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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**

3  
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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 km<sup>2</sup> (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  $\delta^2H$ ) 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  $\delta^2H$  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 ( $\delta^{18}\text{O}_{\text{xy}}$ ) 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  $\pm 2$  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 ( $\Delta_e$ ) in the leaf, is  
 285 fundamental in determining the recorded variability in  $\delta^{18}O_{cell}$  values. The  $\Delta_e$  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  $T+1$  (°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  $\varepsilon_k$ , 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  $\delta^{18}O$  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 ( $\delta^{18}O_{sw}$ ) (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  $\delta^{18}O_{msw}$  in response to  
333 variations in  $T$ ,  $RH$ ,  $\delta^{18}O_{ww}$ , 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,  $\Delta_e$  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  $\Delta_e$  on  $\delta^{18}O_{wv}$  can be shown using equation (2). If  $e_a/e_i = 1$  (saturated conditions), then  $\Delta_e$   
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,  $\Delta_e$  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  $\Delta_e$  over this large temperature range, compared to that of  $\delta^{18}O_{wv}$  and  $RH$ . As  $T_l$  increases, the  
354 equilibrium fractionation factor,  $\epsilon^*$ , is reduced causing a decline in  $\Delta_e$ . 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<sup>-2</sup>s<sup>-1</sup>) and  $E$  (mmol m<sup>-2</sup>s<sup>-1</sup>), 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  $\Delta w$  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/](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<sup>-2</sup>s<sup>-1</sup> at 101.3 kPa to generate paired  
363 estimates of  $E$  with a subsequent range of 0.40 – 4.20 mmol m<sup>-2</sup>s<sup>-1</sup>. 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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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

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673

674

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675

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1114

## 1115 **FIGURE CAPTIONS**

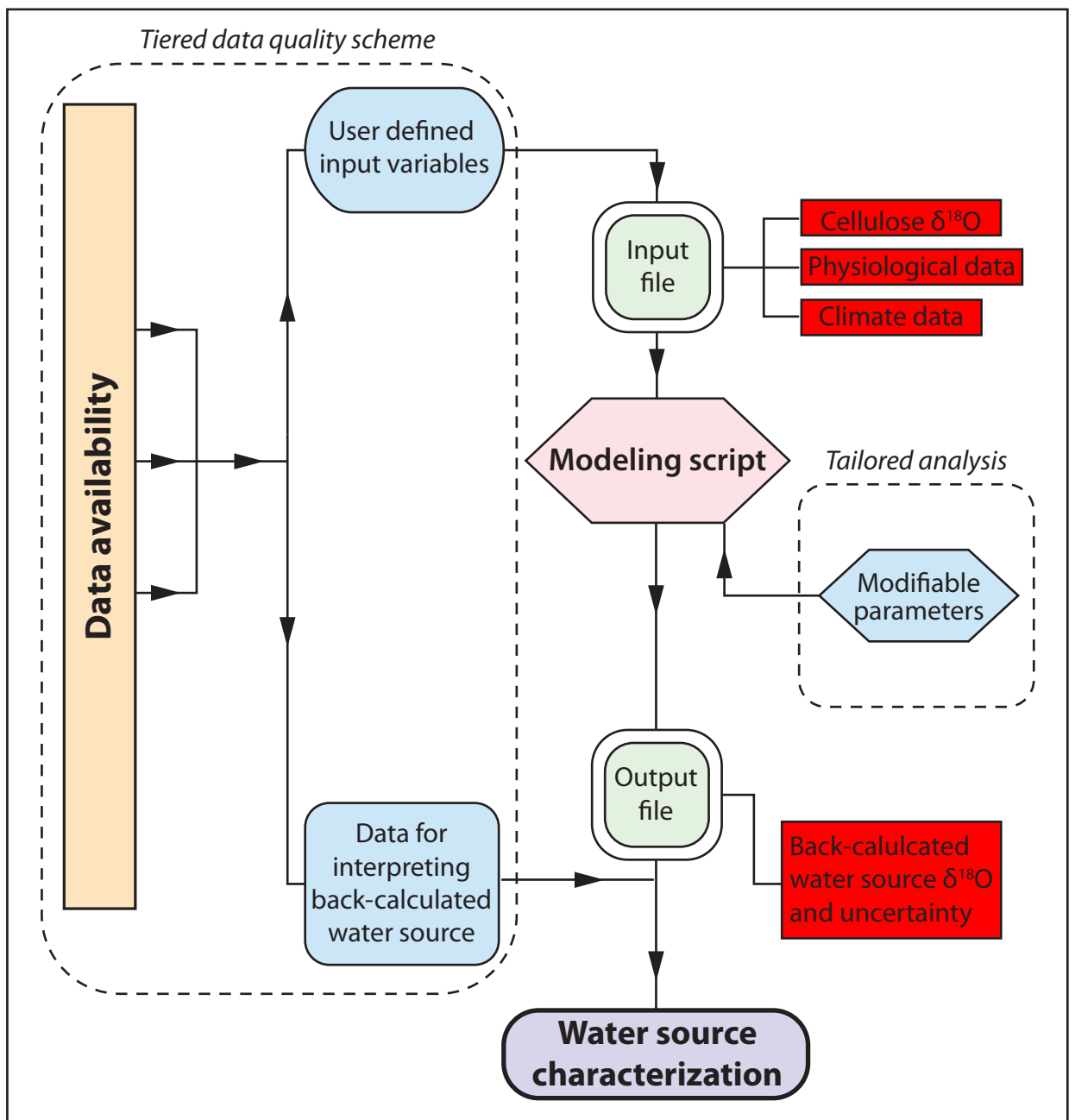
1116 **Figure 1:** Overview of ISO-Tool and the primary components.

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 $^{18}\text{O}$ evaporative enrichment above source water

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

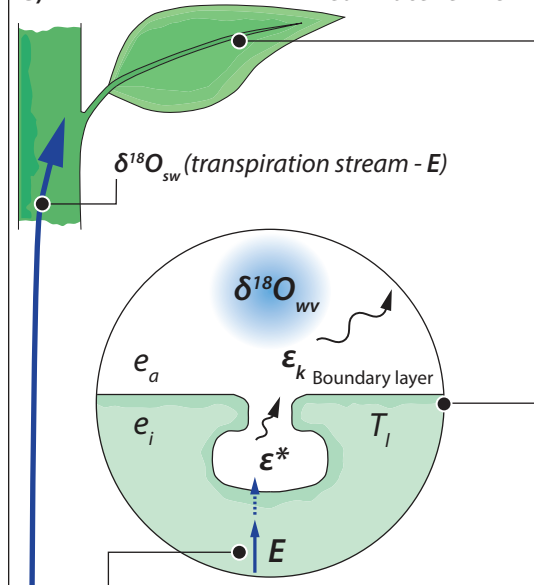
where:

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

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

- $\Delta_e$  Enrichment above source water at sites of evaporation
- $\epsilon^*$  Equilibrium fractionation factor between liquid water and vapor phase
- $\epsilon_k$  Kinetic fractionation factors of  $\text{H}_2^{18}\text{O}$  through the stomata and leaf boundary layer
- $\Delta_v$  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 ( $\alpha_k$ )
- 19 ‰ Kinetic fractionation factor through the boundary layer ( $\alpha_{kb} = \alpha_k^{2/3}$ )

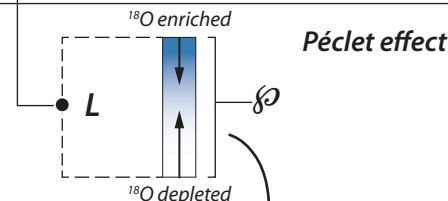
### e) Leaf water enrichment



### b) Péclet effect

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

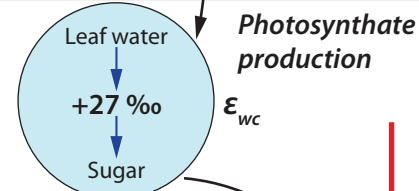
- $\wp$  Péclet effect
- $L$  Effective pathlength (m) ( $L = 2.36 \times 10^{-5} E^{-1.20}$ )
- $E$  Transpiration rate ( $\text{mmol m}^{-2} \text{s}^{-1}$ )
- $C$  Molar density of water ( $55.5 \times 10^3 \text{ m}^{-3}$ )
- $D$  Diffusivity of  $\text{H}_2^{18}\text{O}$  in water ( $2.66 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ )



### c) Lamina water $^{18}\text{O}$ enrichment above source water

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

- $\Delta_L$  Leaf water enrichment above source water

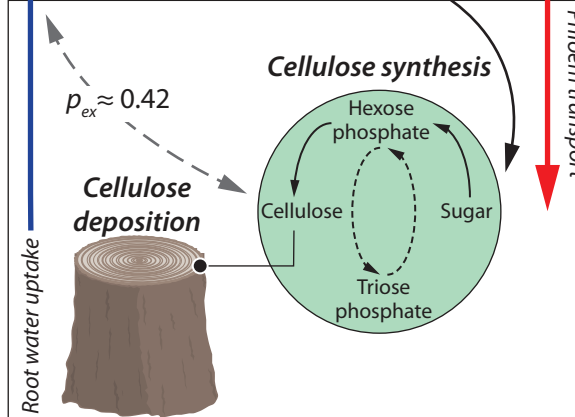


### d) $\delta^{18}\text{O}_{cell}$ enrichment above $\delta^{18}\text{O}_{sw}$

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

$\delta^{18}\text{O}_{cell}$

- $\Delta_{cell}$  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 unenriched water in cell (1)
- $\epsilon_{wc}$  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)

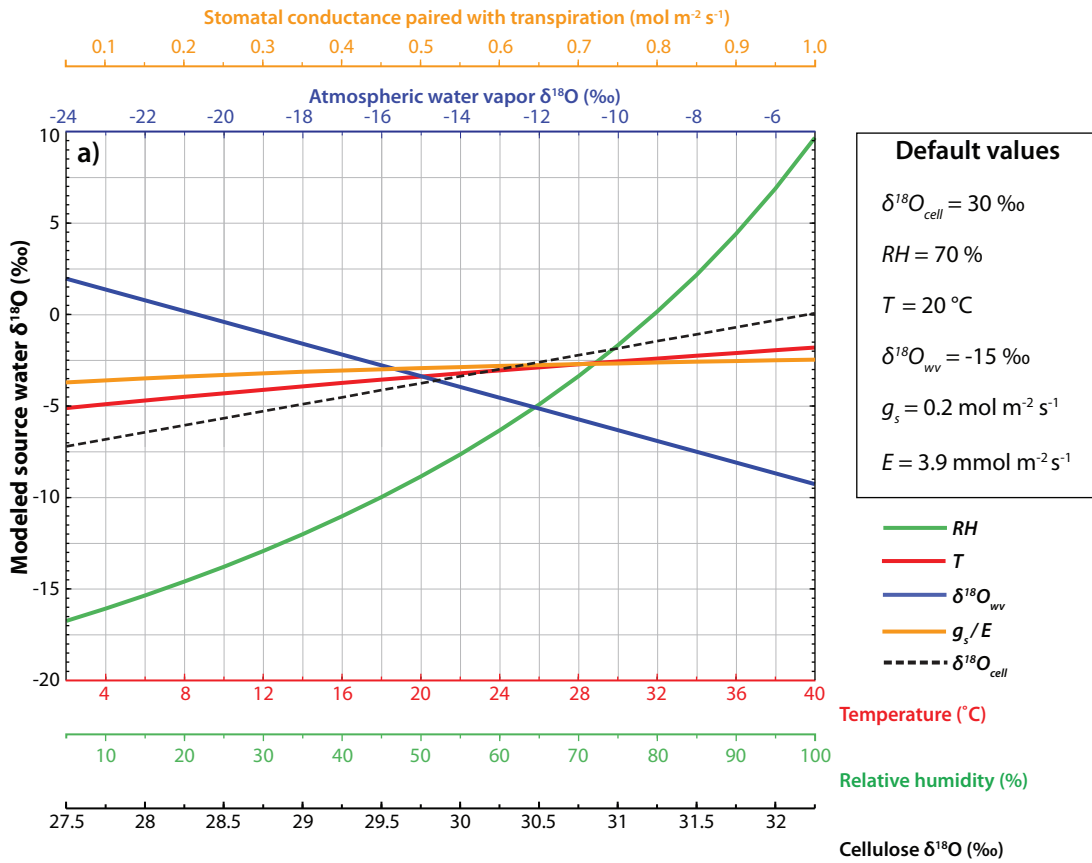
Farquhar & Lloyd (1993)

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

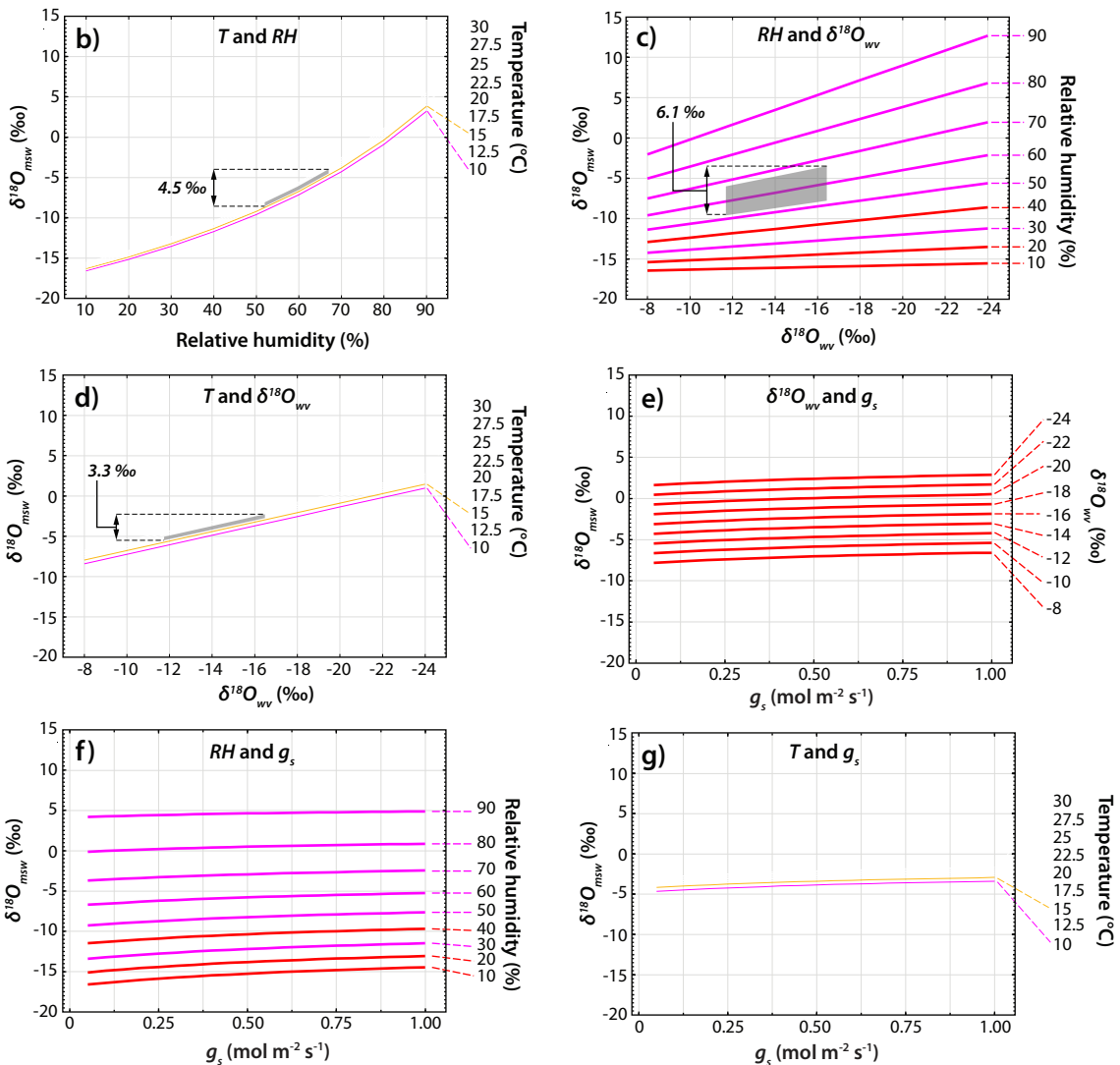
Barbour et al., (2002)

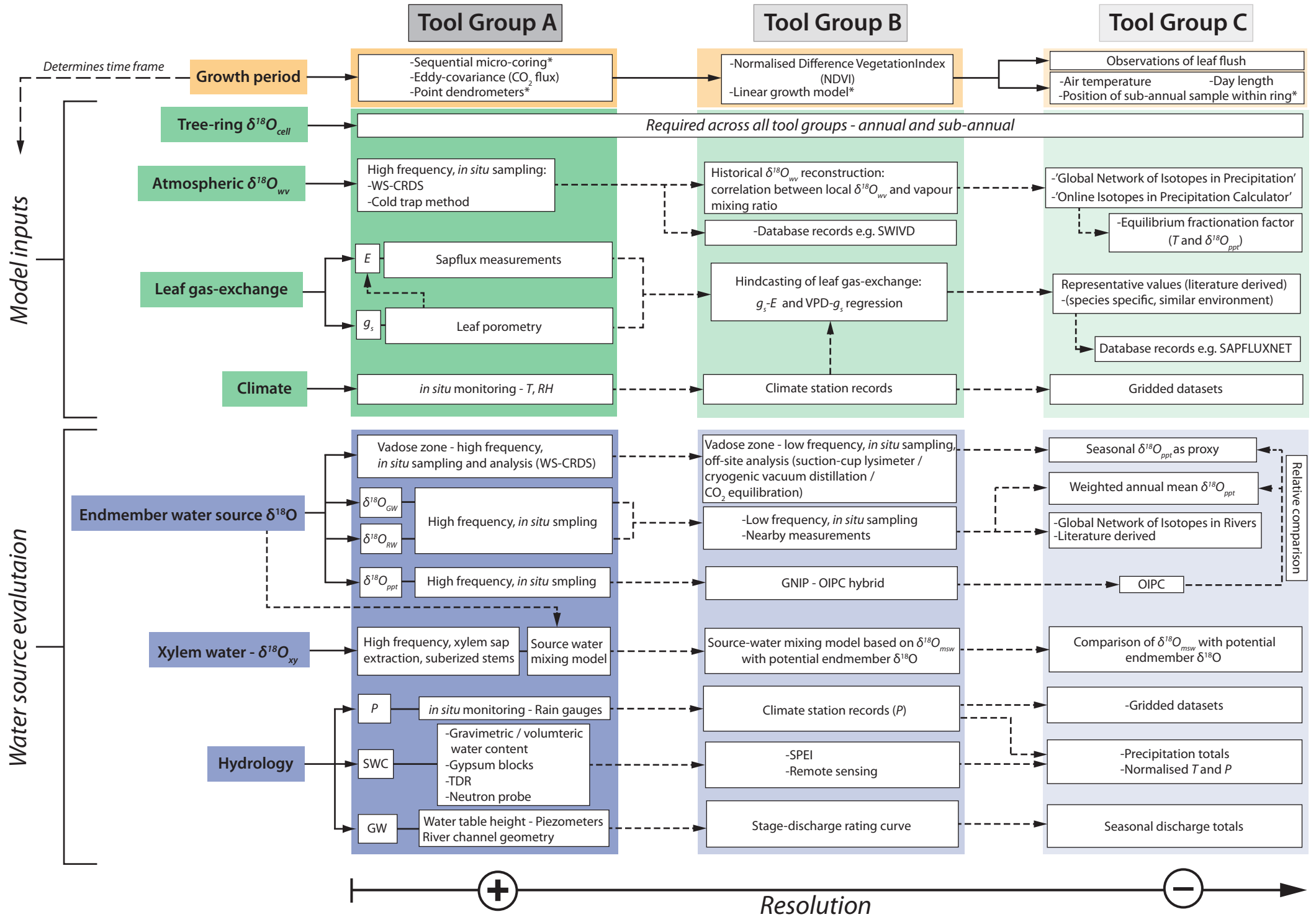
Sternberg et al., (1986), Yakir & DeNiro (1990)

## Independent sensitivity analyses

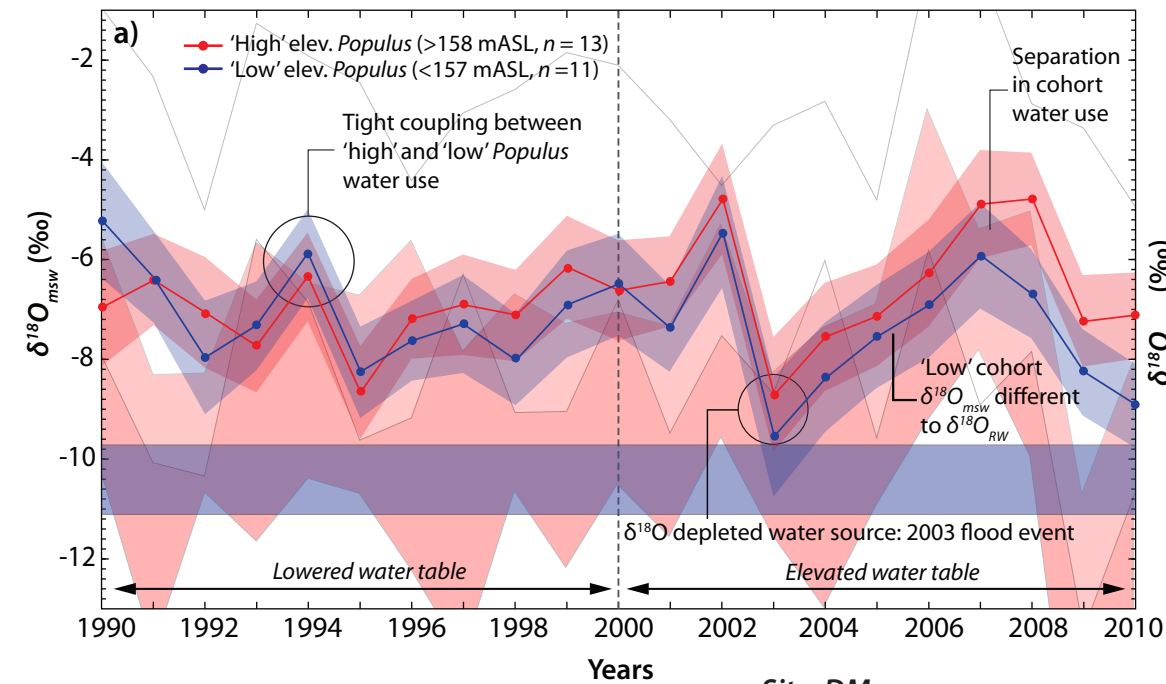


## Co-varied sensitivity analyses

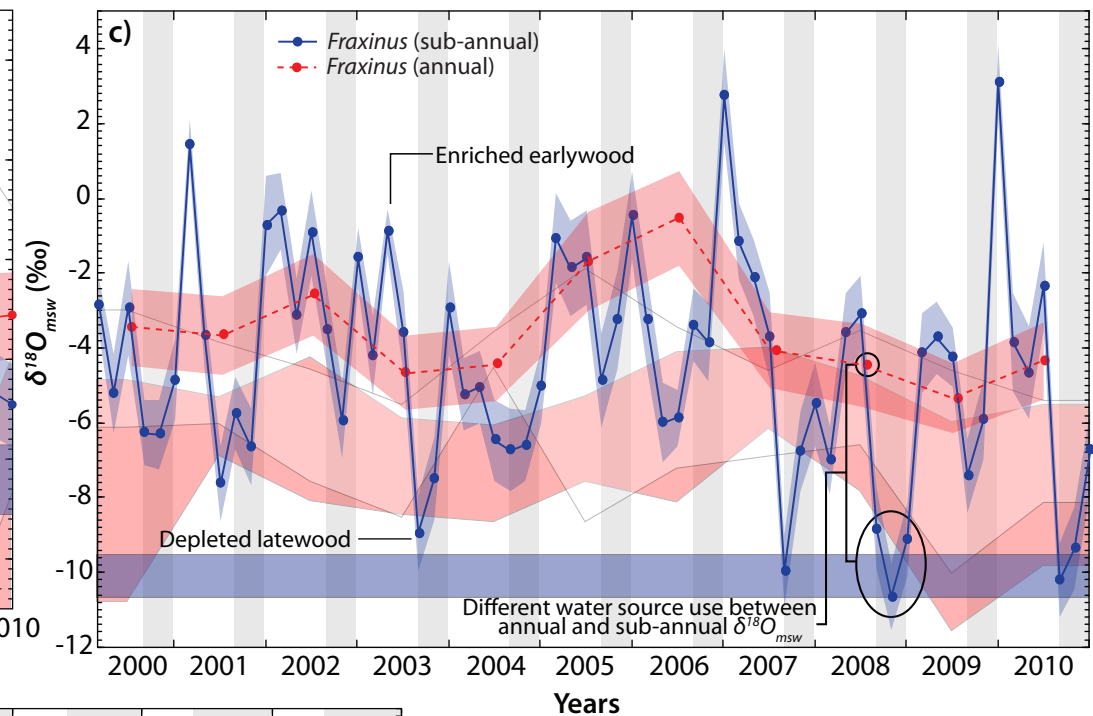




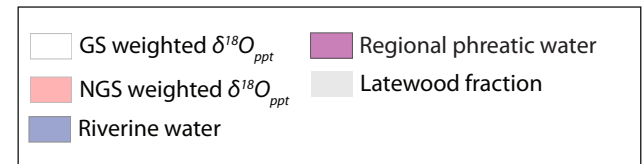
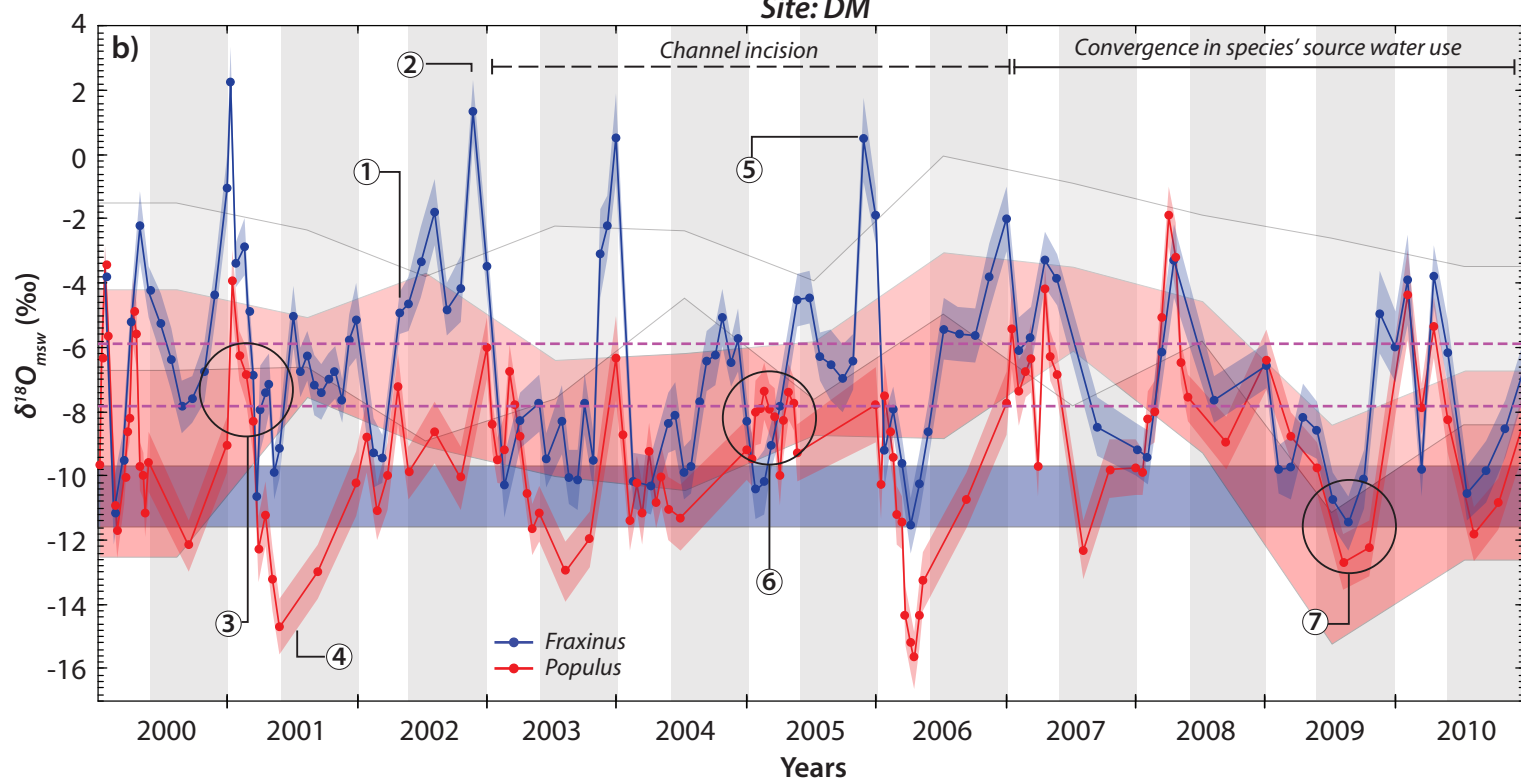
Site: PB



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.)