1 Diurnal variation in the isotope composition of plant xylem

2 water biases the depth of root-water uptake estimates

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21   **Abstract**

22   1. Stable isotopologues of water are a widely used tool to derive the depth of root water

23   uptake (RWU) in lignified plants. Uniform isotope composition of plant xylem water

24   (*i-H$_2$O-xyl*) along the stem length is a central assumption, which has never been properly

25   evaluated.

26   2. We studied the effects of diurnal variation in RWU, sap flux density and various other

27   soil and plant parameters on *i-H₂O-xyl* within a plant using a mechanistic plant hydraulic

28   model and empirical field observations from French Guiana and northwestern China.

29   3. Our model predicts significant *i-H₂O-xyl* variation arising from diurnal RWU

30   fluctuations and vertical soil water heterogeneity. Moreover, significant differences in

31   *i-H₂O-xyl* emerge between individuals with different sap flux densities. In line with

32   model predictions, field data show excessive *i-H₂O-xyl* variation during the day or along

33   stem length ranging up to 25.2‰ in δ^2H and 6.8‰ in δ^{18}O, largely exceeding the

34   measurement error range.

35   4. Our work show that the fundamental assumption of uniform *i-H₂O-xyl* is violated both

36   theoretically and empirically and therefore a real danger exists of significant biases

37   when using stable water isotopologues to assess RWU. We propose to include

38   monitoring of sap flow and soil water potential for more robust RWU depth estimates.

39

40   **Keywords**

41   Deuterium, Ecohydrology, Lianas, Root water uptake, Sap flow, Stable isotope compostion

42   of water, Tropical trees, Water competition

43

44   **1. Introduction**

45   The use of stable isotope composition of water has greatly enhanced ecohydrology studies by

46   providing insights into phenomena that are otherwise challenging to observe, such as depth of

47   root water uptake (RWU) (Rothfuss & Javaux, 2017), below ground water competition and

48   hydraulic lift (Hervé-Fernández *et al.*, 2016; Meunier *et al.*, 2017). Compared to root

49   excavation, the technique is non-destructive, far less labor-intensive and informs on actual

50   RWU while excavation solely informs on root distribution and architecture. Moreover, its

51   flexibility allows use across multiple scales both spatial (i.e. individual to ecosystem) and

52   temporal (i.e. daily to seasonal; Dawson *et al.* 2002). The advantages and wide applicability of

53   this method make it a popular technique that pushes the boundaries of ecohydrology (Dawson

54   *et al.*, 2002; Yang *et al.*, 2010; Rothfuss & Javaux, 2017).

55   A variety of methods exist that infer RWU depth from the isotope composition of plant

56   xylem water (*i-H₂O-xyl)*, but all rely on a direct relationship between the isotopic compositions

57   of plant xylem water and soil water (Ehleringer & Dawson, 1992). More precisely, all have two

58   key assumptions. The first is that the isotope composition of plant xylem water remains

59   unchanged during transport from root uptake to evaporative sites (e.g. leaves and non-lignified

60   green branches). Hence, isotope fractionation - processes that shift the relative abundance of

61   the water isotopolgues during root water uptake and water through non-evaporative tissues - is

62   neglected (Wershaw *et al.*, 1966; Zimmermann *et al.*, 1967; White *et al.*, 1985; Dawson &

63   Ehleringer, 1991; Walker & Richardson, 1991; Dawson *et al.*, 2002; Zhao *et al.*, 2016). Second,

64   all methods assume that xylem water provides a well-mixed isotope composition of water from

65   different soil layers: sampled xylem water instantaneously reflects the distribution and water

66   uptake of the roots independent of sampling time or height.

67   The first assumption is relatively well supported. Fractionation at root level has not

68   raised concerns for most RWU assessments using water isotopologues (Rothfuss & Javaux,

69   2017), with the exception of kinetic fractionation. Kinetic fractionation is a process driven by

70   the differences in molecule mass among the isotopologues that occurs only in extreme

71   environments (Lin & Sternberg, 1993; Ellsworth and Williams, 2007; Zhao *et al.*, 2016).

72   Similarly, isotopic fractionation of water within an individual plant, although possible, is not

73   considered a serious problem (Yakir, 1992; Dawson & Ehleringer, 1993; Cernusak *et al.*, 2005;

74   Mamonov *et al.*, 2007; Zhao *et al.*, 2016). However, the second assumption of time and space

75 invariance of the isotope composition of xylem water has, to our best knowledge, never been

76 assessed.

77   In principle, temporal variance in i-H_2O-xyl within a plant during a day or along its height

78 can be expected on first principles. Here we hypothesize that it is in fact likely that various plant

79 physiological processes, ranging from very simple to more complex mechanisms could

80 influence within plant variance in i-H_2O-xyl at short time scales. For instance, plant

81 transpiration during the course of the day is regulated by atmospheric water demand and leaf

82 stomata which have clear and well known diurnal patterns (Steppe & Lemeur, 2004; Epila *et*

83 *al.*, 2017). This results in changing water potential gradients within the soil-plant-atmosphere

84 continuum and therefore fluctuations in the depth RWU are also expected (Goldstein *et al.*,

85 1998; Doussan *et al.*, 2006; Huang *et al.*, 2017). Hence, as we expect plants capacity to take up

86 water at different soil layers to shift during the day, we should also expect diurnal variation in

87 the mixture of isotope composition from water taken up from various depths. As water moves

88 up along the xylem with velocity proportional to sap flow, different plants and species might

89 respond differently to diurnal variation in RWU. Therefore, from very basic principles we may

90 expect temporal variation in i-H_2O-xy to propagate to different plant heights. As sap flux density

91 depends on plant hydraulic traits in relation to atmospheric water demand and soil moisture

92 gradient, this mechanism could make comparison of isotopic data among individuals and

93 species misleading.

94   In this study we provide a critical assessment of the assumption of i-H_2O-xyl invariance

95 over time and along the length of plant stems. We test the hypothesis that major alterations in

96 the i-H_2O-xyl along the length of lignified plants arise naturally during the day and that this

97 variation in i-H_2O-xyl exceeds the expected measurement error. We test this hypothesis with a

98 twofold approach. First, we build a simple mechanistic model that incorporates basic plant

99 hydraulic realism. We use this model to specifically test that even rudimentary mechanistic

100 models of plant hydraulic functioning predict that diurnal changes in the soil-plant-atmosphere

101 continuum result in shifting mixtures of soil water absorption differing in isotope composition.

102 Second, we test whether the *i-H_2O-xyl* sampled at different plant heights or at different times

103 of the day show large variances with field observations from i) six Neotropical canopy trees

104 and six Neotropical canopy lianas sampled at different heights in French Guiana, and ii) high

105 temporal resolution *i-H_2O-xyl* data of 6 distinct plant species from the Heihe River Basin in

106 northwestern China (Zhao *et al.*, 2014).

107

108 2. Materials and Methods

109 2.1. Part A: Modelling exploration

110 2.1.1. Model derivation

111 The expected *i-H_2O-xyl* at different stem heights within a tree during the course of the day can

112 be derived from plant and physical properties such as root length density, total fine root surface

113 area, water potential gradients and the isotope composition of soil water. We call this the

114 SWIFT model (i.e. <u>S</u>table <u>W</u>ater <u>I</u>sotopic <u>F</u>luctuation within <u>T</u>rees). To derive the SWIFT

115 model, we first describe the establishment of *i-H_2O-xyl* entering the tree at stem base via a

116 multi-source mixing model (Phillips & Gregg, 2003). We subsequently consider vertical water

117 transport within the tree, which relates to the established sap flow pattern. Note that the model

118 presented here, focusses on deuterium but can easily be used to study stable oxygen

119 isotopologues. To ensure consistency and clarity in variable declarations we maintain the

120 following notation in the subscripts of variables: uppercase roman to distinguish the medium

121 through which water travels (X for xylem, R for root, S for soil) and lowercase for units of time

122 and distance (*h* for stem height, *t* for time and *i* for soil layer index). A comprehensive list of

5

123   variables, definitions and units is given in Table 1. A schematic representation of the model is

124   provided in Fig. 1a.

125   *i.   Isotope composition of plant xylem water at stem base.*

126   The deuterium isotope composition of xylem water ($\delta^2 H_{X,0,t}$) of an individual plant at

127   stem base (i.e. height zero; $h = 0$m; Fig. 1a) at time t, can theoretically be derived using the

128   multi-source mixing model approach introduced by Phillips & Gregg (2003). Considering a

129   root zone divided into n discrete soil layers of equivalent thickness Δz, if the deuterium isotope

130   composition of soil water ($\delta^2 H_{S,i}$) in each soil layer is constant over time, a reasonable

131   assumption if the isotopic measurements are conducted during rain-free periods, $\delta^2 H_{X,0,t}$ can

132   be expressed as:

133        $$\delta^2 H_{X,0,t} = \sum_{i=1}^{n} f_{i,t} \cdot \delta^2 H_{S,i}$$ Eq. (1)

134   where $f_{i,t}$ is the fraction of water taken up at the ith soil layer (Fig. 1a) defined as:

135        $$f_{i,t} = \frac{RWU_{i,t}}{\sum_{i=1}^{n} RWU_{i,t}}$$ Eq. (2)

136   and $RWU_{i,t}$ is the net amount of water entering and leaving the roots at time t in the ith soil layer

137   ($RWU_{i,t}$ is defined positive when entering the root). The current representation of the model

138   assumes no water loss via the root system and no mixing of the extracted water from different

139   soil layers within the roots until the water enters the stem base. When tree capacitance is

140   neglected, the sum of $RWU_{i,t}$ across the entire root zone is equal to the instantaneous sap flow

141   at time t, SF_t:

142        $$SF_t = \sum_{i=1}^{n} RWU_{i,t} = \sum_{i=1}^{n} -k_i \cdot A_{R,i} \cdot \left[\Psi_{X,0,t} - (\Psi_{S,i,t} - z_i) \right]$$ Eq. (3)

143   Where k_i is the plant specific total soil-to-root conductance, $\Psi_{X,0,t}$ is the water potential at the

144   base of the plant stem and $\Psi_{S,i,t}$ is the soil water matric potential (Fig. 1a). Total plant water

145   potential is generally defined as the sum of the pressure, gravity and matrix potential. Hence,

146    $\Psi_{X,0,t}$ represents the xylem pressure potential. The term z_i is the gravimetric water potential

147   necessary to lift the water from depth z_i to the base of the stem, assuming a hydrostatic gradient

148   in the transporting roots. The model considers z_i to be a positive value (zero at the surface), thus

149   z_i is subtracted from $\Psi_{S,i,t}$. $A_{R,i}$ is the absorptive root area distribution over soil layer i (Fig. 1a).

150   This parameter can be derived from plant allometric relations (Čermák *et al.*, 2006) which is

151   subsequently distributed over the different soil layers via Jackson *et al.* (1995).

152   The total soil-to-root conductance is calculated assuming the root and soil resistances are

153   connected in series (Fig. 1a):

154    $$k_i = \frac{k_R \cdot k_S}{k_R + k_S}$$ Eq. (4)

155   where k_R is the effective root radial conductivity (assumed constant and uniform), and $k_S =$

156    $K_{S,i}/\ell$ is the conductance associated with the radial water flow between soil and root surface.

157    $\ell = 0.53/\sqrt{\pi \cdot B_i}$ represents the effective radial pathway length of water flow between bulk soil

158   and root surface (De Jong van Lier *et al.*, 2008)(Vogel *et al.*, 2013). B_i represents the overall

159   root length density distribution per unit of soil. $K_{S,i}$ is the soil hydraulic conductivity for each

160   soil depth. $K_{S,i}$ depends on soil water moisture and thus relates to the soil water potential $\Psi_{S,i,t}$

161   of the soil layer where the water is extracted. $K_{S,i}$ is computed using the Clapp & Hornberger

162   (1978) formulation:

163    $$K_{S,i} = K_{s,max} \cdot \left(\frac{\Psi_{sat}}{\Psi_{S,i,t}}\right)^{2+\frac{3}{b}}$$ Eq. (5)

164   where $K_{s,max}$ is the soil conductivity at saturation and b and Ψ_{sat} are empirical constants that

165   depend on soil type (here considered as constant through all soil layers).

166   Subsequently, $f_{i,t}$ can be restructured as:

167 $$f_{i.t} = \frac{k_i \cdot A_{R,i} \cdot \Delta \Psi_{i,t}}{\sum_{i=1}^{n} k_i \cdot A_{R,i} \cdot \Delta \Psi_{i,t}}$$ Eq. (6)

168 where the root to soil water potential gradient is represented as $\Delta \Psi_{i,t} = \Psi_{X,0,t} - (\Psi_{S,i,t} - z_i)$.

169 Combining Eq. (1) and Eq. (6) then allows derivation of $\delta^2 H_{X,0,t}$ as follows:

170 $$\delta^2 H_{X,0,t} = \sum_{i=1}^{n} \left(\frac{k_i \cdot A_{R,i} \cdot \Delta \Psi_{i,t}}{\sum_{j=1}^{n} k_j \cdot A_{R,j} \cdot \Delta \Psi_{j,t}} \cdot \delta^2 H_{S,i} \right)$$ Eq. (7)

171 This equation requires estimates of $\Delta \Psi_{i,t}$, which is preferably measured instantaneously in the

172 field (i.e. via stem and soil psychrometers for $\Psi_{X,0,t}$ and $\Psi_{S,i,t}$, respectively). However, as

173 measurements of $\Psi_{X,0,t}$ are not always available, estimated $\widehat{\Psi}_{X,0,t}$ can be derived from sap flow

174 by re-organizing Eq. (3) into:

175 $$\widehat{\Psi}_{X,0,t} = \frac{\sum_{i=1}^{n} [k_i \cdot A_{R,i} \cdot (\Psi_{S,i,t} - z_i)] - SF_t}{\sum_{i=1}^{n} k_i \cdot A_{R,i}}$$ Eq. (8)

176 which then allows replacement of $\Psi_{X,0,t}$ with $\widehat{\Psi}_{X,0,t}$ in Eq. (7).

177   *ii.*   *Height-dependent isotope composition of plant xylem water*

178   In our model, from the stem base, the water isotopologues simply move upwards with

179 the xylem sap flow, hence diffusion and water fractionation during transportation are not

180 considered. The isotope composition in xylem water at height h and time t ($\delta^2 H_{X,h,t}$) is then the

181 isotope composition of xylem water at stem base at time $t - \tau$.

182 $$\delta^2 H_{X,h,t} = \delta^2 H_{X,0,t-\tau}$$ Eq. (9)

183 where τ is the lag before $\delta^2 H_{X,0,t}$ reaches stem height h (Fig. 1a) which depends only on the

184 true sap flux density in the xylem (SF_V). True sap flux density indicates the real speed of vertical

185 water displacement within a plant, derived by dividing SF_t over the lumen area of the plant (A_x;

186 Fig. 1a) i.e. the total cross-sectional area of the vessels. τ was derived from the mass

187 conservation equality:

188     $$h \cdot A_x = \int_{t=0}^{\tau} SF_t \, dt$$ Eq. (10)

189   Note that since most scientific studies express sap flux density as the sap flow over the total

190   sapwood area (SF_S), rather than over the total vessel lumen area (SF_V), for consistency, we will

191   present the model outputs as functions of SF_S.

192   *iii.   Model parameterization and analyses*

193   We adopted the basic plant parameters from Huang *et al.* (2017) for a loblolly pine

194   (*Pinus taeda L.*) (Table S1). We started with synthetic sap flow patterns and volumes extracted

195   from the model runs of Huang *et al.* (2017) for a typical day (day 11 of the 30 days sequence),

196   and assumed no variation between days. Sap flow follows the plant's water demand which is

197   the result of daily cycles of transpiration driven by photosynthetic active solar radiation (PAR),

198   vapor pressure deficit (VPD) and optimal stomatal response (Epila *et al.*, 2017). Secondly, both

199   the soil water potential ($\Psi_{S,i,t}$) and deuterium isotope composition of soil water ($\delta^2 H_{S,i}$)

200   profiles with soil depth were adopted from Meißner *et al.* (2012) (Fig. S2, see Table S1 for

201   equations) and were assumed to stay constant over time. Since measurements of Meißner *et al.*

202   (2012) are derived from a silt loam plot in the temperate climate of central Germany, soil

203   parameters were selected accordingly from Clapp & Hornberger (1978). Subsequently, the

204   following model simulations were executed (see Fig.1a):

205   1) **Analysis A1: impact of temporal SF$_t$ variation on the isotope composition of**

206   **xylem water at a fixed stem height.** Temporal patterns in deuterium isotope

207   composition in xylem water ($\delta^2 H_X$) were evaluated for a typical situation, i.e.

208   measurement at breast height (*h*=1.30 m), conforming to standard practice of RWU

209   assessment.

210   2) **Analysis A2: impact of temporal SF_t variation at different tree heights.**
211   Temporal patterns in $\delta^2 H_{X,i}$ within a tree at various sampling heights (5, 10 and 15
212   m).

213   3) **Analysis A3: impact of temporal SF_t variation on the isotope composition of**
214   **xylem water and the timing of sampling.** Representation of the profile of $\delta^2 H_X$
215   along the full height of a tree, measured at different sampling times (9:00, 11:00 and
216   13:00), with the standard parameterization given in Table S1.

217   4) **Analysis B: variation in $\delta^2 H_X$ due to differences in absolute daily average sap**
218   **flow speed.** Diurnal patterns in the deuterium isotope composition of xylem water
219   in trees that differ solely in daily averaged SF_V, which are set to 0.56, 0.28 and 0.14
220   m h^{-1} (respectively corresponding to SF_S values of 0.08, 0.04 and 0.02 m h^{-1}).

221   All parameters (e.g. RWU) of the four analyses are given in Table S1.

222   Model runs for each analysis were compared to a null model. The null model adopts the

223 standard assumption of zero variation in $\delta^2 H_X$ along the length of the plant body. We used

224 extraction protocol related measurement errors with an accepted maximum error range of 3‰

225 when water extraction recovery rates are higher than 98% (Orlowski *et al.*, 2013). In our null

226 model, this is represented by a normal distribution with a mean of 0‰ and a standard deviation

227 of 1‰ , i.e. N(μ=0‰, σ=1‰), which makes the probability of an error of ≥3‰ highly unlikely

228 (p ≤ 0.0027). Analytical errors introduced by the measurement device, i.e. a Picarro (California,

229 USA), are considered negligible relative to the extraction error. Note that SF_V, which

230 normalizes sap flow over total vessel lumen area, is correlated with plant diameter at breast

231 height (DBH) which enables comparison with field measurements without the need for explicit

232 consideration of DBH in the model. SWIFT was implemented in R version 3.4.0 (R Core Team,

233 2017), and is publicly available (see GitHub repository HannesDeDeurwaerder/SWIFT).

234

2.1.2. Estimation of rooting depth

235

236 RWU depths were derived from the simulated $\delta^2 H_X$ values by use of both the direct inference

237 method and the end-member mixing analysis method, together representing 96% of the applied

238 methods in literature (Rothfuss & Javaux, 2017). We refer readers to Rothfuss & Javaux (2017)

239 for a complete discussion of these techniques. Here, average rooting depth is assumed to be the

240 depth obtained by relating the simulated $\delta^2 H_X$ with the $\delta^2 H_{S,i}$ depth profile. We compared

241 rooting depth estimates from simulated $\delta^2 H_X$, as described in the analyses above, with the true

242 average rooting depth. The true average rooting depth was defined as the depth corresponding

243 to the daily weighted average $\delta^2 H_X$, calculated as the weighted sum of $\delta^2 H_{X,i,t}$ and the relative

244 fraction of water taken up at each depth.

245

2.1.3. Sensitivity analysis

246

247 We performed two sensitivity analyses to assess the relative importance of all parameters in

248 generating variance in $\delta^2 H_X$ along the length of a plant. In both sensitivity analyses, we varied

249 model parameters one-at-the-time to assess the local sensitivity of the model outputs. The

250 sensitivity analysis provides insight into the design of field protocols, revealing potential key

251 measurements in addition to any caveats.

252   We first assessed model sensitivity to (bio)physical variables by modifying model

253 parameters of soil type, sap flow and root properties as compared to the standard

254 parameterization (given in Table S1). The following sensitivity analyses were considered:

255 **Soil type:** The soil moisture content over all soil layers ($\theta_{S,i,t}$) can be deduced from the

256   considered Meißner et al. (2012) $\Psi_{S,i,t}$ profile (see Fig. S2 and Table S1) using the Clapp

257   & Hornberger (1978) equation:

258 $$\theta_{S,i,t} = \theta_{sat} \cdot \left(\frac{\Psi_{S,i,t}}{\Psi_{sat}}\right)^{-1/b}$$ Eq. (11)

259   Where θ_{sat}, Ψ_{sat} and b are soil-type specific empirical constants that correspond to

260   sandy loam soil textures in the standard model parameterization (Clapp & Hornberger,

261   1978). The derived soil moisture profile ($\theta_{S,i,t}$), in turn, then provides a basis to study

262   the impact of other soil textures. A new soil texture specific $\Psi_{S,i,t}$ profile can then be

263   deduced by using θ_{sat}, Ψ_{sat} and b values corresponding to different soil texture types

264   (values from Table 2 of Clapp & Hornberger (1978)). This enabled us to study $\Psi_{S,i,t}$

265   profiles for four distinct soil types, i.e. (i) sand, (ii) loam, (iii) sandy clay and (iv) clay

266   soils, in relation with the original silt loam $\Psi_{S,i,t}$ profile.

267   **Volume of water uptake:** We varied the total diurnal volume of water taken up by the

268   tree. New SF_t values are scaled using algorithms from literature that provide an estimate

269   of the daily sap flow volume of a tree based on its DBH (Andrade *et al.*, 2005; Cristiano

270   *et al.*, 2015).

271   **Root conductivity:** We varied the root membrane permeability (k_R) to match multiple

272   species specific values found in literature (Sands *et al.*, 1982; Rüdinger *et al.*, 1994;

273   Steudle & Meshcheryakov, 1996; Leuschner *et al.*, 2004).

274   The second set of sensitivity analyses test the impact of root hydraulics, sap flux density

275 and sampling strategies on the sampled $\delta^2 H_X$. We obtained 1000 samples per parameter from

276 corresponding distributions and ranges (given in Table S2) with a Latin hypercube approach

277 (McKay *et al.*, 1979; McKay, 1988). This is a stratified sampling procedure for Monte Carlo

278 simulation that can efficiently explore multi-dimensional parameter space. In brief, Latin

279 Hypercube sampling partitions the input distributions into a predefined number of intervals

280 (here 1000) with equal probability. Subsequently, a single sample per interval is extracted in an

281   effort to evenly distribute sampling effort across all input values and hence reduce the number

282   of samples needed to accurately represent the parameter space.

283

284   **2.2.   Part B: Empirical exploration**

285   **2.2.1.   Data on variation in i-H$_2$O-xyl with plant height**

286   We used data for six canopy trees and six canopy lianas sampled on two subsequent dry days

287   (24-25 August, 2017) at the Laussat Conservation Area in Northwestern French Guiana. The

288   sampling site (05°28.604'N-053°34.250'W) lies approximately 20 km inland at an elevation of

289   30 m a.s.l. This lowland rainforest site has an average yearly precipitation of 2500 mm yr^{-1}

290   (Baraloto *et al.*, 2011). Average and maximum daily temperatures of respectively 30°C and

291   36°C were measured during the sampling period. Sampled individuals are located in the white

292   sands forest habitat (Baraloto *et al.*, 2011), on a white sandy ultisol with typically high

293   percentage of sand.

294   Individuals (Table 2) were selected based on assessment of climbable tree, intactness of

295   leafy canopy vegetation and close vicinity with one another to optimize similarity in

296   meteorological and edaphic characteristics. Liana diameters were measured at 1.3 m from the

297   last rooting point (Gerwing *et al.*, 2006), tree diameters were measured at 1.3 m (Table 2).

298   Sampling was performed between 9am and 2pm to assure high sap flow. Liana and tree

299   sampling allowed highly contrasted sap flux density (Gartner *et al.*, 1990).

300

301   **2.2.2.   Sampling strategy**

302   The stem xylem tissue of individual plants was sampled at different heights (1.3, 5, 10, 15 and

303   20 m where possible) at the same radial position of the stem, between 8:00 and 15:00. The order

304   of sampling, i.e. ascending versus descending heights, was randomized. Tree stem xylem

305   samples were collected with an increment borer (5 mm diameter), resulting in wooden cylinders

306   from which bark and phloem tissues were removed. Coring was performed within the horizontal

307   plane at the predefined heights, oblique to the center of the stem to maximize xylem and

308   minimize heartwood sampling, and slowly to avoid heating up the drill head and kinetic

309   fractionation. Taking one sample generally took between 5 and 10 minutes. Since coring lianas

310   was not possible, we collected cross-sections of the lianas after removing the bark and phloem

311   tissue with a knife. All materials were thoroughly cleaned between sampling using a dry cloth

312   to avoid cross-contamination. Upon collection, all samples were placed in pre-weighed glass

313   collection vials, using tweezers, to reduce contamination of the sample. Glass vials were

314   immediately sealed with a cap and placed in a cooling box, to avoid water loss during

315   transportation.

316

2.2.3. Sample processing

318   Sample processing was performed as in De Deurwaerder *et al.* (2018). Specifically, all fresh

319   samples were weighed, transported in a cooler and frozen before cryogenic vacuum distillation

320   (CVD). Water was extracted from the samples via CVD (4 h at 105°C). Water recovery rates

321   were calculated from the fresh weight, weight after extraction and oven dry weight (48 h at

322   105°C). Samples were removed from the analysis whenever weight loss resulting from the

323   extraction process was below 98% (after Araguás-Araguás *et al.*, 1998). The isotope

324   composition of the water in the samples was measured by a Wavelength-Scanned-Cavity Ring-

325   Down Spectrometer (WS-CRDS, L2120-i, Picarro, California, USA) coupled with a vaporizing

326   module (A0211 High Precision Vaporizer) through a micro combustion module to avoid

327   organic contamination (Martin-Gomez *et al.*, 2015; Evaristo *et al.*, 2016). Internal laboratory

328   references were used for calibration, with measurement precision of ±0.1‰ and ±0.3‰ for $\delta^{18}O$

329   and δ^2H, respectively. Post-processing was performed using SICalib (version 2.16; Gröning,

330   2011)

331   Isotopic composition, expressed in terms of $[^{18}O]/[^{16}O]$ and $[^2H]/[^1H]$ ratios, is

332   represented by δ-values (in our case, $\delta^{18}O$ and δ^2H), which indicate the deviation from a

333   designated standard (i.e. V-SMOW, Vienna Standard Mean Ocean Water) in parts per thousand

334   (expressed in ‰):

335    $$\delta_{sample(‰)} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \cdot 1000$$ Eq. (12)

336   where R is the heavy to light isotope ratio measured in the sample or standard. We calculate

337   normalized *i-H2O-xyl* for each individual at every sampled height h (ε^2H_X and $\varepsilon^{18}O_X$) as being

338   the deviation of each sample from the stem mean (derived from all stem samples of that

339   individual):

340    $$\varepsilon^2H_{X,h} = \delta^2H_{X,h} - \frac{1}{N}\sum_{h=1}^{N}\delta^2H_{X,h}$$ Eq. (13)

341   with N the number of heights sampled per individual.

342

2.2.4. Data on high temporal-resolution variation in *i-H2O-xyl*

344   We used data from three extensive field campaigns by Zhao *et al.* (2014) who sampled plant *i-*

345   *H2O-xyl* at high temporal resolution in the Heihe River Basin (HRB), northwestern China. Four

346   distinct study locations differing in altitude, climatological conditions and ecosystem types

347   were selected. At each location, the dominant tree, shrub and/or herb species was considered

348   for sampling. In August 2009, *Populus euphratica* was sampled in the Qidaoqiao riparian forest

349   (42°01'N-101°14'E) and *Reaumuria soongorica* in the Gobi desert ecosystem (42°16'N-

350   101°17'E; 906-930 m a.s.l). In June–September 2011 *Picea crassifolia*, *Potentilla fruticose,*

351   *Polygonum viviparum* and *Stipa capillata* were measured in the Pailugou forest ecosystem

352   (38°33'N-100°18'E; 2700-2900 m a.s.l). All species were samples 2-hourly, with the exception

353   of P. crassifolia which was measured hourly. Stem samples were collected for trees and shrubs,

354   while root samples were obtained for the herb species (more details in Zhao *et al.* (2014)).

355   Upon collection, all samples were placed in 8 mL collection bottles and frozen in the

356   field stations before transportation to the laboratory for water extraction via CVD (Zhao *et al.*,

357   2011). Both $\delta^{18}O$ and δ^2H were assessed with an Euro EA3000 element analyzer (Eurovector,

358   Milan, Italy) coupled to an Isoprime isotope ratio mass spectrometer (Isoprime Ltd, UK) at the

359   Heihe Key Laboratory of Ecohydrology and River Basin Science, Cold and Arid Regions

360   Environmental and Engineering Research institute. Internal laboratory references were used for

361   calibration, resulting in measurement precision of ±0.2‰ and ±1.0 ‰ for $\delta^{18}O$ and δ^2H,

362   respectively.

363

364   **3. Results**

365   **3.1. Part A: Modelling exploration**

366   **3.1.1. Simulated temporal fluctuation in isotope composition of plant xylem water**

367   *i.   Isotope composition of xylem water at stem base and basic model behavior*

368   At the stem base, simulated $\delta^2H_{X,0,t}$ displays a diurnal fluctuation (Fig. 2) that corresponds to

369   the daily sap flow pattern (Fig. 2c). This pattern is caused by shifting diurnal RWU depth. Early

370   in the morning, when transpiration is low, most of the RWU occurs in deeper layers, where soil

371   water potential is less negative and isotopic composition of soil water is dominated by depleted

372   deuterium (Fig. S2a-b). As transpiration increases during the day, a significant proportion of

373   RWU is extracted from the drier shallow layers, which have an enriched isotopic composition.

374   In the afternoon, as transpiration declines, isotopic composition reflects again the composition

375   of the depleted deep soil and it remains constant throughout the night because SWIFT does not

376   consider mixing of the internal water in stem and roots nor hydraulic lift.

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377   The most enriched δ^2H_X-values (approx.-59‰) are found in alignment with the diurnal

378   minimum of $\Psi_{X,0,t}$ (approx.-0.85 MPa, Fig. 2c). At this moment, $\Delta\Psi_{i,t}$ are maximized, enabling

379   water extracting from the upper and driest soil layers. Most root biomass is located near the

380   surface (cf. Jackson *et al.*, 1995; Fig. S2c) and uptake in these layers will result in relatively

381   high contributions to the total RWU.

382   In contrast, $\Delta\Psi_{i,t}$ are smaller in the early morning and late afternoon causing root water

383   uptake in the upper soil layers to halt. The decreasing $\Delta\Psi_{i,t}$ translates into higher proportions of

384   RWU originating from deeper, more depleted soil layers. This causes δ^2H_X to drop to a baseline

385   of approx. -67‰. This afternoon depletion of δ^2H_X will henceforth be indicated as the δ^2H_X-

386   baseline drop.

387   *ii.   Isotope composition of xylem water at different times, heights and SF_V*

388   Temporal fluctuation in δ^2H_X within a tree at 1.3 m (i.e. the standard sampling height;

389   Analysis A1; Fig. 1a) and at other potential sampling heights (e.g. branch collection; Analysis

390   A2; Fig. 1a), are provided in Fig. 2a-b, respectively. Both analyses show that fluctuations in

391   δ^2H_X depend on the height of measurement and the corresponding time needed to move the

392   water along the xylem conduits. Note that it depends on the selected temporal resolution

393   whether the δ^2H_X-baseline drop at a given height equals the (stem base) minimum (here 1 min,

394   see Fig. S6). The relation between δ^2H_X variance and cumulative sap flow volumes is provided

395   in Fig. 2d. Here, the piston flow dynamics in SWIFT originate from lateral translation of the

396   δ^2H_X fluctuation at $\delta^2H_{X,0,t}$. In addition to sampling height, analysis A3 depicts the importance

397   of sampling time (Fig. 1b).

398   Analysis B outputs predict the occurrence and width of the δ^2H_X-baseline drop as a function

399   of SF_V (Fig. 1c). Moreover, depending on SF_V, the isotopic signal can take hours or days to

400   travel from roots to leaves - as is also observed experimentally (Steppe *et al.*, 2010). Low SF_V

401   allows multiple δ^2H_X-baseline drops over the length of a single tree. This means that sampled

402   δ^2H_X can reflect soil isotopic composition of the past several days. This has direct implications

403   for comparing samples obtained at different times and heights and for species that experienced

404   different SF_V histories.

405

3.1.2.   Potential biases in root depth estimation

407   Both timing of measurement (Fig. 3a) and SF_V (Fig. 3b) influence rooting depth estimates

408   derived via the direct inference and end-member mixing analysis method (Fig. S2) (Rothfuss

409   & Javaux, 2017). Collection of tree samples at 1.30 m can result in erroneous estimation,

410   deviating up to 104% from the average daily RWU depth (Fig. 3). Plotting the relative error in

411   RWU depth as a function of time and SF_V (Fig. 3c) shows that it is possible to time δ^2H_X

412   measurements in a fashion that captures unbiased estimates of the average RWU depth. Xylem

413   water sampling should be timed to capture the δ^2H_X that corresponds to water extracted at peak

414   RWU, and the expected sampling time can be derived by considering the time needed for the

415   water to reach the point of measurement (i.e. at 1.30 m in Fig. 3). In general, SWIFT predicts

416   that plants with slow SF_V should not be measured during the morning hours, as this results in

417   measuring the preceding days' absorbed water. In contrast, trees with higher SF_V support earlier

418   sample collection.

419

3.1.3.   Sensitivity analysis

421   Our sensitivity analyses shows that the expected absolute error in RWU depth assessment is

422   directly related to both 1) maximum variance in and 2) the probability of sampling non-

423   representative δ^2H_X values. The maximum variance depends on the height, while the probability

424   of sampling non-representative areas depends on the width of the "δ^2H_X-baseline drop"

425   respectively (defined above). Hence, bias in δ^2H_X is predominantly a function of the sampling

426   strategy (timing and height of sampling; Fig. S3) in relation to the SF_V of the plant (shown by

427   a strong effect of lumen area and total diurnal RWU volume in Fig. S3) and some biophysical

428   parameter (Fig. S4). We summarized the most important variables as predicted by SWIFT, that

429   should be considering in RWU studies below.

430   Plants on loam soils show larger diurnal δ^2H_X variances (~8‰) in comparison with those of

431   clay soils (~3‰). Larger variances correspond to potentially larger error, but the steeper slope

432   of the δ^2H_X curve results in a thinner δ^2H_X-baseline drop. Hence, loam soil can result in

433   potentially the largest errors but this is mediated by a lower probability of sampling non-

434   representative δ^2H_X values during the day.

435   The volume of water taken up by the plant (SF_t; Fig. S4b) affects xylem water potential of

436   the plant at stem base ($\hat{\Psi}_{X,0,t}$). Higher SF_t requires more negative $\hat{\Psi}_{X,0,t}$, enabling the plant to

437   access more shallow and enriched soil layers. Therefore, an increase in SF_t results in the

438   increase of maximum δ^2H_X values (increased maximum error) but also results in a smaller width

439   of the baseline drop (Fig. 1c). Lower SF_t result in smaller error, but larger probability of

440   sampling an non-representative area (Fig. 1c).

441   Root properties, i.e. root membrane permeability (Fig. S4c) strongly influence both the total

442   range of δ^2H_X variance and the width of the δ^2H_X-baseline drops. Decreasing root permeability

443   results in thinner δ^2H_X-baseline drops, but higher maximum δ^2H_X variance.

444

445   **3.2. Part B: Empirical exploration**

446   The observed normalized deuterium isotope composition in xylem water (ε^2H_X) along the height

447   of lianas and trees showed strong intra-individual variance exceeding the null model by a factor

448   of 3.2 and 4.3 respectively (Fig. 4a-b). Specifically, differences up to 13.1‰ and 18.3‰ in δ^2H

449   and 1.3‰ and 2.2‰ in $\delta^{18}O$ are observed as intra-individual variances for trees and lianas

450   respectively (Table 2).

451   Similarly, excessive diurnal intra-individual δ^2H_X variances emerge in all considered

452   growth forms (Fig. 5). Observed maximums were 18.0‰, 21.0‰ and 25.2‰ in δ^2H_X for trees,

453   shrubs and herbs respectively (Fig. 5; 2.8‰, 6.8‰ and 6.5‰ in $\delta^{18}O_X$ in Fig. S5). The null

454   model expected diurnal variance was exceeded for each species during its measurement period,

455   with the exception of δ^2H_X measurements of _P. euphratica_. The latter is a riparian forest species,

456   living along the river course, where an easily accessible and abundant ground-water reservoir

457   drives its RWU and _i-H₂O-xyl_.

458

459   **4. Discussion**

460   **4.1. Dynamic diurnal isotope compositions of xylem water along plant stems**

461   Our model shows that basic plant hydraulic functioning will result in shifting mixtures of δ^2H_X

462   entering the plant (Fig. 1a-2a). Daily $\Psi_{X,0,t}$ fluctuations interact with the $\Psi_{S,i,t}$ profile causing

463   different parts of the root distribution to be active during the day. The fluctuations in δ^2H_X at

464   the stem base propagate along the xylem with a velocity proportional to the sap flow and this

465   produces variability in sampled δ^2H_X that is much larger than the expected measuring error. In

466   addition, empirical field data show excessive _i-H₂O-xyl_ variance along the stem length (Fig. 4)

467   and over a short time frame (i.e. sub-daily, Fig. 5). Therefore, the assumption of uniform δ^2H_X

468   along the length of a lignified plant is rejected, both theoretically and empirically.

469   Consequently, rather than being static, δ^2H_X values along the height of a plant should be

470   considered a dynamic diurnal process.

471   Importantly, we show that violation of this assumption results in incorrect assessment

472   of differences in RWU depths between plants. Differences do not necessarily result from

473   variability in RWU depth, but may result from monitoring plants at different heights (Fig. 2),

474   at different times (Fig. 1b) or by comparing individuals which have different SF_V (Fig. 1c). Our

475   sensitivity analysis reveals that various soil and plant characteristics have an important role in

476   determining both the daily maximum $\delta^2 H_X$ variance as well as the width of the $\delta^2 H_X$ -baseline

477   drop. These two characteristics directly impact (i) the expected maximum bias in estimates of

478   RWU depth and (ii) the chance of measuring $\delta^2 H_X$ values that do not represent a mixture of all

479   rooting layers during peak RWU (i.e. the baseline drop). Our work supplements the recent

480   overview of Penna *et al.(* 2018) discussing challenges in using stable isotope composition of

481   water to study the terrestrial water fluxes. We additionally advocate that future research should

482   explore the minimum set of (bio)physiological drivers and processes that require quantification

483   to correctly interpret $\delta^2 H_X$ along the length of a plant.

484

485   **4.2. General applicability of model and results**

486   A necessary condition for diurnal shifts in RWU is the existence of a water potential

487   heterogeneity, e.g. more negative water potentials in the upper layers where trees usually have

488   higher root density, which causes a disproportional partitioning of diurnal RWU between deep

489   and shallow roots. Since such a gradient is formed when the upper soil layers undergo

490   evaporation, these conditions are also necessary for the existence of a soil isotopic gradient.

491   Thus, the problem we have identified is intrinsic to the isotopic tracing method for RWU

492   assessment.

493   Plant transpiration results from complex interaction between atmospheric demands (i.e.

494   driven by VPD and radiation) and stomatal conductance which depends on tolerance of drought

495   stress and soil moisture content. We may expect diurnal fluctuation in radiation and VPD, and

496   hence in water transport and depth of water absorption, as modelled here to be a general

497   phenomenon in nature. Hence, there is a real risk of misinterpretation and calculation errors

498   within the existing literature whenever *i-H$_2$O-xyl* are used to asses RWU and water competition

499   strategies. Moreover, much greater fluctuations in VPD and radiation should be expected under

500   natural conditions than the diurnal cycle described here, and these will increase variability of

501   transpiration fluxes, leading to even more complex dynamics of $\Psi_{X,0,t}$. For instance, slight

502   alterations in these variables, i.e. a changing degree in cloud cover, can influence $\Psi_{X,0,t}$ rather

503   abruptly (for e.g. lianas; Chen *et al.*, 2015) and lead to instantaneous changes in $\delta^2 H_X$. Clearly

504   this further complicates the comparison of samples from different plants and sampled at

505   different heights and times, to date overlooked in RWU assessments, and our model certainly

506   illustrates that these considerations are non-trivial.

507   Note that, based on our model, we expect that soil isotopic enrichment experiments will

508   generate extensive $\delta^2 H_X$ variation along the length of trees whenever diurnal RWU fluctuations

509   cause water extraction to shift between labeled and unlabeled soil layers. Furthermore, when

510   enrichment experiments target trees with different hydraulic properties (such as SF_V) care

511   should be taken as to determine when and where to sample these trees in order to assess an

512   enriched isotope composition. Researchers should be certain the signal will be present at the

513   sample height (Fig. 1-2).

514

4.3. Alternative causes of *i-H$_2$O-xyl* fluctuation.

515

516   The SWIFT model provides a simple traceable and mechanistic explanation, using diurnal

517   variations in SF_t and RWU, for the excessive variance and dynamic nature of the *i-H$_2$O-xyl*

518   fluctuations with plant height and time of field samples (e.g. Fig. 4-5) and elsewhere (Cooper

519   *et al.* 1991). We believe that our model provides a plausible simple explanation for diurnal *i-*

520   *H₂O-xyl* variation, which contributes to the variation that is observed empirically. Nevertheless,

521   the model necessarily represents a simplified representation of plant hydraulic functioning and

522   is therefore limited. There may be alternative causes that contribute to the observed intra-

523   individual *i-H₂O-xyl* variances. We discuss these here.

524   *i.   Fractionation at root level*

525   An increasing body of observations show the occurrence of isotopic fractionation at the root

526   level governed by root membrane transport (Lin & Sternberg, 1993; Vargas *et al.*, 2017) or by

527   unknown reasons (Zhao *et al.*, 2016). Brinkmann *et al.* (2019) hypothesize that root level

528   fractionation causes disparity when RWU depth calculations based on $\delta^2 H_X$ measurements are

529   compared with those of $\delta^{18}O_X$. However, it is difficult to imagine a scenario where root

530   fractionation by itself can explain the observed diurnal fluctuations in *i-H₂O-xyl* with height

531   and time. Even if root fractionation significantly contributed to variation in *i-H₂O-xyl,* we would

532   still need to take into account diurnal fluctuation in RWU to explain the observed patterns.

533   ii.   *Temporal and spatial soil dynamics*

534   The dynamics of soil water movement is complex and soil water content can be extremely

535   heterogeneous in the three spatial dimensions and such variation is currently not represented in

536   SWIFT. Hydraulic lift is a process that generates a vertical redistribution of water in the soil

537   through the roots (Dawson & Ehleringer, 1993), which may change the soil water isotopic

538   composition and mixture drawn up by roots. However, hydraulic lift should redistribute and

539   mix the depleted isotopic signal of deeper layers with the enriched signal of shallower layers.

540   This should lead to lower variation in the soil profile, and less variation along plant length, as

541   such hydraulic lift cannot explain the observed patterns. Heterogeneity in horizontal

542   distribution of water pockets may also affect *i-H₂O-xyl* variance. Under these conditions, the

543 horizontal distribution of the absorptive root area becomes more important. However, as the

544 $\Psi_{S,i,t}$ and the isotope composition of soil water of these pockets are interlinked, the mechanistic

545 driver of water extraction is the diurnal fluctuation in water potential gradients in the plant,

546 conform SWIFT.

547 *iii.   Storage tissue and phloem enrichment*

548 Storage tissues release water and sugars in the xylem conduits on a daily basis to support

549 water transpiration demand (Goldstein et al., 1998; Morris et al., 2016; Secchi et al., 2017) or

550 to repair embolism (Salleo et al., 2009; Secchi et al., 2017). Both water and sugars are

551 transported in and out storage tissue via symplastic pathways using plasmodesmata and

552 aquaporins (Knipfer et al., 2016; Secchi et al., 2017), a pathway which has been linked to

553 isotopic fractionation in roots (Ellsworth & Williams, 2007). Moreover, phloem transports

554 photosynthetic assimilates constructed at the leaf level potentially affected by transpiration

555 fractionation (Gessler *et al.*, 2013). Hence, these metabolic molecules might be constructed

556 from enriched ^2H and ^{18}O atoms. Water release from storage or phloem tissue might locally

557 alter *i-H₂O-xyl* (White et al., 1985). Additionally, time between water storage and release could

558 bridge multiple days, and corresponding isotopic composition may reflect different soil

559 conditions. It is evident that such dynamics are complex, and it is hard to predict how storage

560 tissue and phloem enrichment affect the *i-H₂O-xyl* patterns observed here. Xylem isotopic

561 sampling cannot differentiate between water resulting from RWU or storage, and therefore we

562 cannot discount the possibility that tissue and phloem enrichment play a role. At a minimum

563 this adds further uncertainty to RWU assessment.

564 Further studies should determine whether the implementation of additional mechanisms

565 such as tree capacitance, root and stem level fractionation, spatiotemporal soil water dynamics,

566   more detailed root systems or storage tissues impact the intra-individual *i-H₂O-xyl* and should

567   be accounted for to improve RWU assessment and interpretation.

568

569   **4.4. The way forward**

570   Combining a plant hydraulic model with *in situ SF$_V$* and *in situ $\Psi_{S,i,t}$* can help improve

571   the robustness of RWU assessment and interpretation. Measurements of $\Psi_{S,i,t}$ at multiple

572   depths, i.e. by installing multiple soil water potential sensors that measure at high temporal

573   frequency, should be especially valuable since the SWIFT model showed high sensitivity to

574   alterations of this variable and these can be directly supplied as model inputs. At the same time,

575   the availability of *SF$_t$* measurements allows for identifying the moment when water uptake from

576   all root layers is at its maximum, which can be used to determine the optimal timing of sampling

577   at a given height providing a more robust estimation of RWU depth and uptake.

578   Alongside the modeling and theoretical approach presented here, new ways to study

579   δ^2H_X at a high temporal scale are strongly encouraged. For example, pioneering work of

580   Volkmann *et al.* (2016) to the development of an *in situ* continuous isotope measurement

581   technique that offers the possibility for monitoring *i-H₂O-xyl* at a sub hourly resolution. This

582   technique holds strong promise for further elucidating the natural δ^2H_X variances found within

583   plants and the physiology processes from which these variances result. Such high temporal

584   resolution of isotope measurements, coupled with *in situ* monitoring of various environmental

585   and plant biophysical metrics, are needed for both model improvement and further validation.

586   Moreover, these seem inevitable to eventually differentiate all causal mechanisms of the

587   observed intra-individual *i-H₂O-xyl* variance.

588

589   **5. Conclusions**

590   We have demonstrated that the assumption of no intra-individual $i\text{-}H_2O\text{-}xyl$ variation is rapidly

591   violated once models incorporate even basic plant hydraulic functioning. Moreover, the

592   incorrectness of this assumption is confirmed by empirical field data, showing excessive

593   variance and high temporal fluctuations in $i\text{-}H_2O\text{-}xyl$. We expect the observed $i\text{-}H_2O\text{-}xyl$

594   variance and sub-daily fluctuations result, in part, from the mechanisms considered in the

595   SWIFT model, though they likely represent an end product of various physiological processes

596   which impact $i\text{-}H_2O\text{-}xyl$.

597   Our theoretical explorations warn that variability in the isotope composition of plant

598   xylem water can result in erroneous RWU depth estimation and will complicate the

599   interpretation and comparison of data: samples taken at different heights, times or plants

600   differing in SF_V may incorrectly show differences in RWU depth. We further predict that

601   various soil parameters and plant hydraulic parameters affect (i) the absolute size of the error

602   and (ii) the probability of measuring $i\text{-}H_2O\text{-}xyl$ values that do not represent the well-mixed

603   values during the plants' peak RWU. Hydraulic models, such as SWIFT, should be used to

604   design more robust sampling regimes that enable improved comparisons between studied

605   plants. We advocate the addition of SF_t, which indirectly reflects diurnal RWU fluctuations,

606   and $\Psi_{S,i,t}$ monitoring as a minimum in future RWU assessments since these parameters were

607   predicted to be the predominant factors introducing variance in $i\text{-}H_2O\text{-}xyl$ from the SWIFT

608   model exploration. However, soil texture and root permeability are also key considerations to

609   measure especially when comparing across species and sites.

610   Our findings do not exclude additional factors that impact the observed intra-individual

611   $i\text{-}H_2O\text{-}xyl$ variance and temporal fluctuation. Therefore, we strongly emphasize the need for

612   more testing. Directed studies that validate and quantify the relative impact of other plant

613   physiological processes towards variance in $i\text{-}H_2O\text{-}xyl$ are a prerequisite before improved

614   modeling tools can be developed, and bias in RWU assessments eliminated.

627

628 **Author contribution**

629 H.V., M.D.V and P.B. supervised and provided guidance throughout all aspects of the research.

630 H.D.D., M.D.V and H.V. designed the study. H.D.D., L.Z. and L.W. collected the samples and

631 data during the field campaign and performed the processing and analysis of the samples. The

632 model was developed and coded by H.D.D, M.D.V, M.D. and F.M. All authors contributed to

633 interpretation of the results and to the text of the manuscript.

634

635 **Data availability**

636 Both the data and the SWIFT model are available on the GitHub repository

637 HannesDeDeurwaerder/SWIFT

638

639   **Competing interests**

640   The authors declare that they have no conflict of interest.

641

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816 **Tables**

817 **Table 1.** Nomenclature.

Symbol	Description	Unit
$A_{R,i}$	The absorptive root area distribution over soil layer i	m^2
A_{Rtot}	The plants' total active fine root surface area	m^2
$A_{SAPWOOD}$	Sapwood area	m^2
A_x	Total lumen area	m^2
b	Shape parameter for the soil hydraulic properties (Clapp & Hornberger, 1978)	dimensionless
B_i	The overall root length density distribution per unit of soil, not necessarily limited to the focal plant.	$m\ m^{-3}$
$\delta^2 H_{X,0,t}$	Isotope composition of plant xylem water at stem base at time t	in ‰ VSMOW
$\delta^2 H_{X,h,t}$	Isotope composition of plant xylem water at height h and time t	in ‰ VSMOW
$\delta^2 H_{S,i}$	Isotope composition of soil water of the ith soil layer (constant over time)	in ‰ VSMOW
δ_{sample}	Isotope composition of water within a sample	in ‰ VSMOW
$\Delta\widehat{\Psi}_{i,t}$	Estimated water potential gradient between stem base and the ith soil layer at time t derived from Eq. (8)	m
$\Delta\Psi_{i,t}$	Soil water potential gradient between soil and roots at the ith soil layer at time t	$m\ H_2O$
$\varepsilon^2 H_X$; $\varepsilon^{18} O_X$	Normalized isotope composition of plant xylem water	in ‰ VSMOW
$f_{i,t}$	Fraction of water taken up in the ith soil layer at time t	dimensionless
h	Measurement height	m
i	Soil layer index	dimensionless
i-H_2O-xyl	Isotope composition of plant xylem water	in ‰ VSMOW
k_i	Soil-root conductance of the ith soil layer	s^{-1}
K_{max}	Maximum soil hydraulic conductivity	$m\ s^{-1}$
k_R	Effective root radial conductivity	s^{-1}
k_S	The conductance associated with the radial water flow between the soil and the root surface	s^{-1}
$K_{S,i}$	Soil hydraulic conductivity at the ith soil layer	$m\ s^{-1}$
ℓ	The approximated radial pathway length of water flow between bulk soil and root surface	m
LF	Lumen fraction per unit sapwood area	$m^2\ m^{-2}$
n	Number of unique contributing water sources	#
Ψ_{sat}	Soil water potential at soil saturation	m
$\Psi_{S,i,t}$	Soil water potential of the ith soil layer at time t	m
$\Psi_{X,0,t}$	Water potential at the base of the plant stem at time t	m

R	Heavy to light isotope ratio measured in the sample or standard	%
$RWU_{i,t}$	Net amount of water entering and leaving the root tissues per unit of time in the ith soil layer at time t	$m^3\ s^{-1}$
SF_t	Instantaneous sap flow at time t	$m^3\ s^{-1}$
SF_S	Sap flow velocity, calculated as the sap flow per sapwood area	$m\ h^{-1}$
SF_V	True sap flux density, calculated as the sap flow per lumen area	$m\ h^{-1}$
τ	Delay before the isotope composition of xylem water at stem base reaches stem height h	s
θ_{sat}	Soil moisture content at soil saturation	$m^3\ m^{-3}$
$\theta_{S,i,t}$	Soil moisture content of the ith soil layer at time t	$m^3\ m^{-3}$
z_i	Soil depth of the ith soil layer	m

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Table 2. Sampled liana and tree individuals, provided with their species, respective diameter at breast height (DBH, in cm) and their $\delta^2 H$ and $\delta^{18}O$ ranges (in ‰, VSMOW) measured per individual.

Code	Growth form	DBH [cm]	Family	Species name	$\delta^2 H_X$-range [in ‰, VSMOW]	$\delta^{18}O_X$-range [in ‰, VSMOW]
SP1	Tree	15.6	Moraceae	*Coussapoa sp.*	-30.1; -25.5	-2.8; -2.6
SP2	Tree	50.9	Fabaceae	*Vouacapoua americana*	-23.9; -18.1	-3.1; -2.2
SP3	Tree	44.6	Vochysiaceae	*Erisma nitidum*	-27.7; -20.8	-3.2; -1.9
SP4	Tree	26.1	Sapotaceae	*Micropholis guyanensis*	-29.8; -28.0	-3.0; -2.9
SP5	Tree	21.0	Anacardiaceae	*Tapirira guyanensis*	-31.1; -18.0	-3.2; -2.2
SP6	Tree	49.7	Fabaceae	*Albizia pedicellaris*	-26.9; -22.1	-3.2; -2.6
SP1	Liana	2.8	Polygonaceae	*Coccoloba sp*	-27.9; -20.7	-3.9; -2.3
SP2	Liana	2.7	Convolvulaceae	*sp.*	-29.3; -24.0	-4.4; -2.9
SP3	Liana	0.8	Moraceae	*sp.*	-40.8; -22.6	-4.5; -2.3
SP4	Liana	3.8	Combretaceae	*cf. rotundifolium Rich.*	-23.6; -15.2	-2.9; -2.0
SP5	Liana	0.7	Convolvulaceae	*Maripa cf violacea*	-31.6; -19.7	-3.8; -2.7
SP6	Liana	3.8	Convolvulaceae	*Maripa sp.*	-35.3; -24.4	-4.8; -3.1

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824 **Figures**
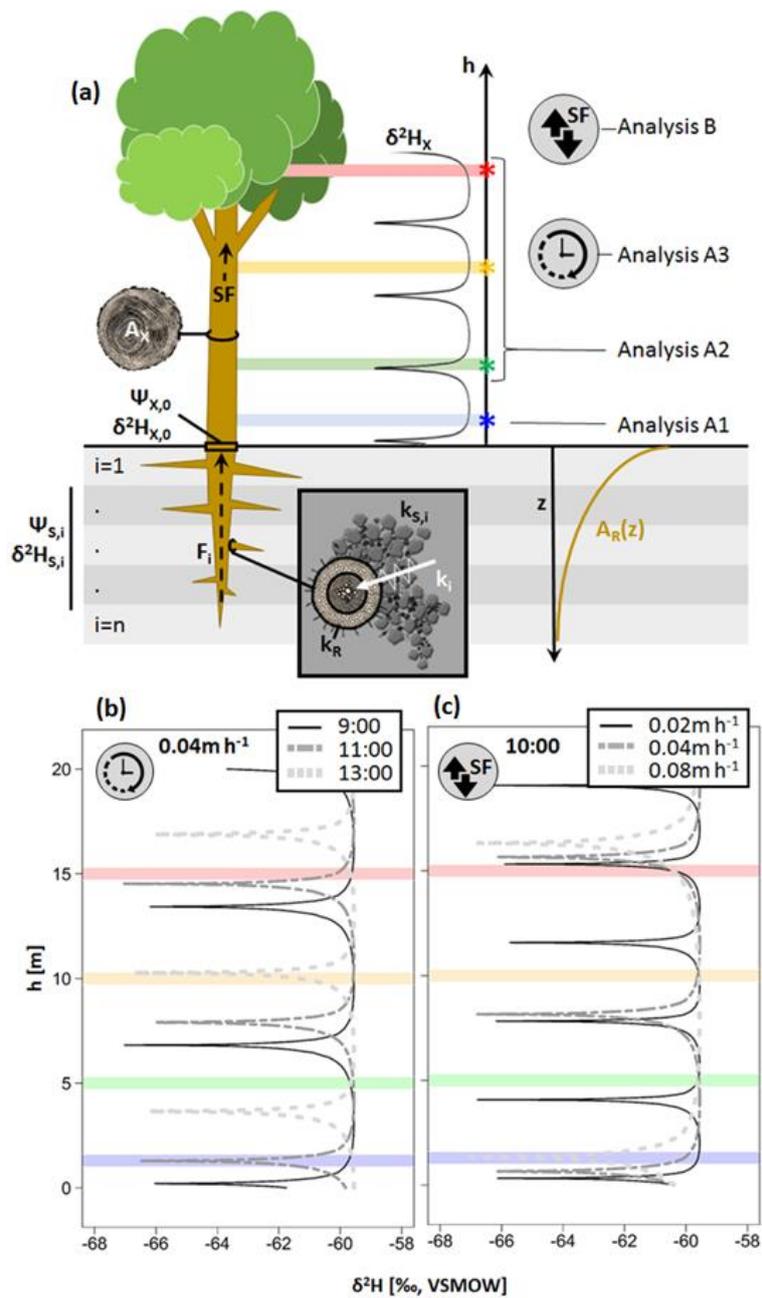
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827 **Fig. 1. Panel a:** Schematic representation of the model and considered analysis detailed in the

828 text. **Panel b:** Model outputs for model analysis A3 representing the deuterium isotope

829 composition of xylem water (δ^2H_X) as a function of the tree height simulated for different

830 sampling times (9:00, 11:00 and 13:00). The modeled tree has an average daily sap flux density

831 of 0.04 m h^{-1} (SF$_S$), which corresponds to an average daily true sap flux density of 0.28 m h^{-1}

832 (SF$_V$). Panel c: Model outputs for model analysis B where δ^2H_X in relation with stem height is

833 shown at 10:00 h, but parameterized with distinct average sap flux density, i.e. 0.08, 0.04 and

834 0.02 m h^{-1} (corresponding to an average true sap flux density SF$_V$ of. 0.56, 0.28 and 0.14 m h^{-}

835 ^{1}, respectively). The standard parameterization used for both study analysis is detailed in Table

836 S1.

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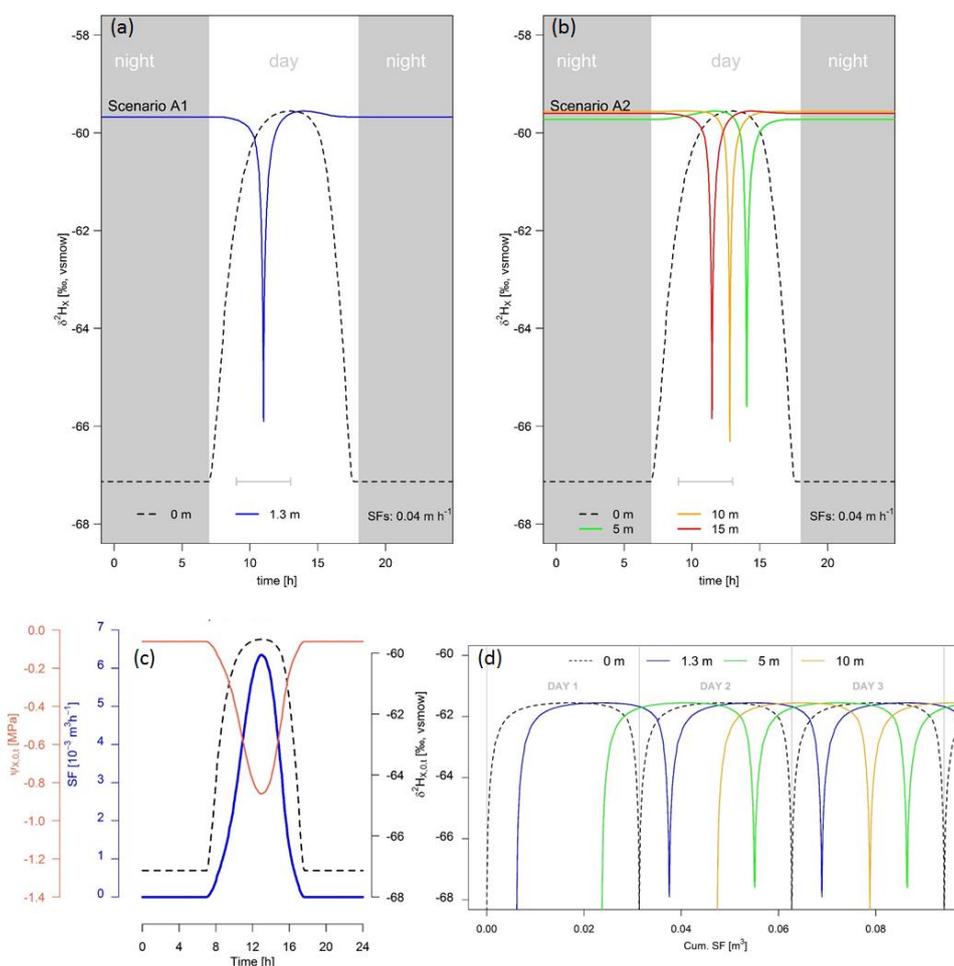
Fig. 2. Panel a & b: Diurnal patterns of simulated in deuterium isotope composition of plant

xylem water (δ^2H_X) fluctuation as a function of time for various tree heights. The modeled tree

has an average daily sap flux density (SF_S) of 0.04 m h^{-1}, which corresponds to an average daily

true sap flux density of 0.28 m h^{-1} (SF_V), and the standard parameterization is detailed in Table

S1. Panel (a) shows analysis A1 output where diurnal δ^2H_X patterns are provided at stem base

(0 m, black dashed line) and at general tree coring height at breast level, i.e. at 1.3 m (blue).

Panel (b) shows analysis A2 outputs demonstrating diurnal patterns in δ^2H_X within a standard

tree at various heights, i.e. at 0 m (black dotted), 5 m (green), 10 m (orange) and 15 m (red).

846   These heights represent random branch sample collection and conform to the standard practice

847   of RWU assessment. Grey lines with whiskers indicate the common sampling period (9:00 until

848   13:00) according to standard practice. **Panel c:** Sap flow rate (SF, blue line), deuterium isotope

849   composition of xylem water ($\delta^2 H_{X,0,t}$ black dashed line) and water potential at stem base

850   ($\Psi_{X,0,t}$, red line) are shown over the period of a single day. **Panel d:** Simulated $\delta^2 H_X$ fluctuations

851   in function of the cumulative sap flow volume measured at various heights: stem base (0 m,

852   black dashed), 1.3 m (blue), 5 m (green) and 10 m (red). Days are delineated by grey vertical

853   lines.

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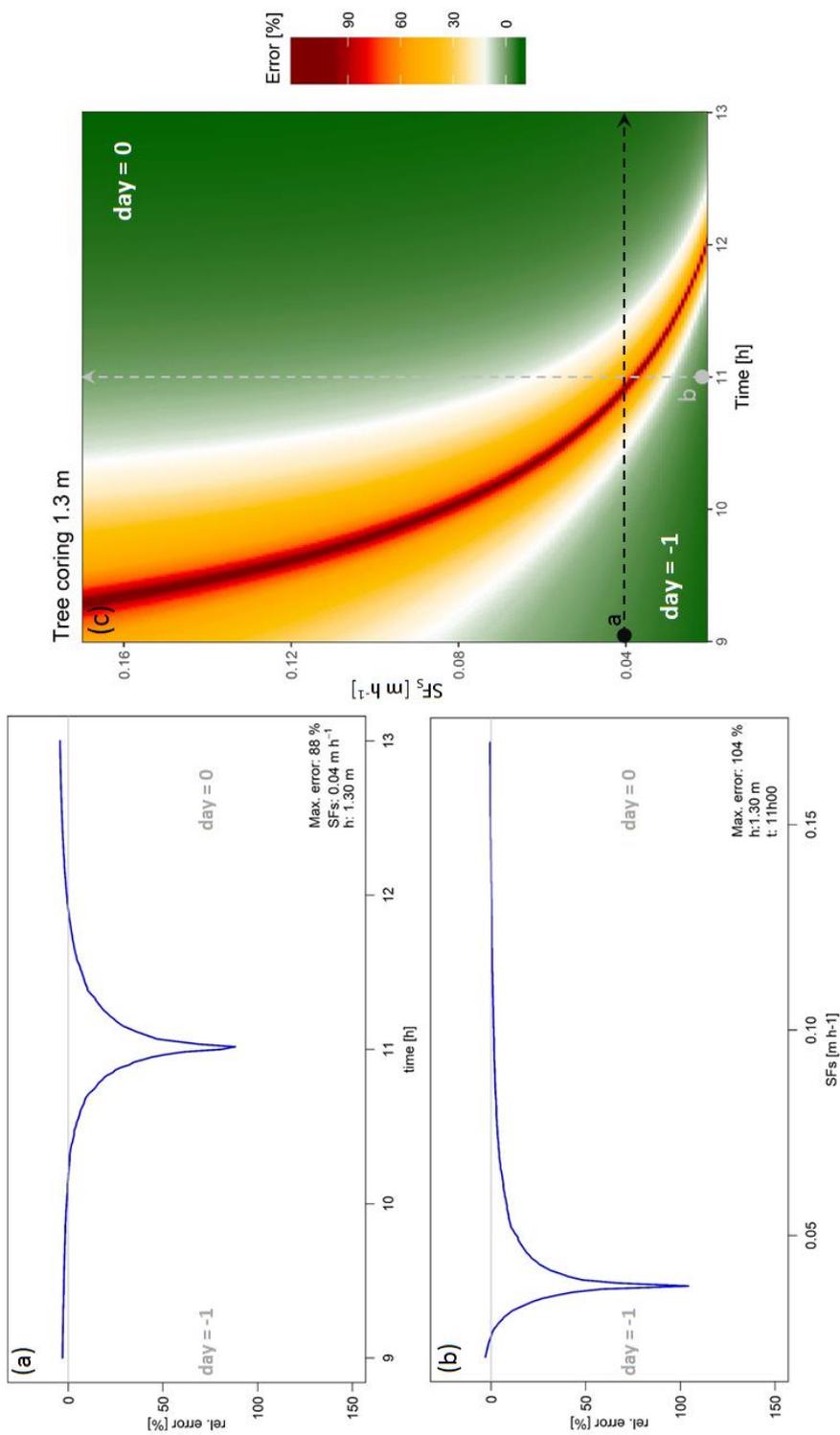
856 **Fig. 3**. a) Relative error on the inferred root water uptake (RWU) depth (i.e. bias between the

857 average daily and the instantaneous derived RWU depth), for a tree measured at standard tree

858 coring height (i.e. 1.30 m) which has a sap flux density (SF_S) of 0.04 m h^{-1} (i.e. $SF_V = 0.28$ m

859 h^{-1}), over the common sampling period (9:00 until 13:00). b) Relative error on the inferred

860 RWU depth considering a tree measured at standard tree coring height (1.30 m) at 11:30, but

861 which differs in SF_S. c) Relative error on the inferred RWU depth over the duration of the

862 common sampling period (9:00 until 13:00) and over a range of potential SF_S (in m h^{-1}) –

863 corresponding to SF_V range of 0.15–1.25 m h^{-1}. Dotted lines a (black) and b (grey) correspond

864 to their respective representation in panel a and b. day= -1 and day= 0 indicate whether the

865 derived RWU depth error corresponds to the previous or current day of measurement.

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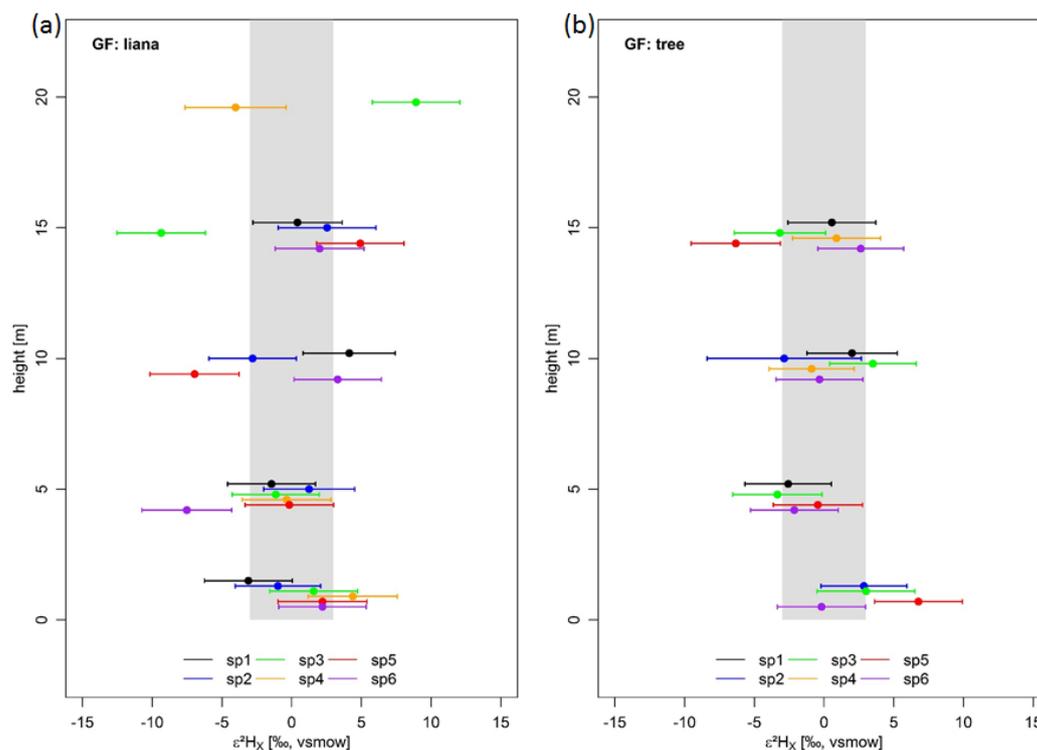
Fig. 4. Field measurements of normalized intra-individual δ^2H_X (ε^2H_X) for six lianas (panel a) and six trees (panel b). Individuals are provided in different colors; species names can be derived from Table 2. Error whiskers are the combination of potential extraction (± 3‰) and measurement errors of the isotope analyzer. The full grey envelope delineates the acceptable variance from the stem mean (i.e. 3‰) according to the standard assumption of no variance along the length of a lignified plant, i.e the null model.

878   **Fig. 5**. High temporal field measurements of deuterium isotope composition of xylem water

879   (δ^2H_X) of two tree (red, stem samples), two shrub (blue, stem samples) and two herb (green,

880   root samples) species sampled in the Heihe River Basin (northwestern China) shown for the

881   respective measurement periods. Timing and location of sampling are provided in the panel

882   titles. The full colored envelope per respective species delineates the acceptable variance from

883   the stem mean (i.e. 3‰) according to the standard assumption of no variance along the length

884   of a lignified plant. Grey vertical lines mark the transition of days. The table provides the

885   maximum measured diurnal δ^2H_X range per species.

886