreatic water for both EW and LW.

563 During the water-use convergence period, *Fraxinus* $\delta^{18}O_{msw}$ is similar to NGS and GS $\delta^{18}O_{ppt}$, ($F_{3,35}$
564 = 2.96, $p = 0.047$) but distinct from $\delta^{18}O_{RW}$ ($U = 737$, $p < 0.001$). Notably, *Populus* underwent a shift in
565 $\delta^{18}O_{msw}$ to that of $\delta^{18}O_{ppt}$ for both parts of the growing season ($F_{3,36} = 8.10$, $p < 0.001$). This convergence
566 water source use between these species indicates *Populus* switched from phreatic to vadose zone
567 moisture uptake (e.g., use of NGS $\delta^{18}O_{ppt}$ in 2009, Figure 5b). The *Populus* water source shift suggests
568 that the phreatic reservoir became inaccessible. An ancillary dataset provides evidence of local river
569 incision of ~1.5 m (Figure S5) between 2003-2007 (Parrot, 2015). Downcutting of the riverbed reduces
570 local river stage and by extension, lateral hyporheic flow into the floodplain, leading to a water table

571 decline. Thus, *Populus* roots apparently became stranded from the phreatic zone by river incision and
572 were subsequently forced to rely on vadose zone water.

573

574 **3.3 Example 3: Annual vs. sub-annual water source use, Mas Thibert (MT)**

575 There are open questions as to whether gravel layers in river floodplains impede access to phreatic
576 water with river floodplains for shallow-rooting species such as *Fraxinus* (Singer et al., 2014), and
577 whether seasonal patterns of water use for such species are consistent across its distribution within the
578 same region.

579 $\delta^{18}O_{wv}$ measurements taken from the nearby (7 km away) monitoring station of Delattre et al., (2015) to
580 reconstruct historical $\delta^{18}O_{wv}$ and $\delta^{18}O_{ppt}$ for our study period (Figure S6) along with climate data (400 m
581 away) sourced from MeteoFrance (meteo france.com, 2017). This dataset and associated reconstruction
582 enabled more accurate (nearly) site-based estimates of $\delta^{18}O_{wv}$ to the $\delta^{18}O_{msw}$ and seasonal $\delta^{18}O_{ppt}$, as
583 opposed to those obtained from the nearest GNIP station (IAEA/WMO, 2017) at Avignon (~46 km north).
584 We used reported values of leaf gas exchange (g_s/E) for each species in similar environmental
585 conditions. The stomatal conductance / transpiration rates for *Fraxinus* were $0.145 \pm 0.065 \text{ mol m}^{-2}\text{s}^{-1}$ /
586 $2.85 \pm 1.05 \text{ mmol m}^{-2}\text{s}^{-1}$ (Lemoine et al., 2001) and for *Populus* were $0.1625 \pm 0.1225 \text{ mol m}^{-2}\text{s}^{-1}$ /
587 $\pm 1.85 \text{ mmol m}^{-2}\text{s}^{-1}$ (Lambs et al., 2006).

588 Figure 5c demonstrates that a high variability in water source use ($\delta^{18}O_{msw}$) is dampened within
589 the whole-ring series. The sub-annual $\delta^{18}O_{msw}$ within a single year of growth can range from 1.7 ‰
590 (2004) to 10.5 ‰ (2009), whilst the range in recorded annual $\delta^{18}O_{msw}$ values is 4.8 ‰. The year 2009
591 exhibits a substantial difference (8.5 ‰) between annual $\delta^{18}O_{msw}$ (-5.4 ‰) and the third LW value of
592 $\delta^{18}O_{msw}$ (+3.1 ‰). This suggests that sub-annual information on water source uses can provide a more
593 robust characterization of water use than annual data, and this information would enable better
594 understanding plant-water relations.

595 The $\delta^{18}O_{msw}$ in EW is generally enriched by +2.0 ‰ compared to that in LW ($T_{52} = 2.91$, $p =$
596 0.005), but see 2006 and 2009 when this pattern is reversed. Interestingly, the general pattern of
597 enriched EW and depleted LW contrasts to the seasonal pattern of water use by the same species at DM
598 (Figure 5b). This suggests differential seasonal availability of water at particular rooting depths between
599 the two sites. River water is indistinguishable from shallow phreatic water ($T_{16} = -1.00$, $p = 0.333$) at MT,
600 indicating that hyporheic flow from the Rhône is the main source of water to the alluvial aquifer. There
601 is also a clear separation between the means of NGS and GS $\delta^{18}O_{ppt}$ ($T_{20} = -2.61$, $p = 0.017$) at this site
602 because of a markedly warmer growing season that delivers isotopically enriched precipitation. Once

603 infiltrated into the vadose zone, this water becomes further enriched by evaporation before uptake by
604 *Fraxinus*.

605 Despite the absence of a gravel layer impeding *Fraxinus* rooting depth at MT, it still apparently
606 relies on infiltrated precipitation. The greater enrichment in $\delta^{18}O_{msw}$ for EW growth compared with LW
607 may be driven by high spring-time evaporative enrichment of shallow vadose zone moisture arising from
608 the strong and dry, northerly 'Mistral' winds, whilst the LW $\delta^{18}O_{msw}$ may correspond to a relatively
609 lessened evaporative enrichment controlled by summer temperatures.

610

611 **4 DISCUSSION AND CONCLUSION**

612

613 The 'Identification of Source-water Oxygen isotopes in trees Toolkit' (ISO-Tool), presented here,
614 is designed to provide the necessary techniques to reconstruct the historical $\delta^{18}O_{sw}$ signatures used by
615 trees for annual or sub-annual periods and to interpret these signatures in terms of hydroclimatic
616 variability. ISO-Tool is designed to be used with three hierarchical 'Tool Groups' with different data
617 requirements (and thus output resolution). These 'Tool Groups' enable investigations of tree water
618 source use under various conditions of data availability. By bringing together techniques from multiple
619 research fields (e.g. dendrochronology, stable isotopes, hydrological data analysis, tree physiology,
620 ecohydrology) into a single and straightforward application framework, our aim is for ISO-Tool to be
621 easily accessible to scientific researchers interested to retrospectively identify water sources to plants
622 for a range of applications (e.g., to relate them to xylem water isotopes). We highlighted the utility of
623 the toolkit under varying scenarios of data availability and provided several applications: restoration
624 assessment (PB); seasonal water source use (DM) and a comparative analysis of low and high resolution
625 $\delta^{18}O_{cell}$ sampling (MT).

626 The underlying principle of ISO-Tool is that the $\delta^{18}O_{sw}$ utilized for any period can be obtained by
627 reversing the fractionation and exchange mechanisms responsible for producing the discrepancy
628 between the $\delta^{18}O$ of root zone moisture and that which is recorded in tree-ring in cellulose. Singer et al.,
629 (2014) and Sargeant and Singer (2016) utilized versions of this inverse modeling technique to determine
630 the isotopic signature of water sources used by riparian trees at annual and sub-annual resolution,
631 respectively. Recent work by Bose et al., (2016) reconstructed the isotopic value of soil water for trees
632 over a larger spatial scale using thirteen $\delta^{18}O_{cell}$ datasets. The present paper formalizes such methods
633 into a set of tools for assessing historical water use by plants.

634 The flexible modeling framework allows the user to define the values of specific parameters
635 (e.g. leaf temperature and boundary layer conductance), circumventing the reliance on default values
636 which may improve in accuracy as research in these topic areas advances. Additionally, if the researcher
637 is able to provide more accurate estimates for such parameters, they can be easily incorporated.
638 Advancement of the modeling component will necessitate the inclusion of post-photosynthetic
639 metabolism and transport mechanisms (Gessler et al., 2014). As such, we acknowledge that the
640 calculated $\delta^{18}O_{msw}$ values may not represent the *absolute* $\delta^{18}O$ values of water utilized by the tree, yet
641 they remain valuable for interpreting ecohydrological interactions as long as isotopically distinct
642 endmembers are present.

643 We have shown the ISO-Tool capability to add value to analyses of restoration efforts, wherein
644 the back-calculated source waters from a particular tree can be used to assess whether it benefited
645 from increased flow support of an elevated floodplain water table (PB). We also applied ISO-Tool to
646 ascertain water use on sub-annual timescales, and this was demonstrated to be much more powerful
647 than annual analyses, providing useful information on the seasonal variability of water sources during an
648 annual growth cycle (DM and MT). This latter work pointed to strong seasonal differences in water
649 usage for the same species at different locations along the Rhône River, a result that would not appear
650 in annual data.

651 The back-calculation of tree $\delta^{18}O_{sw}$, is a more robust technique than $\delta^{18}O_{cell}$ for determining the
652 water source utilized by trees. We suggest that ISO-Tool is well suited for site-based studies and those
653 along climatic transects, as it is capable of shedding light on the nature of hydrological partitioning
654 within the plant rooting zones.

655 We developed a new tool capable of providing estimates of the $\delta^{18}O$ of source-water(s) utilized
656 by trees and incorporating a hierarchical data quality scheme for the constraint of model variables and
657 interpretative techniques of model output, based on available data sources. We foresee ISO-Tool being
658 useful in a wide range of ecohydrological applications with retrospective insights into source-water
659 utilization by trees becoming an increasingly diagnostic tool under future hydroclimatic changes.

660

661 **Acknowledgments**

662 This work was supported financially by the Natural Environment Research Council (NERC) studentship to
663 C. Sargeant, the NERC Facilities Grant (NIGL), Laboratoire d'Excellence (LabEx) Dispositif de Recherche
664 Interdisciplinaire sur les Interactions Hommes-Milieux (DRIHM) and Observatoire Hommes-Milieux
665 (OHM) du Rhône and the School of Earth and Environmental Sciences at the University of St Andrews.

666 We also acknowledge support for MBS from NSF Grants 1700555 and 1660490, and SERDP Grant RC18-
667 1006, and for CS from the Swiss National Science Foundation (SNF 200021_175888). We thank Melanie
668 Leng, Angela Lamb and Andrew Smith at NIGL for sample analysis and advice, Margaret Barbour for her
669 assistance, Cristina Evans for help coding the inverse Barbour model and Angus Calder, Cheryl Wood,
670 Sabrina Wright, Lot Koopmans, Rory Abernethy and Leonie Strobl for laboratory assistance and Rory
671 McDonald, Matthew Holding and Chris Rowan for field assistance. The inverse model can be found at:
672 <https://github.com/blissville71/InverseBarbourModel> , Matlab code (doi: 10.5281/zenodo/1161221).

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1114

1115 **FIGURE CAPTIONS**

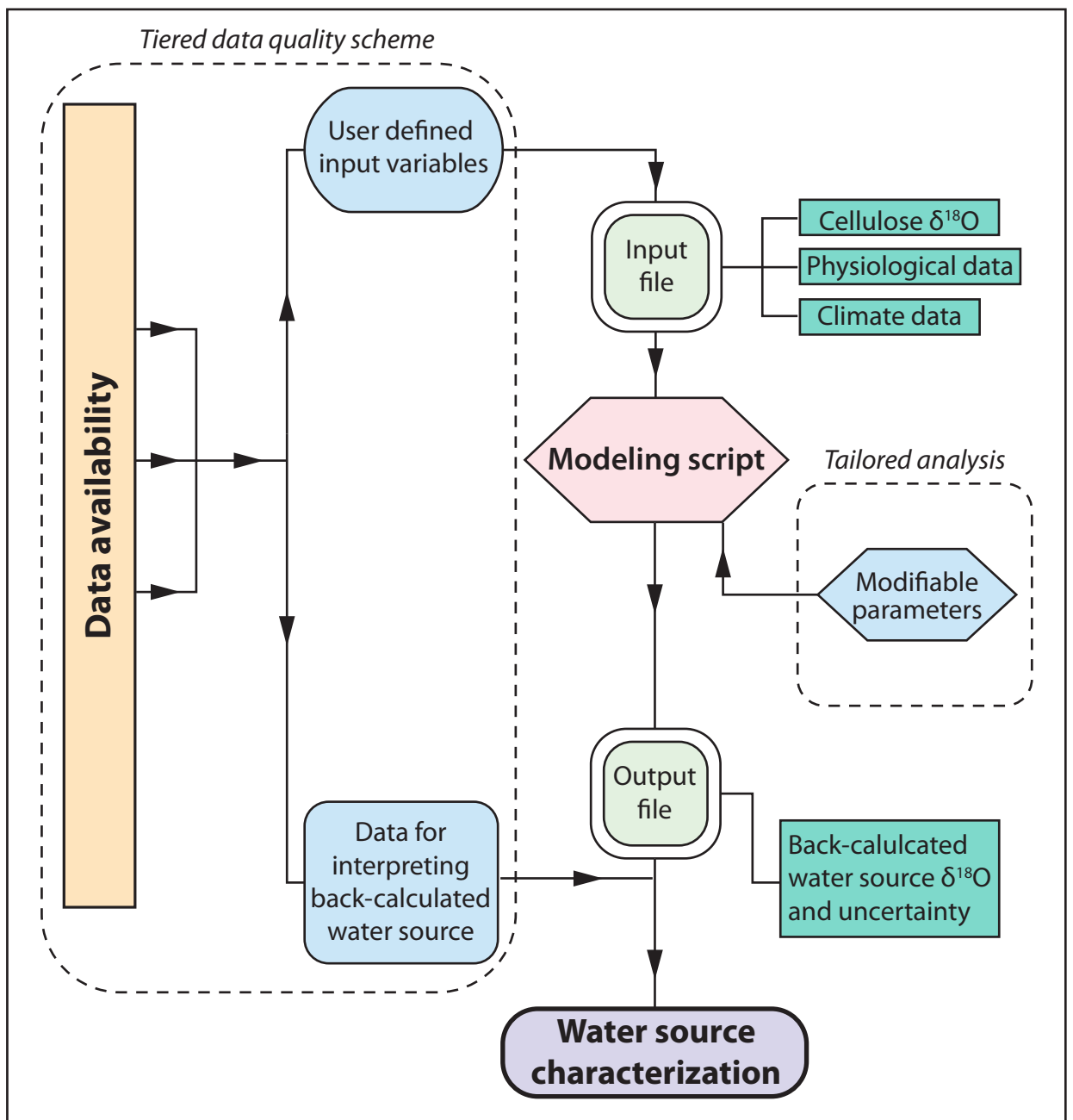
1116 **Figure 1:** Overview of ISO-Tool and the primary components.

1117 **Figure 2:** Key model components and how the required user input values are utilized (a-d), with
1118 associated terminology, and schematics of each process (e).

1119 **Figure 3:** Results of sensitivity analyses of independently (a) and co-varied (b-g) input variables on the
1120 modeled $\delta^{18}O_{sw}$ value. Sensitivity analyses of co-varied model inputs of (b) T and RH , (c) RH and $\delta^{18}O_{wv}$,
1121 (d) T and $\delta^{18}O_{wv}$, (e) $\delta^{18}O_{wv}$ with g_s/E pairing, (f) RH with g_s/E pairing and (g) T with g_s/E pairing. Grey
1122 shading in b-d indicates the range of $\delta^{18}O_{msw}$ results produced by varying the respective input variables
1123 over their observed range of variance for the DM site (May-Sep: 2000-2010).

1124 **Figure 4:** The ISO-Toolkit components and interpretative techniques, divided into tool groups based on
1125 data availability and resolution, with Tool Group A being the most desirable set of data and
1126 interpretative techniques. A review of the mentioned datasets and interpretative techniques is given in
1127 the supplementary text material (S1-3).

1128 **Figure 5:** The mean annual $\delta^{18}O_{msw}$ for *Populus nigra* trees at site PB in relation to potential endmember
1129 water sources mean $\delta^{18}O$ (a), sub-annual $\delta^{18}O_{msw}$ for *Fraxinus excelsior* and *Populus nigra* at site DM
1130 shown in relation to potential endmember water source mean $\delta^{18}O$ (b) and the annual and sub-annual
1131 $\delta^{18}O_{msw}$ for *Fraxinus excelsior* at site MT (c). Colored banding around all reported $\delta^{18}O_{msw}$ is $\pm 1SD$ (based
1132 on the output of 1000 Monte Carlo simulations) and endmember water sources are shown as mean $\delta^{18}O$
1133 $\pm 1SD$.



Model components

Terminology

Conceptualization

Model component references

a) Leaf water ^{18}O evaporative enrichment above source water

$$\Delta_e \approx \epsilon^* + \epsilon_k + (\Delta_v - \epsilon_k) \frac{e_a}{e_i}$$

where:

$$\epsilon^* = \exp \left[\frac{1137}{(T_i + 273.15)^2} - \frac{0.4156}{(T_i + 273.15)} - 2.0667 \times 10^{-3} \right] - 1$$

$$\epsilon_k = \frac{28r_s + 19r_b}{r_s + r_b}$$

Δ_e Enrichment above source water at sites of evaporation

ϵ^* Equilibrium fractionation factor between liquid water and vapor phase

ϵ_k Kinetic fractionation factors of H_2^{18}O through the stomata and leaf boundary layer

Δ_v Difference between $\delta^{18}\text{O}_{sw}$ and $\delta^{18}\text{O}_{wv}$

e_a Vapor pressure outside the leaf

e_i Vapor pressure inside the leaf

T_i Leaf temperature ($^{\circ}\text{C}$)

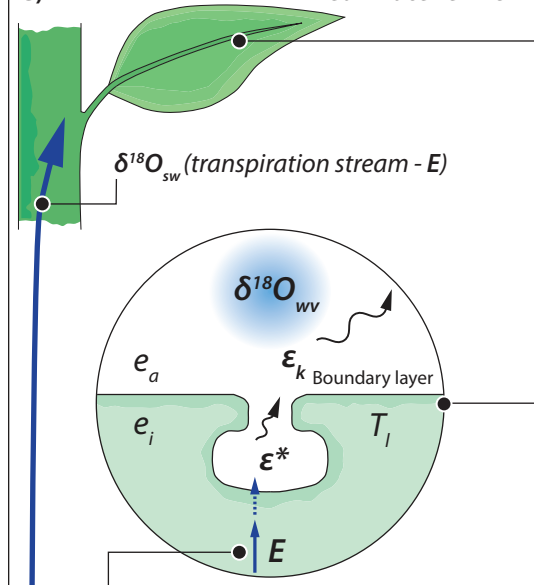
r_s Stomatal resistance (inverse of stomatal conductance [g_s])

r_b Boundary layer resistance (inverse of boundary layer conductance)

28 ‰ kinetic fractionation factor through the stomata (α_k)

19 ‰ Kinetic fractionation factor through the boundary layer ($\alpha_{kb} = \alpha_k^{2/3}$)

e) Leaf water enrichment



b) Péclet effect

$$\wp = \frac{LE}{CD}$$

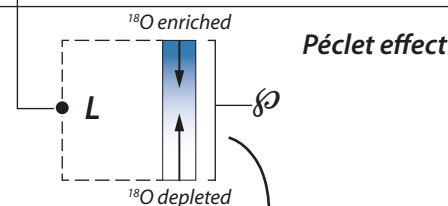
\wp Péclet effect

L Effective pathlength (m) ($L = 2.36 \times 10^{-5} E^{-1.20}$)

E Transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$)

C Molar density of water ($55.5 \times 10^3 \text{ m}^{-3}$)

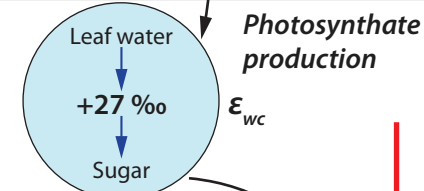
D Diffusivity of H_2^{18}O in water ($2.66 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$)



c) Lamina water ^{18}O enrichment above source water

$$\Delta_L = \frac{\Delta_e (1 - \exp^{-\wp})}{\wp}$$

Δ_L Leaf water enrichment above source water



d) $\delta^{18}\text{O}_{cell}$ enrichment above $\delta^{18}\text{O}_{sw}$

$$\Delta_{cell} = \Delta_L (1 - p_{ex} p_x) + \epsilon_{wc}$$

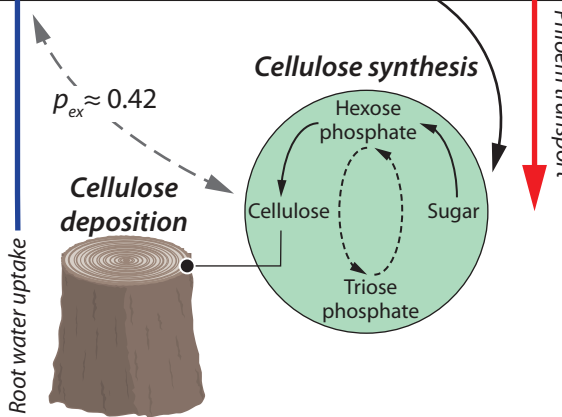


Δ_{cell} Cellulose enrichment above $\delta^{18}\text{O}_{sw}$

p_{ex} Proportion of exchangeable oxygen in cellulose formed from sucrose (0.42)

p_x Proportion of uneriched water in cell (1)

ϵ_{wc} Equilibrium fractionation factor between water and carbonyl oxygen ($+27 \pm 4 \text{ ‰}$)



Required user inputs

$\delta^{18}\text{O}_{wv}$ Atmospheric water vapor $\delta^{18}\text{O}$ (‰) g_s Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$)

T Air temperature ($^{\circ}\text{C}$) E Transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$)

RH Relative humidity (%) $\delta^{18}\text{O}_{cell}$ Cellulose $\delta^{18}\text{O}$ (‰)

Craig & Gordon (1965), Dongmann et al., (1974), Flanagan et al., (1991), Farquhar & Lloyd (1993)

Majoube (1971)

Merlivat et al., (1978), Flanagan & Ehleringer (1991a & b), Luz et al., (2009)

Merlivat et al., (1978), Luz et al., (2009)

Flanagan & Ehleringer (1991a)

Farquhar & Lloyd (1993)

Song et al., (2013)

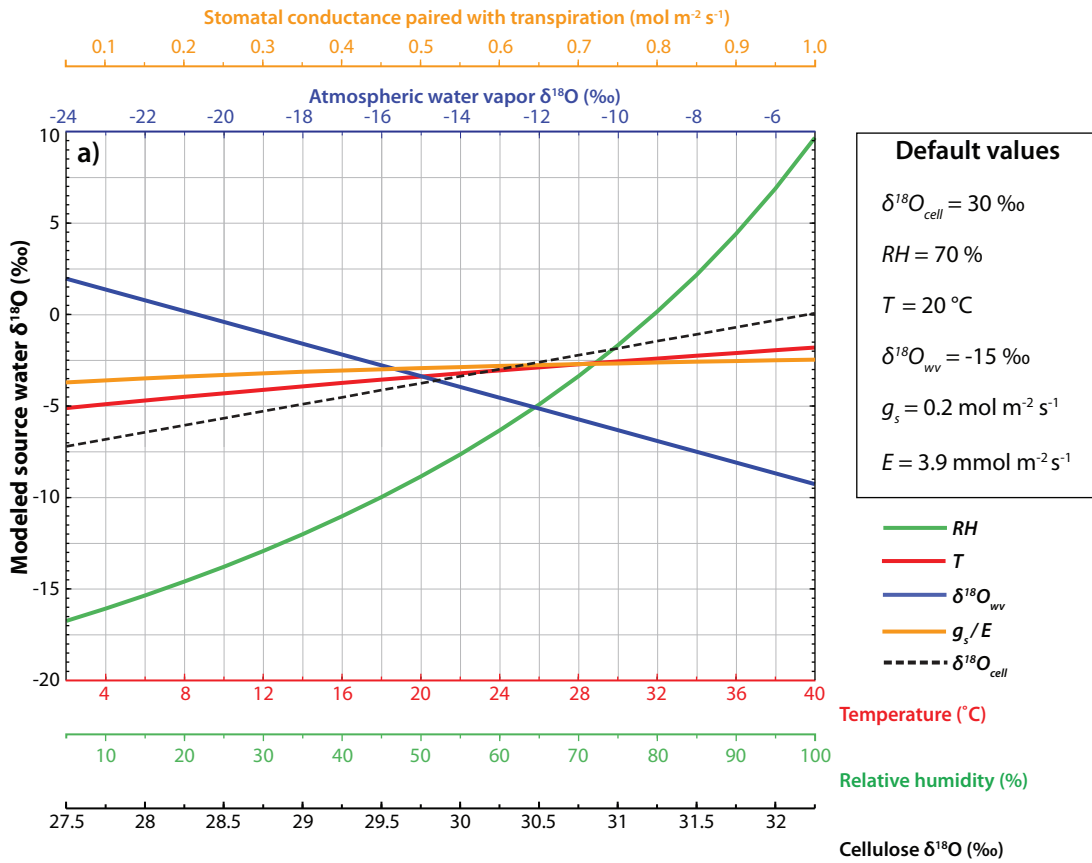
Farquhar & Lloyd (1993)

Hill et al., (1995), Farquhar et al., (1998); Roden et al., (2000)

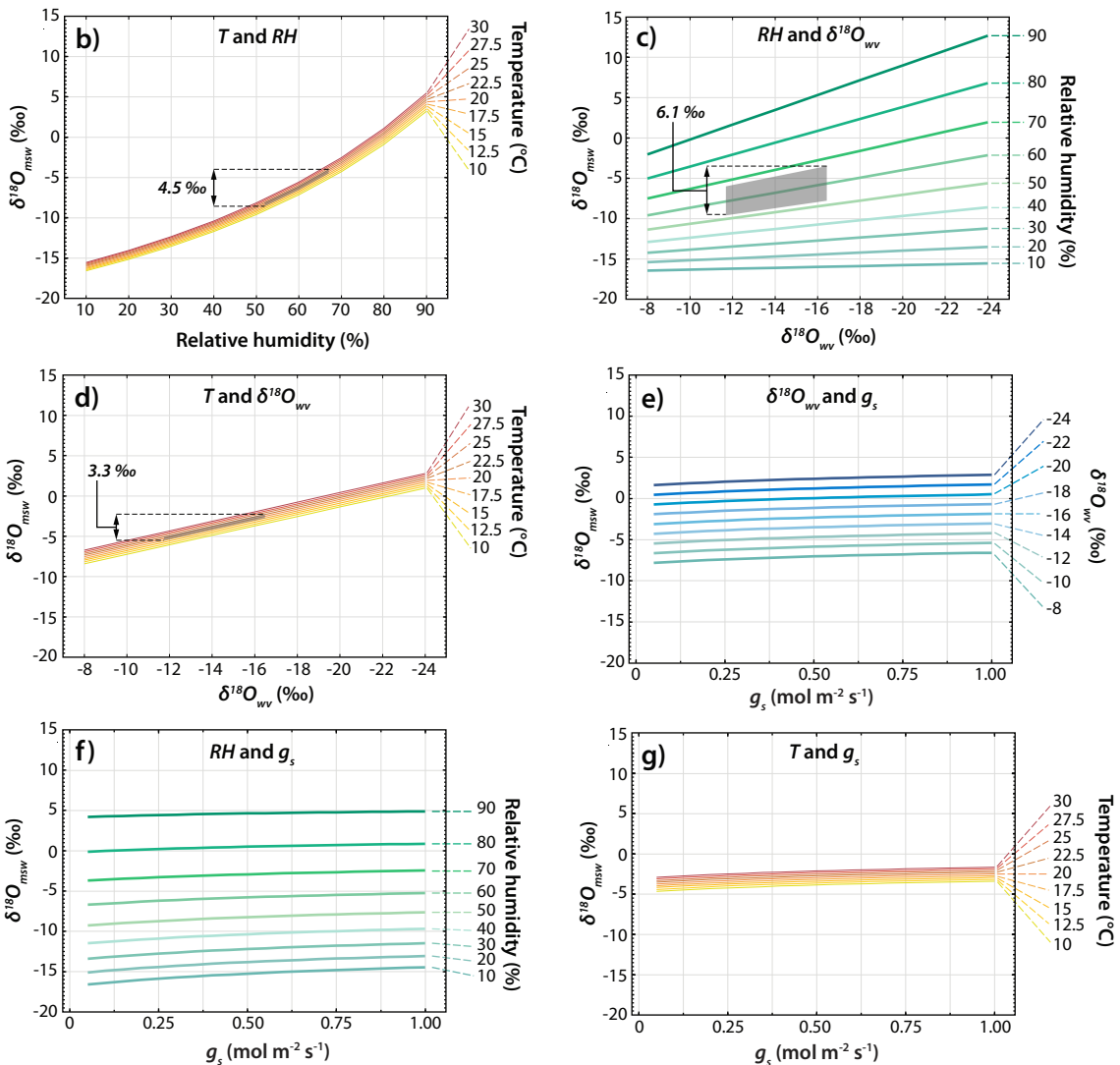
Barbour et al., (2002)

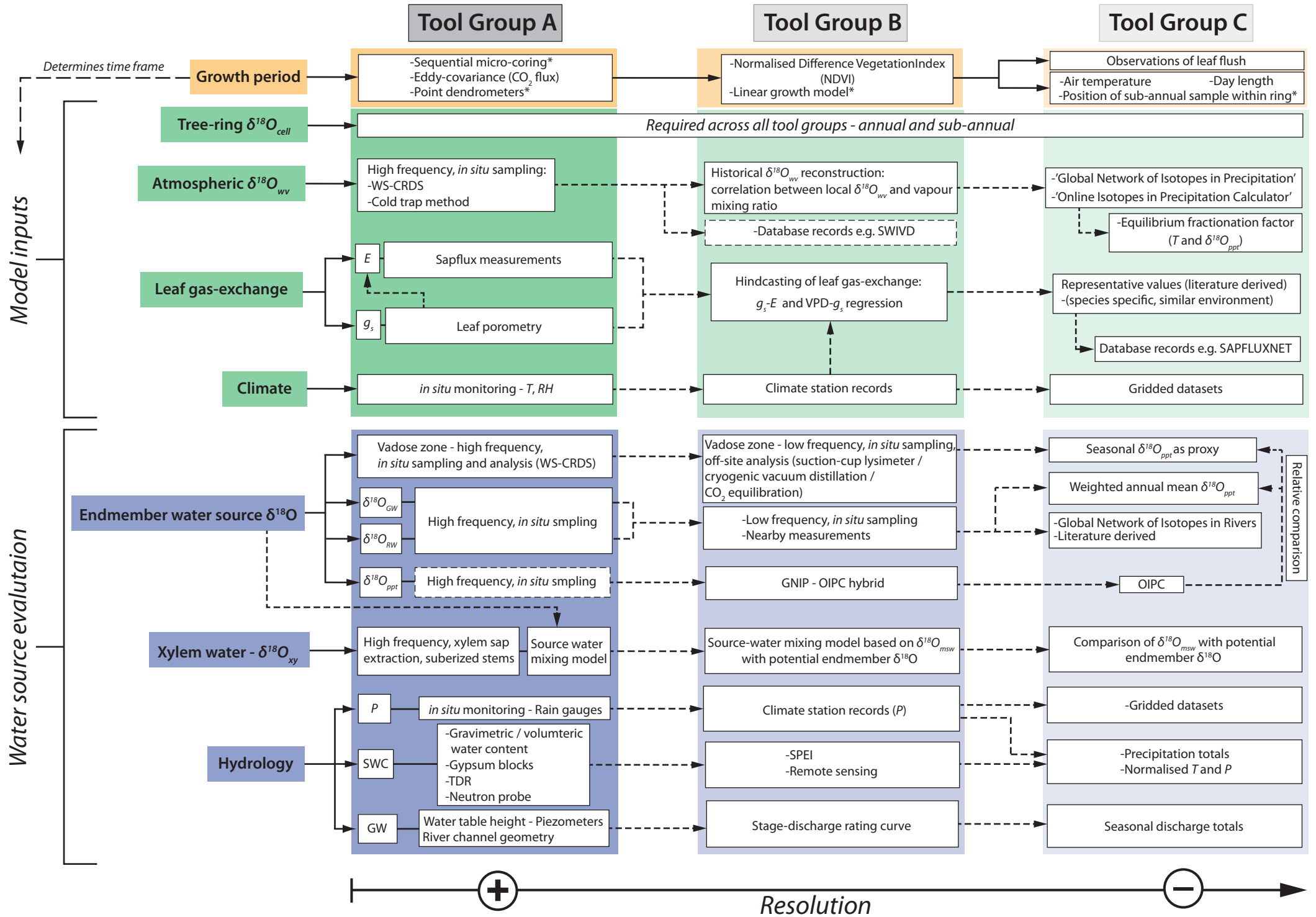
Sternberg et al., (1986), Yakir & DeNiro (1990)

Independent sensitivity analyses

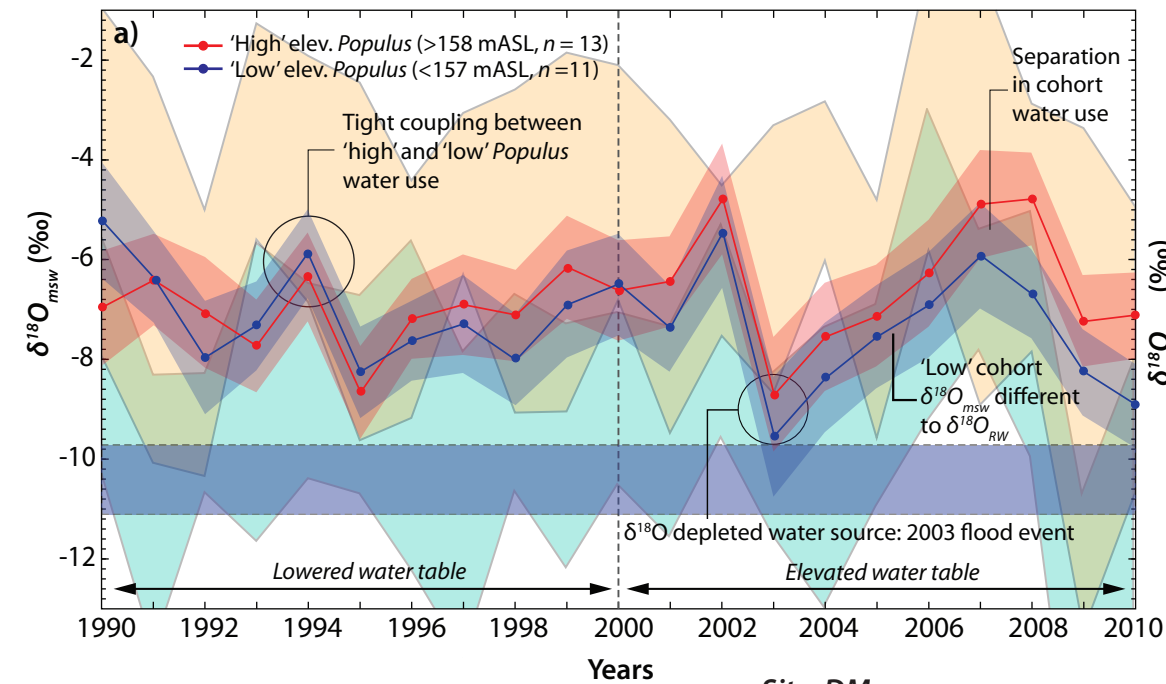


Co-varied sensitivity analyses

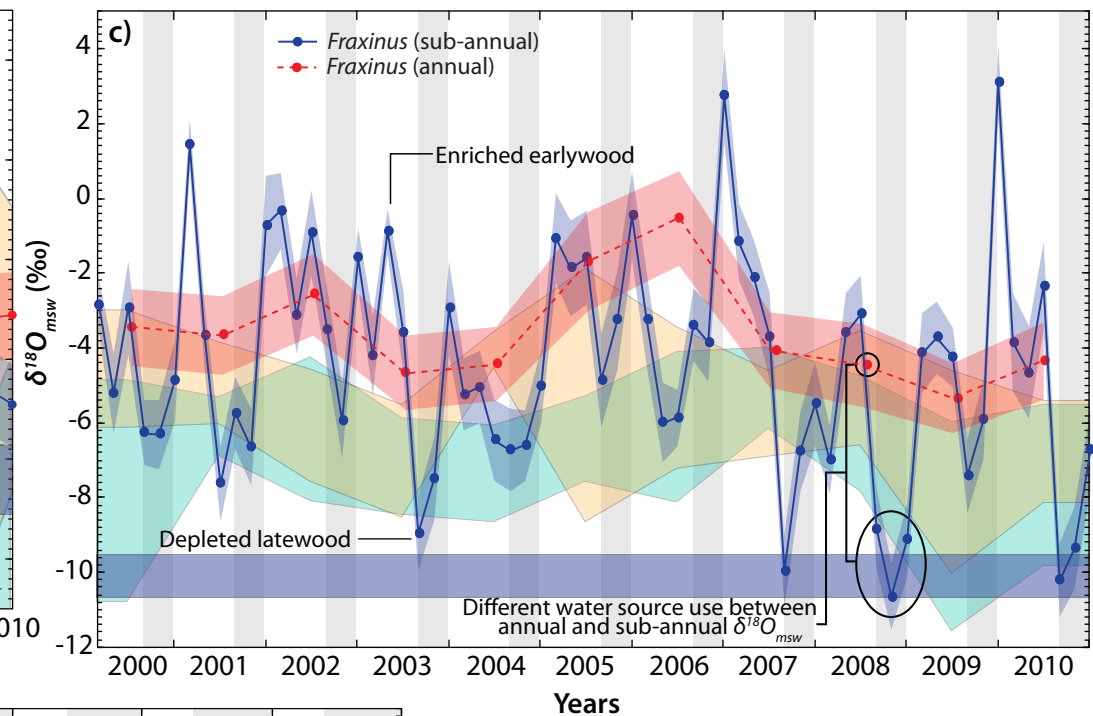




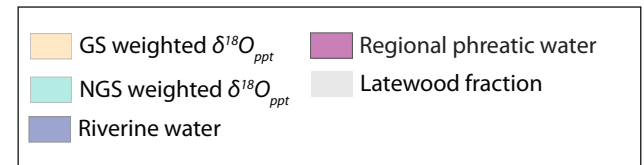
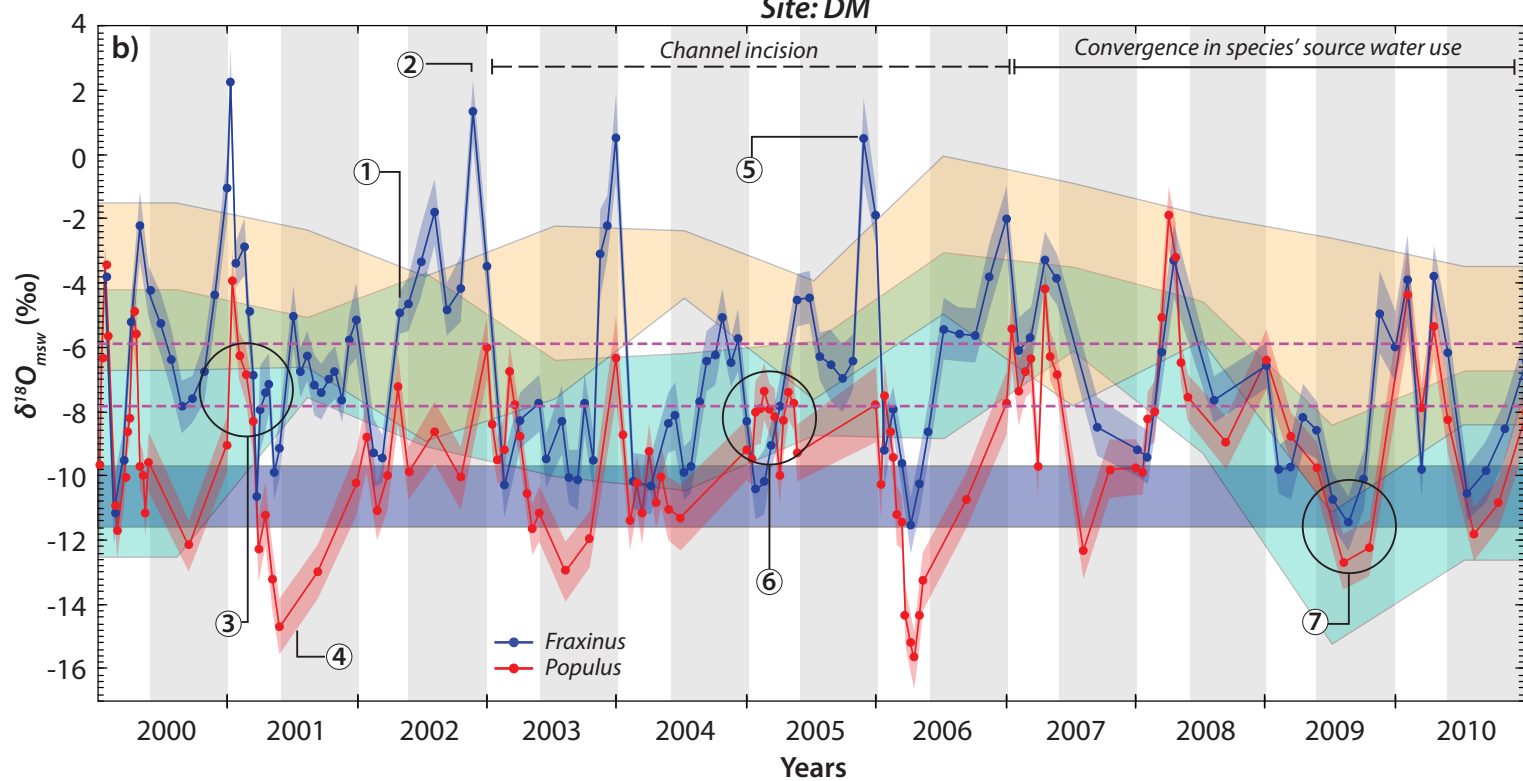
Site: PB



Site: MT



Site: DM



- ① *Fraxinus* EW use of NGS $\delta^{18}O_{ppt}$
- ② *Fraxinus* LW use of GS $\delta^{18}O_{ppt}$
- ③ *Populus* use of regional phreatic water
- ④ *Populus* use of depleted $\delta^{18}O_{RW}$
- ⑤ Evaporatively enriched vadose zone moisture
- ⑥ Potential use of precipitation and riverine water
- ⑦ Inter-species use of $\delta^{18}O$ depleted vadose zone water (heavy NGS precip.)