Dear Editor,

We hereby submit our final response and proposal for improvements to the manuscript "Causes and consequences of pronounced variation in the isotope composition of plant xylem water" to be considered for publication as a research article in *Biogeosciences*.

First, we would like to thank both referees for their thorough assessment of our manuscript, as their suggestions have greatly improved its quality. We are pleased that both reviewers acknowledge the importance of our study (e.g. reviewer #1: *"highlighting the temporal, as well as spatial (longitudinal) dynamics of δxyl, is of evident interest"*, reviewer #2: "*I think it is good that the authors bring forward the point that xylem water may sometimes exhibit rather dynamic variations in its isotope composition*"). We were also happy to notice that the essence of our work (i.e. investigating the diurnal variability in isotopic composition along woody stems) and its merits are not questioned. We noticed that most of the (major) criticisms arise from problems in the presentation, formulation, and overstatement of our work. These oversights are addressed in the new version of the manuscript. In addition, we provide a more balanced presentation of the limitations of our study and of the model we developed as a plausible explanation of the observed variability. In particular,

I. both reviewers indicated that our empirical dataset is not ideal (i) for model validation, and (ii) to support some stronger statements regarding the implications of our findings. We acknowledge these points and have addressed them as follows:

1. **We restructured the manuscript giving more emphasis on the strong points of our empirical datasets,** which are unique in the field, and show pronounced variability (temporal and longitudinal) in the isotopic composition of xylem water (δ^2H_X). Moreover, **a new dataset obtained in Germany** (Magh *et al.*, 2020) extends the original datasets of French Guiana and China. This new dataset describes pronounced intra-individual δ^2H_X variance observed during high temporal resolution monitoring of δ^2H_X in Silver Fir and Beech.
2. **We now emphasize clearly that the model analysis is intended as a theoretical exploration** to build hypotheses and to understand when to expect large variance in δ^2H_X (L35). We clearly indicate that the coupling between the data and the model is only qualitative at this stage (L284:286; L361:363);
3. **We toned down the manuscript title and softened some potentially inflammatory statements** (see details in our response to the reviewer comments), especially regarding the limitations of the isotope method for determining RWU;
4. **We expand the existing discussion section, by elaborating** our existing section on alternative hypotheses that could contribute to the observed variability (L558:564; L575:576; L604:616);
5. **We shed a more positive light on the implications** of diurnal variability in δ^2H_X as this can lead to novel information and opportunities in water acquisition and plant performance studies (L618:622).

II. concerns were raised about the realism of the presented model. Our model considers basic physical and physiological processes, and we agree that it is inevitably - as every other model - a simplification of reality. We stress, however, that the suggested implementations, while improving realism, will not change the conclusion of the paper: pronounced changes

in δ^2H_X can be expected along the stem of woody plants (or similarly at one vertical position over time). We highlighted this by implementing some suggestions, and providing further details in our discussion where alternative hypotheses seemed appropriate – we note that some of the reviewers' concerns were already included in the discussion section and are now elaborated on. In particular, changes include:

1. Reviewer 2 suggested including a molecular diffusion term in the transport equation as it can lead to the homogenization of δ^2H_X within the plant. We explored this possibility using analytical solutions of the advection-diffusion equation. **These simulations show that the impact of diffusion is negligible when sap flux densities are high** (see figure 1, below)**, as is the case for our experimental examples.** Diffusion will - very slowly, i.e. over multiple days - reduce the absolute range of variability in δ^2H_X by smearing the isotopic composition (See figure: ±5cm in 24h), but it also leads to broader δ^2H_X-baseline drops. This implies that while the absolute range of variability might slightly decrease over time (or with tree height). The probability of sampling in the δ^2H_X-baseline drop will in fact increase, **strengthening the importance of our main message. However, we also indicate that diffusion could become more important at very low sap flux densities** as this implies an accumulated effect over multiple days. This results in a time-lag between δ^2H_X and isotopic composition of soil water, presenting another complication for RWU assessment**. Diffusion is now extensively discussed** in the manuscript (L466:482; L603:615; and supplementary methods B).
2. The impact validation of molecular diffusion did not show strong impacts on δ^2H_X dynamics along the length of the stem. However, this suggestion of reviewer 2 instigated **a more in-depth assessment if other processes besides molecular diffusivity might contribute to isotope transport through the plant** (e.g. variable flow velocities within vessels and among vessels of the xylem network). We extend our study with a new analysis comparing the xylem transport in the model against a recent ^2H enrichment study of Marshall *et al.* (2020) (L474:483; L604:616; Fig 6; Supplementary methods B). Marschall *et al.* (2020) applied a novel *in situ* borehole equilibration technique for continuous monitoring δ^2H_X dynamics in a *Pinus pinea* individual. This new analysis highlights the need for an improved understanding of δ^2H_X uptake and transport along trees. It further emphasizes the current lack in understanding various important processes, besides diurnal fluctuations in RWU-activity, that might alter δ^2H_X. These processes are currently ignored in the usual approach of using stable water isotopes for RWU assessment. **Therefore, we further highlight and discuss the need for more intra-individual physiological and hydrological understanding via targeted studies,** for the betterment of the current implementation of the stable water isotopic technique for RWU as well as the presented model (L604:616; L664:668).
3. There is potential for water exchange between storage tissues and xylem water, we discuss this implication in the discussion (L580:603), but decided not to include such a process in the present model version as (i) it depends on the assumption that storage water is representative of soil water uptake by the roots, (ii) we do not have information on storage water isotopic signature and dynamics, and (iii) we are not aware of any existing dataset that could parameterize this model process. Moreover, we highlight that no homogenization is visible in the presented empirical data despite the likely exchange from storage cells (Fig 3c and supplementary methods B). Furthermore, in the discussion, we particularly highlight that, if storage water is not representative of soil water uptake by the roots, then exchange with storage water **likely exacerbates potential bias in the isotope tracing technique, strengthening the main issues raised in our paper.**

4. Similarly, including variations of soil water isotopic composition and water potential over time may improve the model realism and affect the absolute range and the dynamics of xylem water isotopic composition **but would not lead to homogenization.** (L565:579)

Finally, we like to highlight that Kathrin Kuehnhammer, Ruth-Kristina Magh and John D. Marshall have been added to the author list, as they provided (a) the empirical data in Germany and (b) the dataset used in the new validation of δ^2H_X transport dynamics through the plant at very low sap flow velocities, and (c) helped with the corresponding analysis and revision.

Please find our detailed responses to reviewer comments below (responses to reviewers in bold).

We hope that the implemented adjustments, inspired by the reviewers' suggestion, improved the manuscript allowing publication in *Biogeosciences*.

The authors

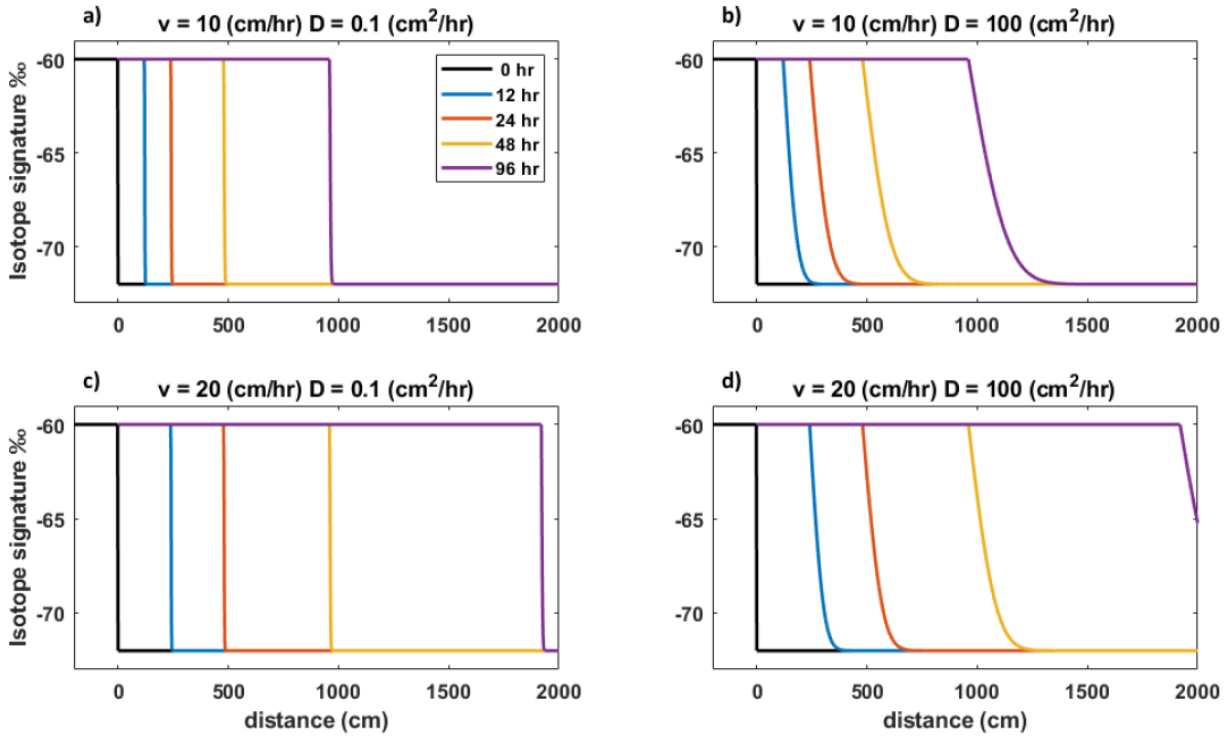

Fig 1: Analytical solutions of advection-diffusion equation on a semi-infinite 1-D domain with 12 ‰ step-change in isotope signature for different values of flow velocity and diffusivity. The plots show the impact of diffusion on the isotopic composition of xylem water. Colored lines show the solution at different time intervals: 0, 12, 24, 48, and 96 hr. Note that the values of diffusivity are much higher than these reported for heavy water (e.g. D=0.1 cm^2 h^{-1}; Meng et al., 2018)

Anonymous Referee #1

De Deurwaerder and colleagues present a composite work where they (i) run a model simulating diurnal variations and vertical heterogeneity in xylem water isotopic composition (δxyl) and perform a multivariate sensitivity analysis. They also (ii) present results of sampling campaigns where δxyl temporal and spatial variations were observed in twelve tree and liana species. The authors explain these variations and thus the departure of the generally accepted hypothesis of homogeneous δxyl on account of their model output. Finally, they warn the isotopic community against the "danger" in using water stable isotopes as tracers for RWU analysis.

The manuscript is well written, figures and tables are of good quality and appropriate referencing supports the text. Finally, the manuscript content falls within the scope of BG.
We thank the reviewer for his/her appreciation of the quality of our work, and the detailed assessment of the study and constructive feedback on the manuscript. We feel that the paper improved considerably thanks to his/her suggestions.

My general comments are listed below:

1- I note that the authors do not confront their model results to collected data, nor thoroughly test their model hypotheses on independent data. I do not see a particular problem, but it should be mentioned clearly that aforementioned items (i) and (ii) are only "softly" coupled in the study.
The reviewer makes a fair point. As our field data is unique but limited, they do not allow direct validation. Our model presents a theoretical exploration of one of the potential causes of the observed high variability in isotopic composition in xylem water (δ^2H_X) along the stem of a woody plant. For illustrative and interpretative purposes, our model explores ideal, simplified environmental conditions. The empirical data present a much more complex situation, which we were unable to characterize fully due to financial and logistical restrictions. The new version of the manuscript mentions that our study presents only a qualitative coupling between model and data, as suggested by the reviewer (L283:285; L360:362);

2- highlighting the temporal, as well as spatial (longitudinal) dynamics of δxyl, is of evident interest. However, the prevalence of such dynamics may not put in "danger" – as the authors say – the determination of fractional root water uptake for other non-wooden species. The abstract should be rewritten accordingly. The isotopic community should be on the "safe" side if researchers extract water from a plant tissue for which it has been proven that its stable isotopic composition reflects that of RWU. Of course, this should be investigated for each investigated plant species, preferably under controlled conditions (see for example Barnard et al., 2006).
The abstract of the paper is rewritten accordingly (see L33:38). We agree that a distinction between woody and non-woody plants should be considered as described by Barnard *et al.*, (2006), as highlighted by the reviewer. Our model targets woody species (i.e. > 70% of all isotopic studies, Rothfuss and Javaux (2017)), and it is therefore not appropriate to speculate about non-woody species. We re-formulate our statements in the new version of the manuscript (i.e. expressions such as "to put in danger" is dropped and replaced by more informative and appropriate formulations).

3- The authors provide no information about the soil compartment; what about the soil water isotopic composition profile temporal and spatial variabilities? Are the isotopic differences in xylem water reflected by the span of isotopic composition values in soil water? This would offer the possibility to rule out possible evaporation effects mostly during sampling and transport (which is not listed as other reasons for the observed diurnal variations of δxyl). If soil water isotopic information is not available, it should be stated as a limitation of the study;
The empirical data collection indeed has the limitation of the absence of adequate soil characterization during field setup (i.e., soil water potential and isotopic composition of soil water), which is now clearly stated in the new version of the manuscript (supplementary methods A; Fig S1).

In addition, evaporation effects during sampling and transport can never be excluded in field studies but have been minimized by the applied protocol, as detailed in the manuscript. However, we expect evaporation effects to be low because of (a) the imposed strict protocol. Specifically, fast sampling with cautious care to avoid heating the extraction instruments was followed by fast capping of the sample vials (sealing caps with rubber and glass vials having a minimum of two full closing coils), immediate cooling of the vials in the field, and freezing of vials upon return in the lab; and, (b) cross-validation of the obtained δ^2H_X with potential source water isotopic composition performed during the dry season at Laussat do suggest that our δ^2H_X lie within the natural span of the soil and precipitation water sources. These observations, although not of adequate quality as most samples did fail the 98% recovery validation, is now provided as a supplementary figure (Fig S1, and supplementary method A). In addition, we highlight that the pronounced intra-individual δ^2H_X variance is now observed in 3 independent datasets collected by 3 independent research groups.

4- I found on several occasions that the authors did not fully understand basic principles driving isotopic fractionation (see my specific comments);
We regret that we left the impression of a less than full understanding of the basic principles behind isotopic fractionation. We removed all instances of careless representation and wording in the new version of the manuscript (see our answers to the specific comments for more details).

5- In general, I do not think that such field experiments, where a significant number of environmental driving factors are unknown, should be used to question the entire isotopic research methodology. I urge the authors to discuss this point as well and measure their words.
We agree with the reviewer and have toned down the message to be more in line with the uncertainties in the data. We now realize that our original tone could have been perceived as questioning the entire isotopic research methodology – which was not our intention. Therefore, in the revised manuscript, we use more appropriate statements, as well as included some positive aspects of diurnal variability in δ^2H_X (L618:622). These could present new opportunities in water acquisition and plant performance studies. However, we remain convinced that our findings indeed show the need for caution when applying isotopic research methodology in multiple situations and configurations, as large variability of stem isotopic composition are expected and could plausibly lead to significant bias in average RWU depth determination. Our main objective remains to (a) build increased awareness of the potential of diurnal variability to bias future isotope

endeavors, and (b) to advocate for more targeted intra-individual physiological and hydraulic studies to further our understanding in how isotopes are taken up and transported throughout the tree and how these processes might impact the current RWU assessment approach using stable water isotopes.

The authors will also find a list of specific questions/remarks/corrections/issues:

L24. What does "i-H_2O-xyl" refer to? To "plant xylem water uniform isotope composition" or "plant xylem water isotope composition"? In either case, "δ" is to be preferred over "i-H_2O-"
This is a good suggestion. The "δ"-notation suggested by the reviewer is adopted, as i-H_2O-xyl originally referred to the plant xylem water isotope composition.

L32-33. "field data show pronounced i-H2O-xyl variation during the day or along stem length ranging up to 25.2‰ in δ2H and 6.8‰ in δ18O" does not read well. I propose something like: "the hydrogen (oxygen) isotope composition of plant xylem water showed strong temporal (i.e., daily) and spatial (i.e., along the stem) variation ranging up to 25.2 (6.8) ‰´'
This sentence is adjusted accordingly (L39:42).

L36. Please rephrase: "danger" is not the proper word.
This is adjusted accordingly (L46:49).

L46-47. There is no such thing as the depth of root water uptake in the case of several soil water sources. Only in the context of direct inference is this true. But the authors do not refer to the later (and outdated) technique.
We agree that the terminology: 'average root water uptake depth' is more appropriate. This is implemented accordingly throughout the manuscript.

L49. This is not true: the isotopic technique is of course destructive (you have to take a soil core), very labor-intensive (e.g. extraction of soil and plant xylem water).
This statement presents a comparison with root excavation endeavors, which are extremely time-consuming, laborious, and destructive. We adjusted the statement to read that in comparison to root excavation, isotope techniques are far less destructive and time-consuming, and are hence definitely preferred when studying multiple individuals at once. (L63:67)

L50 (also L47). You should mention that it is fractional RWU and not absolute RWU you are talking about. You cannot solve for water mass balance with the isotope technique, which constitutes its greatest limitation when compared to other techniques.
This is adjusted accordingly (L66).

L52. How would you determine fractional root water uptake at the ecosystem level?
This statement, which is taken from Dawson *et al.*, (2002), can - for instance - embody δ_{xyl} analysis performed on the dominant tree species of a forest stand (i.e. forest stands on Mount Kilimanjaro - Bodé *et al.*, 2019), or on classified groups of plant individuals (juvenile versus adult – Stahl *et al.*, 2013; liana versus tree – De Deurwaerder *et al.*, 2018). These measurements inform on the average depth of water acquisition (i.e., "strategy") of

the species/group, which can then be extrapolated to estimate the expected dynamics/strategies at the ecosystem scale.

L56. This should be "δxyl". Why the "i" instead of "δ" here? Also, why the "H2O"? (is there another molecule investigated here?)
As indicated in previous comment, we now adopted the "δ_{xyl}"- notation suggested by the reviewer.

L58-60. Peclet effect is measurable in the xylem vessels upstream of the evaporative sites. This assumption is not systematically made. Instead, authors investigate the prevalence of isotopic fractionation depending on the plant tissues they sample, e.g. in Barnard et al. (2006). Please revise.
This remark of the reviewer is addressed by (i) emphasizing that this study targets woody plants, as non-woody plant are indeed subjected to "stem" fractionation processes (Barnard et al, 2006, a reference which will be included), and notify that (ii) the Péclet effect might be observed in branches upstream of evaporative surfaces (L29-31 in Supplementary method A). The later presents a rather local phenomenon and should not, or very limitedly, impact stem samples at distance from the evaporative surface, as performed in this study.

L67-69. There can only be kinetic fractionation playing a role during the transport of water through the root membranes since there is no liquid-vapor phase change that would involve equilibrium fractionation. Please revise.
The reviewer is correct, and this is revised accordingly.

L69-72. Not only kinetic fractionation is a result of the difference in mass of the water isotopologues, but fractionation in general (e.g., equilibrium and kinetic fractionations).
This is correct and was unfortunately dropped out during the editing of the manuscript. We revise our definition accordingly emphasizing that this entails the transport of water through a root membrane.

L94-95. Why would you make the assumption that δxyl is constant over time (over which period of time anyway)? At this point of the MS, it is not clear. Actually, no one makes this assumption in the field, rather they sample from e.g. the base stem among individuals at e.g. a sub-hourly temporal resolution and sub-daily temporal extent.
We agree with the reviewer that we should be more precise in our formulation of the hypothesis and the time-frequency considered (sub-daily and even sub-hourly). This is now addressed in the new version of the manuscript (L99:100, but see Fig 1). We also note that there may be a misunderstanding here regarding the assumption made in the field.

It is indeed correct that a few high-frequency measurements of δ_{xyl} exist. However, it should be noted that these are (a) rather rare at the moment; and (b) predominantly target sampling of the leaves. Sampling of leaves, however, is less relevant to the 'isotopic tracing technique for RWU assessment' as multiple other processes impact the isotopic composition of leaf water (i.e. the aforementioned Péclet effect). In this study, we do not address leaf water monitoring because of the decoupling between source water and measured signature. To date, most studies where the isotopic composition of xylem is used for RWU assessment have - at best - a daily, but more often a monthly or seasonal temporal sequence. Moreover, many of the studies (including ours) consider only one-time sampling (including ours, see e.g. De Deurwaerder *et al.*, 2018). These studies do assume

a constant δ_{xyl} over time. Hence, sub-hourly/daily δ_{xyl} variances are generally not accounted for in studies on lignified stem sampling.

Finally, we acknowledge that coring close to the base of an individual stem is generally applied in non-woody, herbaceous plants as recommended by Barnard et al., 2006. However, to the best of our knowledge, this is not standard practice in woody plants. We acknowledge that it might be more general than we know, as implied by the reviewer, but this is not reflected in the existing literature where the height of coring is rarely provided, and when so, coring is generally performed where stem diameter is measured (i.e. 1.3m in the metric system, and at 4.5 feet in the imperial system) (e.g. White *et al.*, 1985; Meinzer *et al.*, 1999; Goldsmith *et al.*, 2012; Hervé-Fernández *et al.*, 2016; De Deurwaerder *et al.*, 2018; Muñoz-Villers *et al.*, 2019) (L302:304)

100-101. What do you mean by "diurnal changes in the soil-plant-atmosphere continuum"? Which changes?
This statement indeed needs further clarification, which is pursued in the new version of the manuscript. In short, with "diurnal changes in the soil-plant-atmosphere continuum" we imply: changes in water potential differences between leaf and soil along the day (L102:103, but also see Fig 1). These gradients will determine the vertical distribution of root water uptake.

L113. What exact "water potential gradients" do you refer to?
Here, we refer to the water potential gradient between soil and the evaporative surfaces (leaves) of the plant. This is added to the manuscript (L102:103, see Fig 1).

L114-116. Why would you need to use a mixing model, especially since you did not sample soil water and determine its isotopic composition? You may as well simulate a sinusoidal pattern for the δxyl. Please elaborate/explain.
We did measure soil water ourselves in this study, however, the obtained recovery rates of the extraction mostly did not reach the requested benchmark of 98% recovery (see Fig S1). This data is therefore not considered for further analysis (L53 in supplementary method A). The soil water isotopic composition used in our theoretical exploration target a model representation that is practically implementable and repeatable. Here, the approach of Phillips and Gregg (2003) presents a widely used and implementable approach to mathematically represent fractional water uptake in the soil. The apparent sinusoidal pattern of the xylem water isotopic composition observed by the reviewer results from the diurnal fluctuations in leaf water potentials and corresponding changes in the distribution of the root water uptake in the various soil layers. Specifically, here, the pattern in leaf water potential is imposed by a bell-shaped sap flow curve obtained from Huang *et al. (*2017). Hence, this apparent sinusoidal pattern naturally emerges from the source mixing model approach and was not hardcoded as such in the model.

L117-119. You should write the isotopic equations with "δ" instead of "δ2H" as the model does not focus on 1H2HO, to the contrary of what the authors say. For the model to focus on 1H2HO, it would mean that 1H2HO and 1H218O would follow different physical processes, which is not the case (both isotopologues undergo mass dependent fractionations, i.e. $\varepsilon_{eq}(2H)/\varepsilon_{eq}(18O) \approx 8$ and $\varepsilon_{k}(2H)/\varepsilon_{k}(18O) \approx 0.88$). Also write 2H instead of Deuterium and do it consistently throughout the MS. The latter is just an element's isotope and does not deserve (anymore) its own letter (see IAEA tech reports guidelines).

We adopted the notation suggested by the reviewer throughout the manuscript, and we rephrased the statement as we indeed focus on the water isotope element instead of the water isotopologue as was inaccurately implied in the original manuscript. [L 198-199]

L130-131. This assumption is only reasonable when soil water redistribution no longer occurs, e.g., this does not stand shortly after a rain event.
For the sake of simplicity, we present a model that assumes rain-free periods and prevents soil redistribution, as indicated by our statement L130-132 (original manuscript lines): *"… a reasonable assumption if the isotopic measurements are conducted during rain-free periods, …".* We acknowledge that this assumption was not presented clearly, which we addressed in the new version of the manuscript. (L205:207)

L125ff. Report the dimension of each variable and parameter throughout the MS.
For the readability of the text, we prefer the use of a dedicated table listing the variable/parameter dimensions altogether, as done in Huang *et al.* (2017) (here Table 1, as indicated in the text L197).

L142. From Eq. (3), I understand that the water "potentials" are in fact "hydraulic heads". This should be clarified.
This is now clarified in the new version of the manuscript by indication the generic potential term equals hydraulic head or soil matric potential (L219).

L143-144. Add here that k_i and $\Psi_{S,i,t}$ are also specific to the ith soil layer.
This is adjusted accordingly.

L193-196. I am missing background information to understand what the "30 days sequence" of the "model runs of Huang et al. (2017)" refers to. Please elaborate.
We agree that our statement is unclear for readers that are not familiar with the paper of Huang *et al.* (2017) in which a 30-day drought simulation study of loblolly pine was conducted. An average day within this representation was selected based on both representativeness and data availability. We now elaborate on this topic in more detail in the new version of the manuscript (L285:290).

L201-203. Why would you need external data (Meissner et al. 2012) and not simply do your model exploration on basis of a synthetic experiment?
The reviewer is correct, as applying a complete synthetic experiment is indeed possible. We chose to use the Meißner *et al.*, (2012) data as this presents a realistic dataset (in terms of range and variation in both soil water potential and soil water isotopic composition) obtained during field studies, and therefore find it relatable for both interpretation as well as providing insights for model requirements guiding field setups.

L208-209. I did not hear of such standard practice and I doubt there is. Could you add a reference for this?
Here, we assume researchers followed standard procedure in using an increment borer in forest inventory, i.e., coring where stem diameter is typically measured (i.e. breast height: at 1.3m according to the metric system, in at 4.5 feet according to the imperial system). This method has been applied multiple times (e.g. see White *et al.*, 1985; Meinzer *et al.*, 1999; Goldsmith *et al.*, 2012; Hervé-Fernández *et al.*, 2016; De Deurwaerder *et al.*, 2018; Muñoz-Villers *et al.*, 2019), but we agree that it does not need to be presented as a standard practice, as several isotope tracing studies applying an increment borer to collect xylem

cores do not specify the height of coring. We also acknowledge that (a) many studies sample branches (ignoring the effect of evaporative enrichment from the leaves to upstream plant organs), and (b) that our assumption that researchers follow the standard increment borer approach could be incorrect. We rephrased this statement, providing the here presented references in support of the followed approach (L302:304).

L223-225. Split the sentence and add detail. It is hard to understand. Also following the Rayleigh distillation model, the error should always be negative in the case of incomplete water recovery, which does not match to your normal distribution of error in the null model.
The indicated sentence is split up and clarified. We thank the reviewer for this excellent suggestion. It is true that the expected error should be negative, which we now have implemented in the model structure by using a skew-normal distribution instead of a normal one (L327:332).

L228-229. How so? And why would it be relevant to take into account the analyzer systematic error at this point of your model testing?
Analyzers always have an embedded error which is generally very small. But, if known, the user can opt to implement these in SWIFT model. In this study, we consider these errors negligible and have indeed ignored them as it has little relevance in the model testing at this point, as indicated by the reviewer. For sake of clarity, this sentence is now removed.

L235-244. Are you talking about RWU depth of rooting depths here? How do you define the latter term? Why would you use the direct inference model (which is a very simplistic view on RWU, i.e., one single root sampling from on single layer at a time) if you use a multi-source mixing model (Phillips and Gregg, 2003), which allows the plant to sample simultaneously from different layers? Please explain this apparent contradiction. Overall this section is quite difficult to read and I ask that the authors simplify it.
Here we are talking about the average depth of RWU (i.e. a weighted mean of the depths of root water uptake, with the root flows at the different depths as weights (now included L335:336), and hence, this section/title will be changed accordingly for clarification (L334). The paragraph was further simplified by textual alteration (L335-345). We applied both methods for completion of the presented study, as combined, they embody 96% of all applied methods (Rothfuss and Javaux, 2017). While direct inference might be considered as very simplistic, to date, it remains the most applied technique in the literature (46% according to Rothfuss and Javaux, 2017). In short, the direct inference approach compares hydrogen and oxygen isotopes between the soil water profile and the xylem water of the stem. The depth of soil water having similar isotope values to the stem water indicate the main depth of soil water sources used by the plant (e.g. see Wang *et al.*, (2010)). This approach does not exclude that the plant can take up water from multiple soil layers but just assumes that the signature found in the xylem reflects the dominant signature of bulk water uptake. It is therefore unclear to us what the reviewer exactly means with "the apparent contradiction".

L258. Is there a specific reason why you did not use Van Genuchten's soil retention curve?
There is no specific reason not to use the van Genuchten's soil water retention curve. As we do not know the soil hydraulic properties at the site (soil retention and conductivity curves), we do not have any reason to prefer Clapp and Hornberger (1978) closed-form equation instead of the Mualem-van Genuchten model. We will implement more soil hydraulic models (including Mualem-van Genuchten) in future model versions.

L309. Delete “kinetic”. It is not even sure that you would have fractionation at all, considering that you may boil (==fractionation free process) the water here rather than evaporate it.
This is adjusted accordingly.

L321. Fresh weight does not take into account possible loss of water during transport/ storage. You should have weighted the samples prior extraction again.
We agree with the reviewer that measuring before extraction itself could provide extra information on whether or not water was lost during transport/storage of the samples. We did not do this, and can therefore not provide such insights to the reader.

However, as we used glass vials with sealing caps (including sealing rubber, and at least two complete loops of closing coil), water losses during transport and storing should be negligible/absent. Besides, it should be noted that measuring samples after storage, i.e. before extraction, might itself impose sample contamination and inaccurate assessment of the percentage of water extracted by the cryogenic water extraction method. Specifically, frozen vials will attract frost and condense water onto the vial exterior, which can substantially impact the weight of the vial itself. This then should be accounted for, for instance by warming the samples to room temperature before weighing, a practice which arguably is also not recommended.

L331-336. Since you are measuring with a Picarro, which does not give ratio (but performs already the delta conversion), you need to say that you “corrected the Picarro raw delta readings into calibrated delta values thanks to the values of the aforementioned ‘internal laboratory References’ expressed on the international V-SMOW scale”. No need to display the equation (12) but you may detail these “internal laboratory References” (e.g., value).
The suggestion of the reviewer is implemented in the new version of the manuscript. (L58:62 in supplementary method A)

L336-334. Still at this point, I do not know what the difference is between i-H2O-xyl and δxyl: : :If there is none, please use the latter term. In addition, use another letter than ε for the normalized “i-H2O-xyl”: it usually stands for isotopic fractionation, defined as the deviation of the fractionation factor to unity. It seems even odd that you would consider such a letter…
As indicated above, we replaced the symbol of i-H_2O-xyl by δ_{xyl} as suggested by the reviewer. We agree with the reviewer that our choice to use ε here was unfortunate. While ‘ε’ is commonly used in statistics to indicate bias in the sample set, we now see that this indeed can result in confusion for the isotope community. Hence, another Greek letter (i.e. ‘β’) is used in the revised manuscript.

L369. I still do not understand what is the concept of RWU depth if you consider the multi-source mixing model approach.
As indicated above, we have clarified this definition. Throughout this study, we consider ‘average depth of root water uptake’ (i.e. a weighted mean of the depth of root water uptake, with the root flows at the different depths as weights), as is now adjusted in the new version of the manuscript (L335:336).

Fig. 2. Panel (a): how do you come up with a night δxyl at 1.3 m above –60‰ Also, I don’t see why panels (a) and (d) look so different for day 1, since if I understand correctly, the cumulative SF is a function of time (if sap flow remains constant).

The patterns in δ^2H_X results of both the isotopic composition of the water taken up by the roots at any time – and – the volume displacement of water moving as a slab along the tree stem. At each time step considered, a specific volume of water and isotopic composition is extracted by the plant. This presents δ^2H_X at stem base which is not limited by the volume of the tree yet. However, this quantity of water is subsequently pushed, as a slab, upwards in the limited volume of the stem, i.e. our model presents a piston-flow approach. At this point, the quantity of water taken up by the plant also impacts the observed δ^2H_X pattern.

Specifically, the water movement within the tree can be visualized by 'a stack of disks' of water each having a time-specific δ^2H_X, where stack-height is defined by the quantity of water taken up and the stack area corresponds to the lumen area of the tree. Step by step, new disks are introduced at the bottom, pushing previous disks upwards, i.e. water moves as a slab through the stem. When root water uptake activity stops, i.e. sap flow is zero, the stacks remain at their respective position. When measuring at 1.3m height, the entire volume of water taken up in the late afternoon, with values of -66‰ is simply too small to reach the measurement height. For this reason, δ^2H_X at 1.3m represents the water isotopic composition of water taken up earlier during the day (i.e. around midday), which has a more enriched isotopic composition.

L371. "isotopic composition of soil water is dominated by depleted deuterium". Please correct phrasing: soil water can be depleted in 2H in comparison to another water volume, but there is no such thing as "depleted 2H".
This is corrected accordingly.

L373. An isotopic composition, which is a number, cannot be "enriched". Please correct.
This is corrected accordingly.

L375. "depleted deep soil water"
This is corrected accordingly.

L384. ": : :RWU originating from deeper, more depleted soil layers". Please correct: water from a given soil layer might be depleted, not the soil layer in itself.
This is corrected accordingly.

L399-400. This belongs to the discussion section.
This sentence is moved to the discussion section.

L407-418. Nowadays no study is published where RWU depth is investigated with the direct inference method. Analyses are performed with Bayesian mixing models. So I wonder if this section, although interesting theoretically, would benefit practically to the community.
Indeed, Bayesian mixing approaches become more commonly used in current literature. However, we argue that the potential issues in RWU assessment unraveled in our research apply to all existing literature, of which the direct inference method still embodies the majority of studies (see Rothfuss and Javaux, 2017). For this reason, we are convinced that this section can be relevant when a critical assessment of former studies is pursued.

L446 and Fig. 4. See my previous comment on the use of "ε". The caption of a figure should not point to another figure or table. Write here the name of the species (no need to write them in the figures though).

This suggestion is implemented in the figure, and the notation "ε" is replaced by "β".

L452. Add in the text that growth forms refer to lianas and trees.
This is adjusted accordingly

L455-457. This belongs to the discussion section. Also the link between "easily accessible and abundant groundwater reservoir" and the fact that the diurnal intra-individual variance is minimized is not clear. I suggest moving to the discussion and elaborating on this.
This sentence is no longer included in the paper, as it distracted from the main storyline of the study.

L471-472 and Table 2. How many individuals (which you could consider as replicates) of each species were sampled during the experiment? Discuss the implication of having n=1 with respect to δ_{xyl} variance.
It is true that only one replicate per species was obtained for this study. That was because we did not target the intra- or interspecific variances in δ_{xyl} in our experimental protocol but instead we investigated the intra-individual δ_{xyl} variability and the theoretical exploration of a likely cause of this phenomenon.

L486-492. The authors say that the intrinsic problem of the "isotopic tracing method" is that there is a soil water isotopic gradient in case there is evaporation and under heterogeneous soil water potential gradient? I don't understand this at all (!) The isotopic methodology for studying plant RWU relies on heterogeneous isotopic gradients in soil water. This is a solution, not a problem here…
We acknowledge that the text was not clearly formulated in support of the argument envisioned. What we wanted to convey is that the soil water conditions required to perform the 'isotopic tracing method', also facilitate a large variance in δ^2H_X, which could have important consequences for the RWU assessment. An altered, non-ambiguous discussion is now presented in the new version of the manuscript. (L518:521, see Fig 1)

L493-506. I disagree. There is a clear problem in determining fractional RWU profiles on basis of measurements of the transpiration isotopic composition, which is highly temporally dynamic and spatially heterogeneously distributed; many observation of leaf water confirm the non-reaching of isotopic steady state. In addition, how would a "change of cloud cover degree" have an "instantaneous" influence on δxyl? This contradicts the results of your synthetic experiments, where depending on sap flow rate, there is a marked isotopic memory effect of the antecedent water moving upward it the xylem vessel.
Cloud cover will result in reduced water environmental demand and thus impact the sap flow velocity (and thus the water and isotope dynamics in the stem). Hence, the cloud cover of a tree will reflect in distinct patterns of RWU uptake dynamics and the bulk isotopic composition of water extracted from different soil layers. The statement does not contradict our model findings but we acknowledge that the presentation could have been more clear. What we wanted to say is that the intra-individual variability of δ^2H_X, according to our model simulations, reflects indeed the past changes of root water uptake dynamics (including due to dynamic changes of environmental demands). This is now clarified in the text (L531:334)

L516-523. The model provides an explanation, sure, but does not validate your hypotheses from the confrontation with experimental data. This is missing from your study and should be mentioned.

We fully agree with the reviewer and acknowledge that we have overstated our findings. This version of the manuscript more clearly describes the limitations of our study (L284:286; L361:363);**. This is also clarified by the change in paper structure: we now present our model simulations as a potential explanation of the isotopic composition variability observed in the field.**

L534-546. My understanding from the literature is that hydraulic redistribution is intermittent and localized, thus does not affect that much the bulk soil water isotopic composition, rather it affects the direct environment of the roots.
Correct, we agree with the reviewer that hydraulic redistribution will predominantly impact the rhizosphere of the plant, rather than the isotopic composition of the water in soil layers. This paragraph is rewritten as such (L566:572). The main message within this paragraph, suggesting that hydraulic lift will reduce the δ_{xyl} variance, remains valid as the variance of the isotopic composition of water accessed by the plant can be reduced.

L578-587. Not to forget we need to monitor soil water isotopic composition to verify if δxyl spreads within the range of isotopic values observed in the soil profile.
Correct, and we add this suggestion to the new version of the manuscript (L623:626).

Anonymous Referee #2

The manuscript by De Deurwaerder and colleagues challenges the idea that, in absence of precipitation or other rapid changes in climate, the water isotope composition of plant xylem should stay fairly constant over diurnal time scales or along stem height. Their analysis is based mostly on a model (!) of root water uptake and isotopic transport within the roots, up to the stem base. Their model considers that (1) the isotope composition of stem water at the base of a tree ($\delta^2H_{x(0;\,t)}$) is the average isotope composition of soil water over the root zone, weighted by the fractional root water uptake rates at each depth (Eqs. 1 or 7) and (2) the isotope composition of stem water at any height h ($\delta^2H_{x\,(h;\,t)}$) is the isotope composition of stem water at the base, delayed by the travel time τ of sap between stem base and height h ($\delta^2H_{x(h;\,t)} = \delta^2H_{x(0;\,t-\tau)}$) (Eq. 9). Soil properties are used as boundary conditions that do not change over the day in terms of soil water potential and isotopic composition. With such model, they predict large diurnal variations of xylem water isotopes at stem base, but also large variations along the stem (see their Figs. 1 and 2). Based on this modeling exercise, and separate observations of the $^2H=^1H$ ratio in water extracted from tree stems and lianas at different heights within a tropical forest canopy, and showing some scatter sometimes larger than 3‰ (the estimated error from water extraction and isotope analysis), they conclude that (1) the common assumption that the isotope composition of stem water is fairly constant over time is violated and (2) it can cause significant biases when using water isotopes to identify plant water origin.

I think it is good that the authors bring forward the point that xylem water may sometimes exhibit rather dynamic variations in its isotope composition. However, I am afraid the proposed model is inadequate and the dataset is too limited for illustrating this point. To me, the study does not prove anything; it shows that there are variations in the data and that there are variations in the model but there is no model-data comparison. Besides, variations in the data are not very large and can be explained by lots of other processes, and variations in the model are mostly caused by its lack of realism. These two points are explained in more detail below.

We thank the reviewer for his/her detailed assessment of the study and helpful feedback on the manuscript. We do want to stress that our study is based on both a theoretical model exploration and empirical, novel field data. Moreover, we now present 3 independent datasets (datasets collected in French Guiana, in China, and Germany) on a variety of species, which all show pronounced variability in isotopic composition. We do not agree that all these data can be dismissed easily.

Additionally, we believe that all models can be criticized due to a lack of realism but their value depends on the insights they bring. In fact, process-based model explorations are proven tools in many scientific fields, because of the insights they provide and not because of their subjective realism. We hope to convince the reviewer that adding supplementary processes would indeed improve model realism and might impact the dynamics and absolute range of δ^2H_X, but it will not alter our conclusion: large variability of isotopic composition along woody stems is expected in many situations. Moreover, including some of the suggested realism strengthened our results.

We agree with the reviewer, however, that the message brought in our original manuscript had to be toned down to better reflect the limitations of the analysis and data. Therefore, we revised our manuscript, providing a more appropriate message by (i) down toning our statements and by (ii) including the positive aspects of diurnal variability in δ^2H_X, which could present new opportunities in water acquisition and plant performance

studies (l618:622). At the moment we are unable to fully validate the model as such data does not yet exist to our knowledge. Moreover, the presented model serves as a theoretical exploration of one possible explanation that could cause the observed variance in δ^2H_X. Here we apply generally accepted plant hydraulic processes which show that large variance in δ^2H_X is expected under the simulated (and realistic) conditions. We remain convinced that our findings, though not conclusive, can help build increased awareness of the potential of diurnal variability which can bias future isotope endeavors. At a minimum, it calls for more research. To meet the concerns of the reviewer, the new version of the manuscript more clearly mentions the limited coupling between model and data (L284:286; L361:363), as also suggested by reviewer 1. We acknowledge that other mechanisms could contribute to the observed δ^2H variance, and have extended the sections discussing other potential processes (see discussion and new performed analysis assessing if processes other than molecular diffusivity might contribute to the isotope transport along the xylem). Future model developments and targeted datasets are encouraged and are highlighted more in the new version of the manuscript (i.e. L600:603; L623:642; L663:667).

In short, we agree with most of the reviewer's comments, but we do not share his/her conclusion on the data and model.

- **The French Guiana dataset (i.e. measuring isotopic composition along stems of lianas and trees) is indeed limited but is the first of its kind and does show intra-individual variations that are too large to be explained by extraction error only. We do not think that variances up to 20‰ δ^2H in a natural system should be considered as negligible.**
- **The model lacks realism for certain processes. It however does provide the insight that naturally arising changes in evaporative demand should lead to isotopic composition variability in woody stems due to their coupling to variable isotopic and soil water potential gradients (which are the basis of the isotopic studies). Adding model complexity, as the reviewer suggests, would allow us to refine both the ranges and the dynamics of the variation but will not prevent it. As we illustrate using the reviewer's suggestions below.**

Hence our main objective (to illustrate the fact the xylem water isotopic composition does exhibit dynamic variations) still stands.

The dataset accompanying this study only consists of a few water isotope data from tree stems and lianas collected over a couple of days. No soil water data is shown, or even sap flow or rooting depths. I doubt it is the best dataset to test the proposed theory, or draw any conclusion about plant water uptake. The data shown in Fig. 5 is interesting but it comes from another study (Zhao L, Wang L, Liu X, Xiao H, Ruan Y, Zhou M (2014) The patterns and implications of diurnal variations in the d-excess of plant water, shallow soil water and air moisture. Hydrology and Earth System Sciences,18, 4129–4151). Many processes (stem evaporation, different proportions of storage tissues or even atmospheric vapour use) and measurement artefacts (during sampling and transport, water extraction, isotopic analysis…) could explain significant variations in the water composition of stems from trees and lianas of different statures. Accounting for uncertainties in the extraction and analysis is certainly not enough.

We more clearly discuss such limitations in the new version of the manuscript (L284:286; L361:363).

This study indeed presents data collected by Zhao *et al.*, (2014). We note that this study is in collaboration with the authors who collected the data (please see the author list). The data we present show high temporal xylem water observations not presented in Zhao *et al.* (2014). This type of data is very rare in literature. Zhao *et al.* (2014) focused their paper on d-excess variability throughout the day, which is a derivative of δ^2H and $\delta^{18}O$ data. In our paper, we provide the raw δ^2H and $\delta^{18}O$ temporal data. Now also an additional dataset, collected in Germany is included in our study, also **showing pronounced variance in δ^2H and $\delta^{18}O$ data.**

Finally, we note that the factors the reviewer mentions were included in our discussion section. Not all of them will be an issue, while others will exacerbate bias. We will expand this discussion section to address the concerns of the reviewer. Here in short:

- **<u>Stem evaporation</u>: This is indeed a good suggestion for non-woody plants. However, we target woody/lignified plants (this might have not been stated clearly enough, but should be now in the new version of the manuscript). Stem evaporation, especially when measuring relatively low in the stem (at 1.3m), should be negligible (see supplementary method A, L29-31).**
- **<u>Different proportions of storage tissues</u>: We fully agree with the reviewer that this presents a potential explanation of observed patterns, as discussed in the discussion section "iii. *Storage tissue and phloem enrichment*" (L580:603).**
- **<u>Atmospheric vapor use</u>: The reviewer presents another excellent argument of why applying the 'tracer isotopic technique' and sampling protocols should be re-assessed, addressing large variances in δ^2H_X and all potential contributing factors.**
- **<u>Sampling protocol and extraction procedure</u>: While sampling protocols and extraction procedures are never perfect, our extraction protocol is based on best practice as suggested by Orlowski *et al.*, (2013). Hence, considering extraction error rates of 3‰ are very cautious estimates, and actual error is most likely much less. Here we remark that despite these cautious estimates, we observe significant variability (as compared to the null model), which is remarkable and should be reported.**

More importantly, I find the modelling analysis flawed and totally unrealistic. As explained above, the proposed model simulates water isotope gradients along the stem based on the average travel time of sap between two stem points (i.e. assuming the water isotope composition of xylem water at height h is that at stem base at an earlier time corresponding to the travel time between stem base and height h). By doing so, the model neglects the mixing of water isotope by diffusion during water transport. If we neglect pit structure and consider vessels as regular pipes, the Péclet number $\wp$ that compares advection and diffusion is, using their notations: $\wp = SFV\ h=D_l$. Taking an average sap flow velocity of SFV = $0.3mh^{-1}$ (see caption of Fig. 2) a typical height (diffusion length) h of 1m and a self-diffusion of liquid water of $D_l = 2.5\ 10^{-9}m^2s^{-1}$ this leads a Péclet number $\wp$ around 30000, i.e. high enough to justify neglecting (a posteriori) water mixing by diffusion. However, mixing with storage tissues also occurs and tree sap does not move like a slab. In their model, as soon as transpiration stops, root uptake stops and sap flow at any height stops too so that the δ^2H of xylem water at any height remains to its value at dusk over the entire night until the following morning plus the time delay τ (see for example Fig. 2a, the curve for h=1.3m). In reality, at night, sap flow does not stop immediately because plant elastic tissues need to be replenished. Root uptake will continue until full replenishment of the elastic tissues is done. This will contribute to homogenisation of xylem water over night.

Also when sap flow becomes small diffusion is not negligible anymore (low Péclet), which will reinforce the isotopic mixing by water diffusion. In other words, in the real world, xylem water should not exhibit large isotopic gradients along the stem such as shown in their Figs. 1 or 2. Mixing with storage tissues is briefly discussed (section 4.3.iii) but not in the same direction as above. If night-time mixing of xylem water in roots and stems was accounted for, this should strongly minimize the predicted diurnal variations of _2Hx(h; t), even at stem base. Not accounting for diurnal variations in soil conditions (water potential and isotopic composition) is also a strong limitation of the model.

We thank the reviewer for this excellent suggestion to use the Péclet effect to support neglecting diffusion in the model when sap flow is large enough. We have implemented diffusion in the model and have performed an analysis to evaluate the impact of molecular diffusion at night, when sap flow is zero, as assumed by the model at night (i.e. Péclet number becomes low as advective flow rate goes to zero, while diffusive flow rate remains constant, hence flow is dominated by diffusion at night). As the diffusion coefficient is low (i.e. $D_l = 3\ 10^{-5}\ cm^2\ s^{-1}$) the impact of diffusion at night is mostly negligible (12 hours of diffusion results in a smearing of the signature ± 4 cm). Diffusion causes an increase in the width of the δ^2H_X-baseline drop, which means that the probability of sampling a non-representative section within this δ^2H_X-baseline drop will increase. Including more realism hence increases bias in RWU estimates. It should be noted that diffusion will indeed reduce the absolute range of variability in δ^2H_X over time (but very slowly and very little), and hence with the height of the plant. However, it will not lead to homogenization overnight (which is by the way not observed in our supporting datasets, Fig 3c). This would require the accumulated impact of diffusion over many days, creating a time-lag between the measured isotopic composition of xylem and soil water, causing a decoupling of the signatures. These results and corresponding figures are implemented in the manuscript (supplementary method B, L466:472). In addition, we like to stress that for simplicity of the theoretical exploration, we deliberately chose for zero sap flow at night, as indicated in the model description. However, the model is flexible, and direct sap flow data complying with the wishes of the user can be implemented without a problem.

The impact validation of molecular diffusion did not show strong impacts on δ^2H_X dynamics along the length of the stem. However, this suggestion instigated a more in-depth assessment if other processes besides molecular diffusivity might contribute to isotope transport through the plant, especially when considering very low sap flow velocities (e.g. variable flow velocities within vessels and among vessels of the xylem network). We extend our study with a new analysis comparing the xylem transport in the model against a recent ^2H enrichment study of Marshall *et al.* (2020) (L466:482; L603:615, Fig 6). Marschall *et al.* (2020) applied a novel *in situ* borehole equilibration technique for continuous monitoring δ^2H_X dynamics in a *Pinus pinea* individual. This new analysis highlights the need for an improved understanding of δ^2H_X uptake and transport along trees. It further emphasizes the currently lack in understanding various important processes, besides diurnal fluctuations in RWU-activity, that might alter δ^2H_X. These processes are currently ignored in the usual approach of using stable water isotopes for RWU assessment. Therefore, we further highlight and discuss the need for more intra-individual physiological and hydrological understanding via targeted studies, for the betterment of the current implementation of the stable water isotopic technique for RWU as well as the presented model.

As we explored in the discussion section (see section "*Storage tissue and phloem enrichment*"; L580:603), homogenization of xylem water overnight depends on the assumption that storage water is representative of the water taken up by the roots. In fact, for several reasons, the isotopic composition of storage tissues is likely to deviate from the isotopic composition in soil water. This decouples the isotopic signature observed in xylem from the isotopic composition of the water mixture obtained by RWU and exacerbates potential bias in the isotope tracing technique. Unfortunately, empirical data on the isotopic composition of storage tissue is absent in literature to our knowledge, and this hampers the theoretical exploration of such a hypothesis. We highlight more clearly the importance of research targeting evaluation of the impact of storage water use by future studies, which would then allow the implementation of storage tissue in the model (L600:603). However, the presented empirical data do not show any indications of complete homogenization despite obtained from lianas and trees during the dry season (Fig 3c). This might suggest that storage tissue does not completely succeed in homogenizing δ^2H_X overnight as suggested by the reviewer. Therefore, in our opinion, the diurnal root water uptake fluctuation remains a convincing explanation for the observed variability in δ^2H_X.

Finally, the reviewer is correct in pointing out that the absence of diurnal variations in soil conditions (water potential and isotopic composition) presents a limitation of the model. But this is already discussed in the discussion section "*Temporal and spatial soil dynamics*" (L565:579). However, temporal and spatial soil dynamics are generally very small given (a) the timeframe and (b) conditions in which stable isotopic tracing technique are studied, i.e., one-day sampling during dry conditions without rain are generally preferred. Hence, for all these conditions, the simplification of our model is acceptable in our opinion. Besides, our model implementations are flexible and if variable soil condition data are available, they can easily be implemented.

In conclusion, I find the argument raised by De Deurwaerder and colleagues not supported by their data nor by their model simulations. More realism would need to be brought to the model and the dataset should be complemented with additional information before drawing any conclusion on how variable the isotopic of xylem water in tree stems and lianas is over diurnal time scales or with height.

We understand the reservations of the reviewer for this study, as the coupling between data and the model exploration was not fully possible. Therefore, we toned down statements in the manuscript to better represent the limitations of the data and models. We do not agree that our data can be dismissed so easily: we stress that these are now three independent datasets that show pronounced variability in δ^2H_X, which is illustrated for the first time. We also stress that the processes that reviewer suggested to increase realism do not change our conclusion itself: along the stem of woody plants, we can expect changes in water isotopic composition. We believe that arguments raised by both reviewers present additional incentives to re-assess and therefore further refine and improve the stable isotopic tracer technique.

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Causes and consequences of pronounced variation in the isotope composition of plant xylem water

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Abstract

1. Stable isotopologues of water are widely used to derive root water uptake (RWU) profiles and average RWU depth in lignified plants. Uniform isotope composition of plant xylem water (δ_{xyl}) along the stem length of woody plants is a central assumption within the isotope tracing approach, which has never been properly evaluated.

2. Here we evaluate whether strong variation in δ_{xyl} within woody plants exists using empirical field observations from French Guiana, northwestern China, and Germany. In addition, supported by a mechanistic plant hydraulic model, we develop hypotheses on how variation in δ_{xyl} can form through the effects of diurnal variation in RWU, sap flux density, diffusion, and various other soil and plant parameters on the δ_{xyl} of woody plants.

3. The hydrogen and oxygen isotope composition of plant xylem water shows strong temporal (i.e., sub-daily) and spatial (i.e., along the stem) variation ranging up to 25.2‰ and 6.8‰ for δ^2H and $\delta^{18}O$ respectively, greatly exceeding measurement error range in all evaluated datasets. Model explorations predict that significant δ_{xyl} variation could arise from diurnal RWU fluctuations and vertical soil water heterogeneity. Moreover, significant differences in δ_{xyl} emerge between individuals with different sap flux densities.

4. This work shows a complex pattern of δ_{xyl} transport in the soil-root-xylem system, which can be related to the dynamics of RWU by plants. These dynamics complicate the assessment of RWU when using stable water isotopologues, but also open new opportunities to study drought responses to environmental drivers. We propose to include monitoring of sap flow and soil matric potential for more robust estimates of

average RWU depth and expansion of attainable insights in plant drought strategies and responses.

Keywords

Deuterium, Ecohydrology, Lianas, Root water uptake, Sap flow, Stable isotope composition of water, Tropical trees, Water competition

1. Introduction

The use of stable isotope composition of water has strengthened ecohydrology studies by providing insights into phenomena that are otherwise challenging to observe, such as root water uptake depth (RWU depth) (Rothfuss & Javaux, 2017), below-ground water competition and hydraulic lift (Hervé-Fernández *et al.*, 2016; Meunier *et al.*, 2017). Compared to root excavation, the technique is far less destructive and labor-intensive. This makes it more flexible for studying multiple individuals across spatial and temporal scales (i.e. individual to ecosystem, daily to seasonal) (Dawson *et al.* 2002). Besides, the study of stable isotope composition of xylem water measures the real effects of RWU at different depths whereas excavation yields only root distribution and architecture. The advantages and wide applicability of this method make it a popular technique that pushes the boundaries of ecohydrology (Dawson *et al.*, 2002; Yang *et al.*, 2010; Rothfuss & Javaux, 2017).

A variety of methods are used to infer average RWU depth from the isotope composition of plant xylem water (δ_{xyl}), but all rely on a direct relationship between the isotopic compositions of plant xylem and soil water (Ehleringer & Dawson, 1992). All have two key assumptions. The first is that the isotope composition of plant xylem water remains unchanged during transport from root uptake to evaporative sites (e.g. leaves and non-lignified green branches). Hence, isotopic fractionation – i.e. processes that cause a shift in the relative abundances of the water isotopologues, driven by their differences in molecular mass – do not occur during the transport from the uptake to the evaporative site (Wershaw *et al.*, 1966; Zimmermann *et al.*, 1967; White *et al.*, 1985; Dawson & Ehleringer, 1991; Walker & Richardson, 1991; Dawson *et al.*, 2002; Zhao *et al.*, 2016). Second, all methods assume that xylem water provides a well-mixed isotope composition of water from different soil layers: sampled xylem water instantaneously reflects the distribution and water uptake of the roots independent of the timing or height of sampling.

The first assumption is relatively well supported. Isotopic fractionation at root level does not raise concerns for most RWU assessments using water isotopologues (Rothfuss & Javaux, 2017) except for kinetic fractionation that might occur during water transported across the root membrane in extreme environments (Lin & Sternberg, 1993; Ellsworth and Williams, 2007; Zhao *et al.*, 2016). Similarly, isotopic fractionation of water within an individual plant, although possible, is generally not considered a serious problem (Yakir, 1992; Dawson & Ehleringer, 1993; Cernusak *et al.*, 2005; Mamonov *et al.*, 2007; Zhao *et al.*, 2016). This perception was recently contested by Barbeta *et al* (2020), advocating a more general nature of the occurrence of isotopic offsets between xylem water and potential water sources. As the origin of these offsets remains debated, future research should clarify its impact on the applicability of stable water isotopic compositions for RWU assessment. However, the second assumption of time and space invariance of the isotope composition of xylem water has, to our knowledge, never been assessed.

Various plant physiological processes, ranging from very simple to more complex mechanisms, could influence within plant variation in δ_{xyl} at short time scales, i.e. sub-daily to sub-hourly. For instance, plant transpiration during the day is regulated by stomata according to water supply and atmospheric demand, and follows well known diurnal patterns (Steppe & Lemeur, 2004; Epila *et al.*, 2017). This results in a changing water potential gradient between soil and leaves throughout the day (Fig 1a,b), which in turn affects the depth of the average RWU (Goldstein *et al.*, 1998; Doussan *et al.*, 2006; Huang *et al.*, 2017). Hence, shifts in a plant's capacity to take up water at different soil layers during the day can generate diurnal variation in the mixture of isotope composition from water taken up from various depths (Fig 1c). Subsequently, this water mixture moves up along the xylem with the velocity of the sap flux density. As these sap flux densities depend on species and individual-specific hydraulic traits and their responses to atmospheric water demand and soil moisture availability, complex

dynamics in isotopic composition will emerge and propagate through the plant. The above hypothesis, if true, would make the comparison of isotopic data among individuals, species, and studies difficult.

In this study, we provide a critical assessment of the assumption of δ_{xyl} invariance along the length of woody plant stems and over short time periods. We first show that variation in δ_{xyl} along the length of lignified plants exceeds the expected measurement error using three independent datasets including i) canopy trees and lianas sampled at different heights in French Guiana; and ii) plant species from northwestern China (Zhao *et al.*, 2014) and iii) European Beech and Silver firs in south-west Germany (Magh *et al.*, 2020). Second, we build a simple mechanistic model that incorporates basic plant hydraulic transport processes. The model predicts that diurnal changes in water potential gradient between soil and roots result in shifting sources of water absorption that differ in their isotope composition.

2. Materials and Methods

2.1. Part A: Empirical exploration

2.1.1. Field data French Guiana: variation in δ_{xyl} with plant height

Six canopy trees and six canopy lianas were sampled during two subsequent dry days (24-25 August 2017) at the Laussat Conservation Area in Northwestern French Guiana (05°28.604'N-053°34.250'W). Stem xylem tissue of individual plants was sampled at different heights (1.3, 5, 10, 15 and 20 m where possible) at the same radial position of the stem, between 9:00 and 15:00. Stem samples were stripped off bark and phloem tissues. Soil samples were collected at different depths (0.05, 0.15, 0.30, 0.45, 0.60, 0.90, 1.20, and 1.80m) with a soil auger and in close vicinity to the sampled individuals. Samples were placed in glass collection vials, sealed with a cap and frozen awaiting cryogenic vacuum distillation (CVD; 4 h at 105°C). When the

weight loss of a sample resulting from the extraction process was below 98%, the sample was excluded (after Araguás-Araguás *et al.*, 1998) (see Fig S1).

The isotope composition of the water in the samples was measured with a Wavelength-Scanned-Cavity Ring-Down Spectrometer (WS-CRDS, L2120-i, Picarro, California, USA) coupled with a vaporizing module (A0211 High Precision Vaporizer) and a micro combustion module to avoid organic contamination (Martin-Gomez et al., 2015; Evaristo et al., 2016). Post-processing of raw δ-readings into calibrated δ-values (in ‰, v-smow) was performed using SICalib (version 2.16; Gröning, 2011). More details on the sampling site and sampling procedure can be found in supplementary methods A.

2.1.2. Field data China: temporal variation in δ_{xyl}

Plant δ_{xyl} was sampled at high temporal resolution in the Heihe River Basin (HRB), northwestern China during field campaigns described in Zhao *et al.* (2014). Four distinct study locations differing in altitude, climatological conditions, and ecosystem types were selected. At each location, the dominant tree, shrub, and/or herb species were considered for sampling. In August 2009, *Populus euphratica* was sampled in the Qidaoqiao riparian forest (42°01'N-101°14'E) and *Reaumuria soongorica* in the Gobi desert ecosystem (42°16'N-101°17'E; 906-930 m a.s.l). In June–September 2011 *Picea crassifolia*, *Potentilla fruticose*, *Polygonum viviparum* and *Stipa capillata* were measured in the Pailugou forest ecosystem (38°33'N-100°18'E; 2700-2900 m a.s.l). All species were sampled every 2-hours over multiple days (3-4), except for *P. crassifolia* which was measured hourly. Stem samples were collected for trees and shrubs, while root samples were obtained for the herb species. More details are available in Zhao *et al.* (2014)).

Upon collection, all samples were placed in 8 mL collection bottles and frozen in the field stations before transportation to the laboratory for water extraction via CVD (Zhao *et al.*,

2011). Both $\delta^{18}O$ and $\delta^{2}H$ were assessed with an Euro EA3000 element analyzer (Eurovector, Milan, Italy) coupled to an Isoprime isotope ratio mass spectrometer (Isoprime Ltd, UK) at the Heihe Key Laboratory of Ecohydrology and River Basin Science, Cold and Arid Regions Environmental and Engineering Research Institute. Internal laboratory references were used for calibration, resulting in measurement precision of ±0.2‰ and ±1.0 ‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively.

2.1.3. Field data Germany: high temporal variation in δ_{xyl}

Magh *et al.* (2020) conducted an extensive δ_{xyl} monitoring campaign (6-11 July 2017) studying mature Silver firs (*Abies alba*; n=3) and European beeches (*Fagus sylvatica*; n=3) during progressing drought conditions, at the "Freiamt" field site in south-west Germany. Isotopic composition of xylem water was obtained from branch samples, which were collected every two hours between 7:00 and 21:00 at the same height and canopy orientation in the sun crown. Branches were stripped of bark and phloem tissue. A Scholander Pressure chamber (Scholander, 1966), which allowed concomitant registration of water potential of the sampled branches, was used to extract xylem water directly in the field (Rennenberg *et al.*, 1996). Both $\delta^{18}O$ and $\delta^{2}H$ of branch samples were determined with a wavelength scanned cavity ring-down spectrometer (Picarro L2130i, Santa Clara, USA), followed by data correction using ChemCorrect™ (Picarro, 2010). For more details see Magh *et al.* (2020).

2.1.4. Field data normalization

To aid visual comparisons, we use normalized δ_{xyl} – values ($\beta^{2}H_X$ and $\beta^{18}O_X$) which describe the deviation of an individual sample from the average isotopic composition (a) along the height *h* of the stem, or (b) over one day:

$$\beta^{2}H_X = \delta^{2}H_X - \frac{1}{N}\sum_{j=1}^{N}\delta^{2}H_{X,j} \qquad \text{Eq. (1)}$$

With *N* the number of sampled heights or time steps during one day.

2.2. Part B: Model exploration

2.2.1. Model derivation

The expected δ_{xyl} at different stem heights within a tree during the course of the day can be derived from plant and physical properties such as root length density, total fine root surface area, water potential gradients, and the isotope composition of soil water (Fig. 2). We call this the SWIFT model (i.e. Stable Water Isotopic Fluctuation within Trees). To derive the SWIFT model, we first describe the establishment of δ_{xyl} entering the tree at the stem base via a multi-source mixing model (Phillips & Gregg, 2003). We subsequently consider vertical water transport within the tree, which relates to the established sap flow pattern.

To ensure consistency and clarity in variable declarations we maintain the following notation in the subscripts of variables: uppercase roman to distinguish the medium through which water travels (X for xylem, R for root, S for soil) and lowercase for units of time and distance (h for stem height, t for time and i for soil layer index). A comprehensive list of variables, definitions, and units is given in Table 1. A schematic representation of the model is provided in Fig. 2a. Note that the model presented here focuses on hydrogen isotopes (i.e. $^{2}H/^{1}H$) but can easily be used to study oxygen isotopes (i.e. $^{18}O/^{16}O$).

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

The $\delta^{2}H$ composition of xylem water of an individual plant at stem base ($\delta^{2}H_{X,0,t}$) (i.e. height zero; h = 0m; Fig. 2a) at time t, can theoretically be derived by calculating a weighted average of water taken up from different soil depths (Phillips & Gregg, 2003). The root zone is divided into n discrete soil layers of equivalent thickness Δz. Here, we assume a constant $\delta^{2}H$ composition of soil water ($\delta^{2}H_{S,i}$) over time in each soil layer, a reasonable assumption when

isotopic measurements are conducted during rain-free periods, allowing the expression of $\delta^2H_{X,0,t}$ as:

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

where $f_{i,t}$ is the fraction of water taken up at the i^{th} soil layer (Fig. 2a) defined as:

$$f_{i,t} = \frac{RWU_{i,t}}{\sum_{i=1}^{n} RWU_{i,t}} \quad \text{Eq. (3)}$$

and $RWU_{i,t}$ is the net amount of water entering and leaving the roots at time t in the i^{th} soil layer ($RWU_{i,t}$ is defined positive when entering the root). The current representation of the model does not account for water loss via the root system nor for mixing of the extracted water from different soil layers within the roots until the water enters the stem base. When tree capacitance is neglected, the sum of $RWU_{i,t}$ across the entire root zone is equal to the instantaneous sap flow at time t, SF_t:

$$SF_t = \sum_{i=1}^{n} RWU_{i,t} = \sum_{i=1}^{n} -k_i \cdot A_{R,i} \cdot \left[\Psi_{X,0,t} - (\Psi_{S,i,t} - z_i)\right] \quad \text{Eq. (4)}$$

Where k_i is the plant-specific total soil-to-root conductance over soil layer i, $\Psi_{X,0,t}$ is the water potential (i.e. the hydraulic head) at the base of the plant stem and $\Psi_{S,i,t}$ is the soil matric potential at the i^{th} soil layer (Fig. 2a). Total plant water potential is generally defined as the sum of the solute, pressure, gravity, and matric potential. As long-distance water transport through the xylem is studied, the osmotic potential and the kinetic energy head can be assumed negligible (Früh & Kurth, 1999). The xylem pressure potential is represented as $\Psi_{X,0,t}$. And the term z_i is the gravimetric water potential necessary to lift the water from depth z_i to the base of the stem, assuming a hydrostatic gradient in the transporting roots. The model considers z_i to be a positive value (zero at the surface), thus z_i is subtracted $\Psi_{S,i,t}$. $A_{R,i}$ is the absorptive root area distribution over soil layer i (Fig. 2a). This parameter $A_{R,i}$ can be derived from plant

allometric relations with stem diameter (Čermák *et al.*, 2006), and subsequently distributed over the different soil layers, considering the power-law distribution of Jackson *et al.* (1995).

The total soil-to-root conductance is calculated assuming the root and soil resistances are connected in series (Fig. 2a):

$$k_i = \frac{k_R \cdot k_S}{k_R + k_S} \quad \text{Eq. (5)}$$

where k_R is the effective root radial conductivity (assumed constant and uniform), and $k_S = K_{S,i}/\ell$ is the conductance associated with the radial water flow between soil and root surface. $\ell = 0.53/\sqrt{\pi \cdot B_i}$ represents the effective radial pathway length of water flow between bulk soil and root surface (De Jong van Lier *et al.*, 2008; Vogel *et al.*, 2013) with B_i giving the overall root length density distribution per unit of soil. $K_{S,i}$ is the soil hydraulic conductivity for each soil depth. $K_{S,i}$ depends on soil water moisture and thus relates to the soil matric potential $\Psi_{S,i,t}$ of the soil layer where the water is extracted. $K_{S,i}$ is computed using the Clapp & Hornberger (1978) formulation:

$$K_{S,i} = K_{s,max} \cdot \left(\frac{\Psi_{sat}}{\Psi_{S,i,t}}\right)^{2+\frac{3}{b}} \quad \text{Eq. (6)}$$

where $K_{s,max}$ is the soil conductivity at saturation and b and Ψ_{sat} are empirical constants that depend on soil type (here considered as constant over all soil layers).

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

$$f_{i.t} = \frac{k_i \cdot A_{R,i} \cdot \Delta\Psi_{i,t}}{\sum_{i=1}^{n} k_i \cdot A_{R,i} \cdot \Delta\Psi_{i,t}} \quad \text{Eq. (7)}$$

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

Combining Eq. (2) and Eq. (7) then allows the derivation of $\delta^2 H_{X,0,t}$ as follows:

$$\delta^2 H_{X,0,t} = \sum_{i=1}^{n} \left(\frac{k_i \cdot A_{R,i} \cdot \Delta\Psi_{i,t}}{\sum_{j=1}^{n} k_j \cdot A_{R,j} \cdot \Delta\Psi_{j,t}} \cdot \delta^2 H_{S,i} \right) \quad \text{Eq. (8)}$$

This equation requires estimates of $\Delta\Psi_{i,t}$, which is preferably measured instantaneously in the field (i.e. via stem and soil psychrometers for $\Psi_{X,0,t}$ and $\Psi_{S,i,t}$, respectively). However, as measurements of $\Psi_{X,0,t}$ are not always available, estimated $\widehat{\Psi}_{X,0,t}$ can be derived from sap flow by re-organizing Eq. (4) into:

$$\widehat{\Psi}_{X,0,t} = \frac{\sum_{i=1}^{n} [k_i \cdot A_{R,i} \cdot (\Psi_{S,i,t} - z_i)] - SF_t}{\sum_{i=1}^{n} k_i \cdot A_{R,i}} \quad \text{Eq. (9)}$$

which then allows replacement of $\Psi_{X,0,t}$ with $\widehat{\Psi}_{X,0,t}$ in Eq. (8).

ii. Height-dependent isotope composition of plant xylem water

In our model, the water isotopologues simply move upwards from the stem base with the sap flow velocity. Assuming negligible diffusion, the δ^2H isotope composition in xylem water at height *h* and time *t* ($\delta^2 H_{X,h,t}$) is then the isotope composition of xylem water at stem base at time $t - \tau$.

$$\delta^2 H_{X,h,t} = \delta^2 H_{X,0,t-\tau} \quad \text{Eq. (10)}$$

where τ is the lag before $\delta^2 H_{X,0,t}$ reaches stem height *h* (Fig. 2a), which depends on the true sap flux density in the xylem (SF_V). True sap flux density indicates the real speed of vertical water displacement within a plant, derived by dividing SF_t over the lumen area of the plant (A_x; Fig. 2a) i.e. the total cross-sectional area of the vessels. τ can be obtained from the mass conservation equality:

$$h \cdot A_x = \int_{t-\tau}^{t} SF_t \, dt \quad \text{Eq. (11)}$$

Note that since most scientific studies express sap flux density as the sap flow over the total sapwood area (SF_S), rather than over the total vessel lumen area (SF_V), for consistency, we will present the model outputs as functions of SF_S.

Note that SF_V presents the sap flux density normalized over the total vessel lumen area, and as vessel lumen area correlates with plant diameter at breast height (DBH), there is no need for explicit consideration of DBH in the model for comparison among field measurements.

Model analyses show that the impact of the mutual diffusion coefficient of heavy water in normal water on the transport flux is negligible for plants with high sap flux densities, which is the case for the theoretical examples below. However, in plants with low sap flow densities, consideration of diffusion might be required. Diffusion might also be generated by water passing through a complex network of vessels, in analogy to diffusion in a porous media (see supplementary methods B for some analytical results, simulated cases of and a detailed discussion on the role of diffusion). SWIFT was implemented in R version 3.4.0 (R Core Team, 2017), and is publicly available (see GitHub repository HannesDeDeurwaerder/SWIFT).

iii. Model parameterization and analyses

The model's primary purpose is to gain insight into 1) which processes are capable of generating δ_{xyl} variance, and 2) how sensitive the variance in δ_{xyl} along the stem is in response to the modeled plant hydraulic processes. To this end, we adopted the basic plant parameters from Huang *et al.* (2017) who studied soil-plant hydrodynamics of loblolly pine (*Pinus taeda L.*) during a 30-day extended dry down period (Table S1). We started with synthetic basal sap flow patterns and volumes extracted from the model runs of Huang *et al.* (2017) for a typical drought day (day 11). Both basal sap flow patterns and volumes are repeated over the studied period, as no variation between days is assumed. Sap flow follows the plant's water demand which is the result of daily cycles of transpiration driven by photosynthetic active solar radiation

(PAR), vapor pressure deficit (VPD), and optimal stomatal response (Epila *et al.*, 2017). Secondly, both the soil matric potential ($\Psi_{S,i,t}$) and δ^2H composition of soil water ($\delta^2H_{S,i}$) profiles with soil depth were adopted from Meißner *et al.* (2012) (Fig. S8, see Table S1 for equations) as driver data of the model, and were assumed to stay constant over time. Since measurements of Meißner *et al.* (2012) were conducted at a silt loam plot in the temperate climate of central Germany, corresponding soil parameters were selected from Clapp & Hornberger (1978). Subsequently, the following model simulations were executed (see Fig. 2a):

1) **Analysis A1: impact of temporal SF_t variation on the isotope composition of xylem water at a fixed stem height.** Temporal patterns in δ^2H isotope composition in xylem water (δ^2H_X) were evaluated for a typical situation, i.e. measurement at breast height (h=1.30 m) (e.g. White *et al.*, 1985; Meinzer *et al.*, 1999; Goldsmith *et al.*, 2012; Hervé-Fernández *et al.*, 2016; De Deurwaerder *et al.*, 2018; Muñoz-Villers *et al.*, 2019).
2) **Analysis A2: impact of temporal SF_t variation at different tree heights.** Temporal patterns in δ^2H_X within a tree at various sampling heights (5, 10, and 15 m).
3) **Analysis A3: impact of temporal SF_t variation on the isotope composition of xylem water and the timing of sampling.** Representation of the profile of δ^2H_X along the full height of a tree, measured at different sampling times (9:00 and11:00), with the standard parameterization given in Table S1.
4) **Analysis B: variation in δ^2H_X due to differences in absolute daily average sap flow speed.** Diurnal patterns in the δ^2H_X in trees that differ solely in daily averaged SF_V, which are set to 0.64, 0.42, and 0.19 m h^{-1} (respectively corresponding to SF_S values of 0.09, 0.06 and 0.03 m h^{-1}).

All parameters of the four analyses are given in Table S1. The model simulations for each analysis were compared to a null model.

iv. The null model

The null model adopts the standard assumption of zero variation in δ_{Xyl} along the length of the plant body, but allows for potential measurement errors related to the extraction protocol. In reality, empirically obtained data will have some variation as observed values ($Obs.\,\delta_{Xyl}$) are the sum of the true δ_{Xyl}-values and their extraction error ($error_{extraction}$).

$$Obs.\,\delta_{Xyl} = True\ \delta_{Xyl} + error_{extraction} \quad \text{(eq. 12)}$$

Hence, the null model attributes any variance in isotopic composition to extraction errors, with maximum extraction error ranges of 3‰ for δ^2H samples (0.3‰ for $\delta^{18}O$) expected for water extraction recovery rates higher than 98% (e.g. Orlowski *et al.*, 2013). These extraction errors are negatively skewed following the Rayleigh distillation model, which predicts that extraction error for incomplete water recovery will be negative, and therefore Obs. $\delta_{xyl} \leq$ True δ_{xyl}. The null model represents this $error_{extraction}$ by a negative skew-normal distribution (with location parameter ξ = 0‰, the scale ω = 3‰ for δ^2H or 0.3‰ for $\delta^{18}O$, and shape α = -∞) (Azzalini, 2013).

2.2.2. Estimation of average RWU depth

Average RWU depths (i.e. the weighted mean of the depths of RWU, with the uptake fractions at the different depths as weights) were derived from the simulated δ^2H_X values by use of both the direct inference method and the end-member mixing analysis method. Together, these techniques represent 96% of the applied methods in the literature (Rothfuss & Javaux, 2017), and the reader is referred to Rothfuss & Javaux (2017) for a complete discussion of both

techniques. In line with the general approach assessing RWU with stable water isotopes, the average RWU depth is obtained by relating the δ^2H_X with the $\delta^2H_{S,i}$ depth profile. We compared average RWU depth estimates obtained from simulated δ^2H_X, as described in the analyses above, with the true average RWU depth. Here, the true average RWU depth was defined as the depth corresponding to the daily weighted average δ^2H_X, calculated as the weighted sum of $\delta^2H_{X,i,t}$ and the relative fraction of water taken up at each depth.

2.2.3. Transport dynamics and sensitivity analysis

We perform a basic model validation of our model assumption that the propagation of an isotopic signature is driven by diurnal sap flow dynamics and diffusion alone. In essence, the model assumes that once water with a given isotopic signature enters the stem, it moves upwards with the speed of sap flow, and changes only due to the effect of diffusion. The effects of capacitance on δ^2H_X dynamics by the release of storage water in the xylem flow can be ignored. To validate this assumption we compare model predictions against observed δ^2H_X dynamics monitored within a pine tree (*Pinus pinea L.*) following ^2H-enrichment in a controlled greenhouse experiment, as detailed in Marshall *et al.* (2020). δ^2H_X was measured at two heights (0.15 and 0.65m) using a novel *in situ* technique, the borehole equilibration method. Performed model simulations consider the absolute ranges of sap flux densities during the entire monitoring campaign, with the account of tree tapering effect on sap flux densities over the studied stem length (supplementary method C). Validation of diurnal variation in δ^2H_X requires high temporal resolution monitoring of δ^2H_X dynamics in plants stems, with simultaneous high temporal resolution monitoring and characterization of sap flow, soil water potential, and isotopic composition. Such data does not yet exist to our best of knowledge.

In addition, we performed two sensitivity analyses to assess the relative importance of each parameter in generating variance in δ^2H_X along the length of a plant. In both sensitivity analyses,

we varied model parameters one-at-a-time to assess the local sensitivity of the model outputs for soil type, sap flux density, root properties, and sampling strategies. The sensitivity analysis provides insight into possibilities for improving the design of field protocols, by revealing potential key measurements and caveats in field setups. More details on the performed sensitivity analysis and validation of transport dynamics are available in supplementary method C.

3. Results

3.1. Part A: Empirical exploration

The null model assumes constant isotopic composition of root water uptake, with only limited variance in isotopic composition introduced by extraction errors ($\beta^2H_X < 3‰$; $\delta^{18}O_X < 0.3‰$). However, pronounced δ^2H_X variance within individual plants, exceeding the null model ranges, are observed in all three independent datasets. The normalized δ^2H composition in xylem water (β^2H_X) along the stem length of lianas and trees in French Guiana exceeded the null model by a factor of 3.2 and 4.3, respectively (Fig. 3c, Fig. S2). Differences up to 13.1‰ and 18.3‰ in δ^2H and 1.3‰ and 2.2‰ in $\delta^{18}O$ were observed in individuals of trees and lianas, respectively (Supplementary method A, table A,).

Similarly, diurnal intra-individual δ^2H_X variances were found for all considered plant growth forms, i.e. trees, shrubs, and herbs, monitored in China (Fig. 4b-d, Fig S3). Observed daily maximum differences in δ^2H_X were 18.0‰, 21.0‰, and 25.2‰ for trees, shrubs and herbs respectively (2.8‰, 6.8‰, and 6.5‰ in $\delta^{18}O_X$ in Fig. S4). The expected null model variance was exceeded for each species during its measurement period.

Finally, pronounced intra-individual δ^2H_X variance was also observed for all monitored firs and beeches in Germany (Fig 4e, Fig. S5). Here, daily maxima differences in δ^2H_X were 8.2

‰ and 14.2 ‰ for *Abies alba* and *Fagus sylvatica* respectively (2.0‰ and 4.2 ‰ in $\delta^{18}O_X$ in Fig. S6).

3.2. Part B: Model exploration

Isotope composition of xylem water at stem base and basic model behavior

At the stem base, simulated $\delta^2H_{X,0,t}$ displays a diurnal fluctuation (Fig. 2b, Fig S7) that corresponds to the daily sap flow pattern (Fig. S7). This pattern is caused by shifting diurnal average RWU depth. Early in the morning, when transpiration is low, most of the RWU occurs in deeper layers, where soil matric potential is less negative and where soil water is more depleted in δ^2H in comparison with the soil layers above (Fig. S8a-b). As transpiration increases during the day, a significant proportion of RWU can now be extracted from the drier, shallower layers, where the δ^2H-composition of soil water is enriched, hence higher. In the afternoon, as transpiration declines, the isotopic composition reflects again the composition of the more depleted soil water in the deeper soil layers, and it remains constant throughout the night because apart from diffusion SWIFT does not consider mixing of the internal stem water. The mixing effects of diffusion are only noticeable at low sap flow speeds (fig 3b).

The most enriched δ^2H_X-values (approx.-59‰) are found in alignment with the diurnal minimum of $\Psi_{X,0,t}$ (approx.-0.85 MPa, Fig. S7). At this moment, the difference between $\Psi_{X,0,t}$ and $\Psi_{S,i,t}$ is maximal, enabling water extraction from the upper and driest soil layers. Most root biomass is located near the surface (cf. Jackson *et al.*, 1995; Fig. S8c) and uptake in these layers will result in relatively high contributions to the total RWU.

In contrast, differences between $\Psi_{X,0,t}$ and $\Psi_{S,i,t}$ are smaller in the early morning and late afternoon causing root water uptake in the upper soil layers to halt. The decreasing in absolute range of $\Delta\Psi_{i,t}$ translates into higher proportions of RWU originating from deeper, more

411 depleted soil layers. This causes δ^2H_X to drop to a baseline of approx. -67‰. This afternoon
412 depletion of δ^2H_X will henceforth be referred to as the δ^2H_X-baseline drop.

413 *Isotope composition of xylem water at different times, heights and SF_V*

414 Temporal fluctuation in δ^2H_X within a tree at 1.3 m (i.e. the standard sampling height;
415 Analysis A1; Fig. 2a) and other potential sampling heights (e.g. branch collection; Analysis A2;
416 Fig. 2a), are provided in Fig. 2b and 3a. Both analyses show that fluctuations in δ^2H_X depend
417 on the height of measurement and the corresponding time needed to move the water along the
418 xylem conduits. Note that it depends on the selected temporal resolution whether the δ^2H_X-
419 baseline drop at a given height equals the (stem base) minimum (here 1 min, see Fig. S12). In
420 addition to sampling height, analysis A3 depicts the importance of sampling time (Fig. 3a).
421 Outputs of analysis B predict that the occurrence and width of the δ^2H_X-baseline drop are a
422 function of the sap flow velocity SF_V (Fig. 3b). To aid model interpretation and comparability
423 with field data, we (i) provide an illustrative example of normalized δ^2H isotope composition
424 of model-simulated xylem water (β^2H_X) with consideration of extraction error (Fig. 4a), and (ii)
425 display the relation between δ^2H_X variance and cumulative sap flow volumes, for which the
426 piston flow dynamics in SWIFT originate from lateral translation of the δ^2H_X fluctuation at
427 $\delta^2H_{X,0,t}$ (Fig. 2b).

428

429 **3.2.1. Potential biases in average RWU depth estimation**

430 Both timing of measurement (Fig. 5a) and SF_V (Fig. 5b) influence average RWU depth
431 estimates derived via the direct inference and end-member mixing analysis method (Fig. S9).
432 Collection of tree samples at 1.30 m can result in erroneous estimation, deviating up to 104 %
433 from the average daily RWU depth (Fig. 5). Plotting the relative error in average RWU depth
434 as a function of time and SF_V (Fig. 5) shows that it is possible to time δ^2H_X measurements in a

fashion that captures unbiased estimates of the average RWU depth. Xylem water sampling should be timed to capture the δ^2H_X that corresponds to water extracted at peak RWU, and the expected sampling time can be derived by considering the time needed for the water to reach the point of measurement (i.e. at 1.30 m in Fig. 5).

3.2.2. Transport dynamics and sensitivity analysis

Our sensitivity analyses show that the expected absolute error in average RWU depth assessment is directly related to both 1) maximum variance in and 2) the probability of sampling non-representative δ^2H_X values. The maximum variance depends on the height, while the probability of sampling non-representative areas depends on the width of the "δ^2H_X-baseline drop" respectively (defined above). Hence, variation in δ^2H_X is determined by several factors, including the sampling strategy (timing and height of sampling), sap flow velocity (Fig. S10), and below-ground biophysical parameter (Fig. S11). We summarized the most important variables as predicted by SWIFT, which should be considered in subsequent RWU studies.

Plants on loamy soils show larger diurnal δ^2H_X variances in comparison with those on clay soils for a similar prevailing isotope gradient across the soil profile. Larger variances correspond to potentially larger errors, but the steeper slope of the δ^2H_X curve results in a thinner δ^2H_X-baseline drop. Hence, loamy soil can result in potentially the large error but this is mediated by a lower probability of sampling non-representative δ^2H_X values during the day.

The volume of water taken up by the plant (SF_t; Fig. S11b) affects xylem water potential of the plant at stem base ($\Psi_{X,0,t}$). Higher SF_t requires more negative $\Psi_{X,0,t}$, enabling the plant to access more shallow and enriched soil layers. Therefore, an increase in SF_t results in the increase of maximum δ^2H_X values (increased maximum error) but also results in a smaller width

458 of the baseline drop (Fig. 2-3). Lower SF_t result in smaller errors, but a larger probability of
459 sampling a non-representative area (Fig. 3b).

460 Root properties, i.e. root membrane permeability (Fig. S11c) strongly influence both the
461 total range of δ^2H_X variance and the width of the δ^2H_X-baseline drops. Decreasing root
462 membrane permeability, but with no alterations to the sap flow volumes, results in thinner δ^2H_X-
463 baseline drops, but higher maximum δ^2H_X variance.

464 In addition, the true sap flow velocity (SF_t per unit of lumen area) will determine the relative
465 importance of diffusion on the δ^2H_X dynamics. Diffusion can cause a smoothing of the peak and
466 a consequent increase in the width of the δ^2H_X-baseline drop. However, as diffusion is
467 proportional to the time the isotope remains in the xylem, its absolute impact on δ^2H_X is
468 negligible in plants with a high true sap flow velocity. In contrast, the impact of diffusion on
469 δ^2H_X dynamics is substantial for plants with very low velocities, where water takes many days
470 to pass from roots to leaves (see supplementary method B).

471 The role of diffusion was investigated using a stepwise δ^2H enrichment experiment in Marshall *et al.*
472 (2020) (Fig 6). Analytical solutions of an advection-diffusion equation show that at 0.15 cm, a relatively
473 small diffusivity was required to reproduce the initial increase of xylem isotope signature, with values
474 comparable to these reported for diffusivity of heavy water (Meng *et al.*, 2018). However, at 65 cm, the
475 value of diffusivity required to match the observed initial increase was much higher, suggesting other
476 processes besides molecular diffusivity might contribute to the isotope transport (e.g. variable flow
477 velocities within vessels and among vessels of the xylem network). Note also that the analytical solutions
478 were not able to recover the second part of the curve where the isotope reaches the asymptotic enriched
479 value, which is more gradual in the observations (Fig. 6). This also suggests a complex transport of δ^2H_X
480 in the xylem.

481

482 ## 4. Discussion

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

Empirical field data show pronounced δ_{xyl} variance along the stem length (Fig. 3) and over a sub-daily time period (Fig. 4). Our model explorations suggest that basic plant hydraulic functioning can result in shifting mixtures of δ^2H_X entering the plant (Fig. 2-3). Daily $\Psi_{X,0,t}$ fluctuations interact with the $\Psi_{S,i,t}$ profile causing different parts of the root distribution to be active during the day. The fluctuations in δ^2H_X at the stem base propagate along the xylem with a velocity proportional to the sap flow and this produces variability in sampled δ^2H_X that is much larger than the expected measuring error. Consequently, rather than being static, δ^2H_X values along the height of a plant should be envisioned as a dynamic diurnal process.

Importantly, we show that high variance in δ^2H_X can result in an incorrect assessment of differences in average RWU depths between plants. Differences do not necessarily result from variability in average RWU depth, but may result from monitoring plants at different heights (Fig. 2-3), at different times (Fig. 3a) or by comparing individuals which have different SF_V (Fig. 3b) and xylem anatomical properties. For example, depending on SF_V and lumen area, the isotopic signal can take hours or days to travel from roots to leaves - as was also observed experimentally (Steppe *et al.*, 2010; Magh *et al.*, 2020; Marshall *et al.*, 2020).

Low SF_V allows multiple δ^2H_X-baseline drops over the length of a single tree. Sampled δ^2H_X can reflect soil isotopic composition of the past several days. Our sensitivity analysis reveals that various soil and plant characteristics have an important role in determining both the daily maximum δ^2H_X variance as well as the width of the δ^2H_X -baseline drop. These two characteristics directly impact (i) the expected maximum bias in estimates of average RWU depth and (ii) the chance of measuring δ^2H_X values that do not represent a mixture of all rooting layers during peak RWU (i.e. measurements in the baseline drop). Ultimately, these factors will challenge the use of stable water isotope to study the terrestrial water fluxes as recently

reviewed by Penna *et al.* (2018). We additionally advocate that future research should explore the minimum set of (bio)physiological drivers and processes that require quantification to correctly interpret δ^2H_X along the hydraulic pathway length of a plant.

4.2. General applicability of model and results

A necessary condition for diurnal shifts in RWU is the existence of water potential differences, e.g. more negative water potentials in the upper layers where trees usually have higher root density, which can cause a disproportional partitioning of diurnal RWU between deep and shallow roots over a diurnal course. The pronounced variance in δ_{xyl} identified in this study is intrinsic to the isotopic tracing technique for RWU assessment, as this method relies on the existence of a soil water isotopic profile. Such profiles are the result of soil evaporation, a process inextricably coupled to water potential heterogeneity, and hence to variance in δ^2H_X.

Plant transpiration results from a complex interaction between atmospheric demands (i.e. driven by VPD and radiation) and stomatal conductance that depends on tolerance for drought stress and soil moisture content. We may expect diurnal fluctuation in radiation and VPD, and hence in water transport and depth of water absorption, as modeled here to be a general phenomenon in nature. Moreover, much greater fluctuations in VPD and radiation should be expected under natural conditions than the diurnal cycle described here, and these will increase the variability of transpiration fluxes, leading to even more complex dynamics of $\Psi_{X,0,t}$. Specifically, the model simulations suggest that intra-individual variability of δ^2H_X will reflect the past changes of RWU dynamics, including RWU dynamics driven by changes of environmental demands. For instance, a changing degree in cloud cover that impacts sap flow dynamics can influence $\Psi_{X,0,t}$ rather abruptly (e.g. in lianas; Chen *et al.*, 2015) and lead to

instantaneous changes in the δ^2H composition of the water mixture taken up at the root level. This can complicate the comparison of different plants sampled at different heights and times.

Note that, based on our model, we expect that soil isotopic enrichment experiments will generate extensive δ^2H_X variation along the length of trees whenever diurnal RWU fluctuations cause water extraction to shift between labeled and unlabeled soil layers. Furthermore, when enrichment experiments target trees with different hydraulic properties (such as SF_V) care should be taken to determine when and where to sample these trees to assess an enriched isotope composition (Fig 6, but see Magh *et al.*, 2020;).

4.3. Alternative causes of δ_{xyl} fluctuation.

The SWIFT model provides a simple traceable and mechanistic explanation, using diurnal variations in SF_t and RWU, for the pronounced variance and dynamic nature of the δ_{xyl} fluctuations with plant height and time of field samples (e.g. Fig. 3-4) and elsewhere (Cooper *et al.* 1991). However, several other processes might contribute to generate variability, while others can act to damp this variability. In the next section, we will discuss alternative causes, complementary and antagonistic, that contribute to the observed intra-individual δ_{xyl} variances.

i. Fractionation at root or stem level

An increasing body of observations shows the occurrence of isotopic fractionation at the root level governed by root membrane transport (Lin & Sternberg, 1993; Vargas *et al.*, 2017) or by unknown reasons (Zhao *et al.*, 2016). Brinkmann *et al.* (2019) hypothesize that root level fractionation causes disparity when average RWU depth calculations based on δ^2H_X measurements are compared with those of $\delta^{18}O_X$. However, it is difficult to imagine a scenario where root fractionation by itself can explain the observed diurnal fluctuations in δ_{xyl} with height and time. Even if root fractionation significantly contributed to variation in δ_{xyl}, we

554 would still need to take into account diurnal fluctuation in RWU to explain the observed
555 patterns. Isotopic enrichment of xylem water along the stem length was observed in association
556 with stem transpiration (Dawson & Ehleringer, 1993; Barnard *et al.*, 2006). However, this
557 phenomenon is generally restricted to non-suberized plants and in woody branches in close
558 vicinity to the evaporative surface of the plant (Dawson & Ehleringer, 1993). Isotopic
559 enrichment can, therefore, not explain the variances in δ_{xyl} observed in our empirical data, which
560 were sampled within the main stem (data French Guiana) or from lignified branch segment
561 distant from evaporative surfaces (data China and Germany).

562 ii. *Temporal and spatial soil dynamics*

563 Soil water content can be extremely heterogeneous in the three spatial dimensions as well
564 as in time with complex dynamics of soil water movement. For example, hydraulic lift vertically
565 redistributes soil water through the roots (Dawson & Ehleringer, 1993), which may change the
566 water isotopic composition of the water mixture in the rhizosphere that is taken up by roots.
567 Specifically, hydraulic lift redistributes and mixes the depleted isotopic signal of deeper layers
568 with the enriched signal in the rhizosphere in shallower layers. This should lead to lower
569 variation in the soil water accessible to the plant, and hence less variation along plant height.
570 Horizontal heterogeneity of water content may also affect δ_{xyl} variance as soil water potentials
571 and the isotope composition of soil water are interlinked. Under these conditions, it is important
572 to understand how much the radial distribution of roots will naturally average out soil
573 heterogeneity. However, note that heterogeneity in the soil does not automatically translate in
574 variability in the xylem. Differential root water uptake driven by the diurnal fluctuation in water
575 potential gradients in the soil-plant interface is still required to generate variability in the xylem
576 isotopic signature.

577 *iii. Storage tissue and phloem enrichment*

578 Storage tissues release water and sugars into the xylem conduits on a daily basis to support
579 water transpiration demand (Goldstein et al., 1998; Morris et al., 2016; Secchi et al., 2017) or
580 to repair embolism (Salleo et al., 2009; Secchi et al., 2017). Both water and sugars are
581 transported in and out of storage tissue via symplastic pathways using plasmodesmata and
582 aquaporins (Knipfer et al., 2016; Secchi et al., 2017), a pathway that has been linked to isotopic
583 fractionation in roots (Ellsworth & Williams, 2007). Moreover, phloem transports
584 photosynthetic assimilates that were produced in the leaves and are therefore potentially
585 affected by transpiration fractionation (Gessler *et al.*, 2013). Hence, these metabolic molecules
586 might show higher values of δ^2H and $\delta^{18}O$ compared to RWU. Water release from storage or
587 phloem tissue might locally alter δ_{xyl} (White et al., 1985). Additionally, the time between water
588 storage and release could bridge multiple days, and corresponding isotopic composition may
589 reflect soil conditions antecedent a dry spell when the isotopic signature of soil was less
590 vertically stratified. It is evident that such dynamics are complex, and it is hard to predict how
591 storage tissue and phloem enrichment affect observed δ_{xyl} patterns. Importantly, xylem isotopic
592 sampling cannot differentiate between water resulting from RWU or storage, and therefore we
593 cannot exclude the possibility that tissue and phloem enrichment play a role. At a minimum this
594 adds further uncertainty to RWU assessment. Water derived from storage tissues might also be
595 present in larger fraction in higher parts of the plants, especially branches, as contamination
596 accumulates as water moves upwards.

597 Unfortunately, to our best knowledge, empirical data on the isotopic composition of storage
598 tissue and its spatiotemporal dynamics are absent in the literature. Future research should target
599 impact assessment of storage water on intra-individual δ_{xyl}, allowing proper implementation in
600 the model.

601 *Diffusion processes*

Diffusion is a process of net movement of molecules from a region of higher concentration to a region of lower concentration. Consequently, diffusion dampens δ_{xyl} variability, both in time and space within water xylem. Although the mutual diffusion coefficient of heavy water in normal water is very small and flow within vessels is laminar, other processes might still contribute to generating diffusion along the xylem. For example, as the water moves through the complex network of vessels, differences in velocities between vessels of different sizes cause some particles to move faster or slower than average flow. According to the Hagen–Poiseuille law, the flow in each vessel is proportional to the fourth power of the vessel radius and the mean velocity to the square of the radius, thus potentially generating large differences in particle velocities depending on the vessel size distribution and other anatomical properties. Even within a single vessel, velocity is parabolic with a maximum flow velocity in the center and zero at the vessel walls.

4.4. A way forward

The observed large δ_{xyl} variance and temporal dynamics in the empirical data suggest the need for a critical assessment of the stable isotope tracer technique for RWU studies. However, it also creates new opportunities. Since δ_{xyl} variance and temporal dynamics herein likely relate to various plant physiological processes, monitoring of variation in δ_{xyl} can allow a more integrated understanding of plant water transport and hydraulic properties.

Combining a plant hydraulic model with *in situ* SF_V, $\delta^2H_{S,i}$ and $\Psi_{S,i,t}$ can also help improve the robustness of RWU assessment and interpretation. Measurements of $\delta^2H_{S,i}$ and $\Psi_{S,i,t}$ at multiple depths, i.e. by installing soil water suction cups working at a vacuum (i.e. Rennenberg *et al.*, 1996) and multiple soil matric potential sensors that measure at a high temporal frequency, should be especially valuable since the SWIFT model showed high sensitivity to alterations of this variable and these can be directly supplied as model inputs. At

the same time, the availability of SF_t measurements allows for identifying the moment when water uptake from all root layers is at its maximum, which can be used to determine the optimal timing of sampling at a given height providing a more robust estimation of average RWU depth and uptake.

Alongside the modeling approach presented here, new ways to study δ^2H_X at a high temporal scale are strongly encouraged. For example, the pioneering work of Volkmann *et al.* (2016) to the development of an *in situ* continuous isotope measurement technique that offers the possibility for monitoring δ_{xyl} at a sub-hourly resolution. This technique holds strong promise for further elucidating the natural δ^2H_X variances found within plants and the physiology processes from which these variances result. Such high temporal resolution of isotope measurements, coupled with *in situ* monitoring of various environmental and plant biophysical metrics, are needed for both model improvement and further validation. Moreover, these seem inevitable to eventually differentiate all causal mechanisms of the observed intra-individual δ_{xyl} variance.

5. Conclusions

A collection of empirical field data show pronounced variance and high temporal fluctuations in δ_{xyl}. Moreover, these high temporal fluctuations in δ_{xyl} emanate from basic plant hydraulic functioning as model explorations show. We expect the observed δ_{xyl} variance and sub-daily fluctuations result, for a large part, from the mechanisms considered here, though various other physiological processes could also affect δ_{xyl}.

Our theoretical explorations warn that variability in the isotope composition of plant xylem water can result in erroneous average RWU depth estimation and will complicate the interpretation and comparison of data: samples taken at different heights, times or plants

differing in SF_V may incorrectly show differences in average RWU depth. We further predict that various soil parameters and plant hydraulic parameters affect (i) the absolute size of the error and (ii) the probability of measuring δ_{xyl} values that do not represent the well-mixed values during the plants' peak RWU. Hydraulic models, such as SWIFT, could help to design more robust sampling regimes that enable improved comparisons between studied plants. We advocate the addition of SF_t, which indirectly reflects diurnal RWU fluctuations, and $\Psi_{S,i,t}$ monitoring as a minimum in future RWU assessments since these parameters were predicted to be the predominant factors introducing variance in δ_{xyl} from the SWIFT model exploration. However, soil texture and root permeability are also key measurements especially when comparing across species and sites.

Our findings do not exclude additional factors that impact the observed intra-individual δ_{xyl} variance and temporal fluctuation as many processes can act simultaneously and are not mutually exclusive. Therefore, we strongly emphasize the need for more research. Directed studies that validate and quantify the relative impact of other plant physiological processes towards variance in δ_{xyl} are a prerequisite before improved modeling tools can be developed.

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Author contribution

H.V., M.D.V, and P.B. supervised and provided guidance throughout all aspects of the research. H.D.D., M.D.V, and H.V. designed the study. H.D.D., K.K., R.K.M., J.D.M., L.W., and L.Z. collected and processed the empirical datasets. The model was developed and coded by H.D.D, M.D.V, M.D., and F.M. All authors contributed to the interpretation of the results and the text of the manuscript.

Data availability

Both the French Guiana data and the SWIFT model are available on the GitHub repository HannesDeDeurwaerder/SWIFT. For the availability of the data collected in China and Germany, readers are referred to Zhao *et al.* (2014) and Magh *et al.* (2020) respectively.

Competing interests

The authors declare that they have no conflict of interest.

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Tables

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 i^{th} 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 i^{th} soil layer at time *t* derived from Eq. (8)	m
$\Delta\Psi_{i,t}$	Soil matric potential gradient between soil and roots at the i^{th} soil layer at time *t*	$m\ H_2O$
$\beta^2 H_X; \beta^{18} O_X$	Normalized isotope composition of plant xylem water	in ‰ VSMOW
$f_{i,t}$	The fraction of water taken up in the i^{th} soil layer at time *t*	dimensionless
h	Measurement height	m
i	Soil layer index	dimensionless
δ_{xyl}	Isotope composition of plant xylem water	in ‰ VSMOW
k_i	Soil-root conductance of the i^{th} 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 i^{th} 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 matric potential at soil saturation	m
$\Psi_{S,i,t}$	Soil matric potential of the i^{th} 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 i^{th} 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 i^{th} soil layer at time t	$m^3\ m^{-3}$
z_i	Soil depth of the i^{th} soil layer	m

Figures

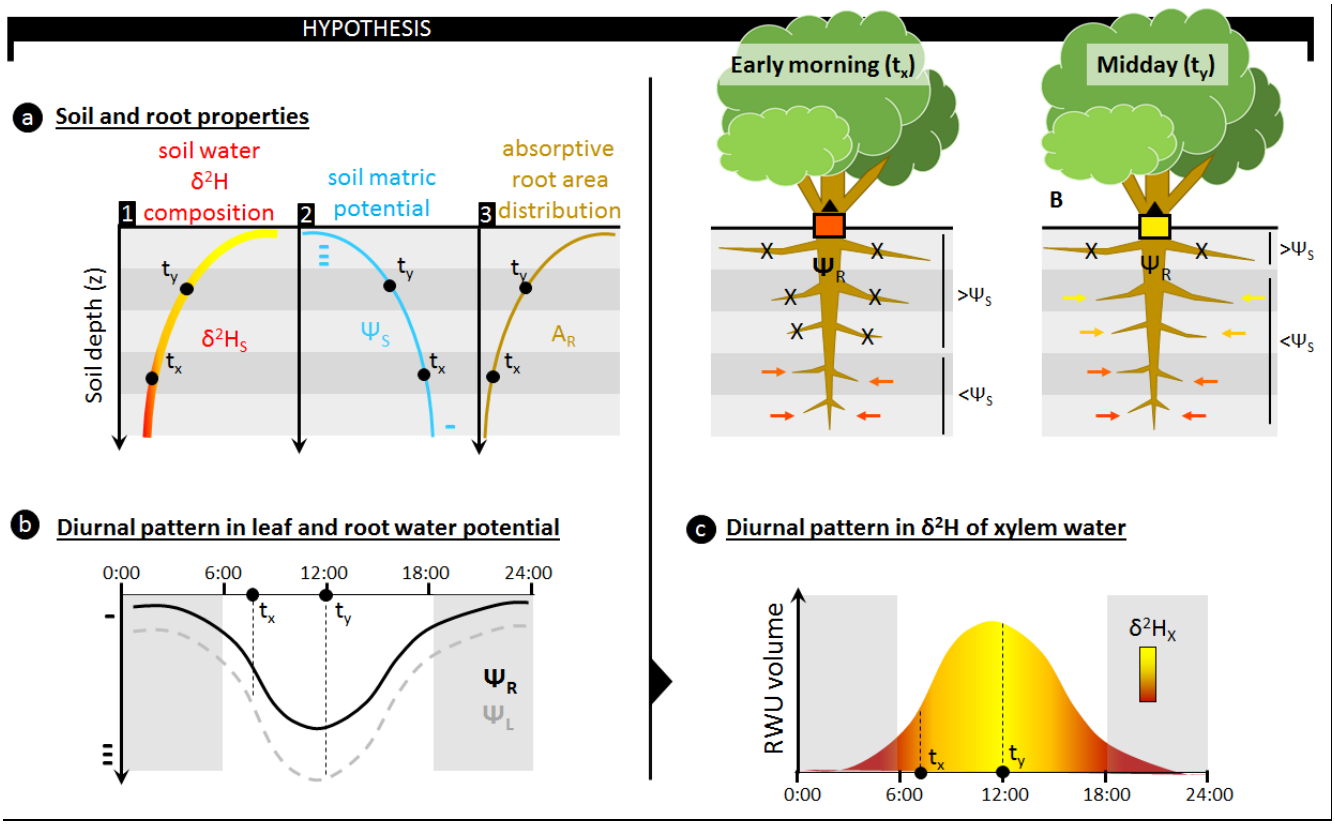
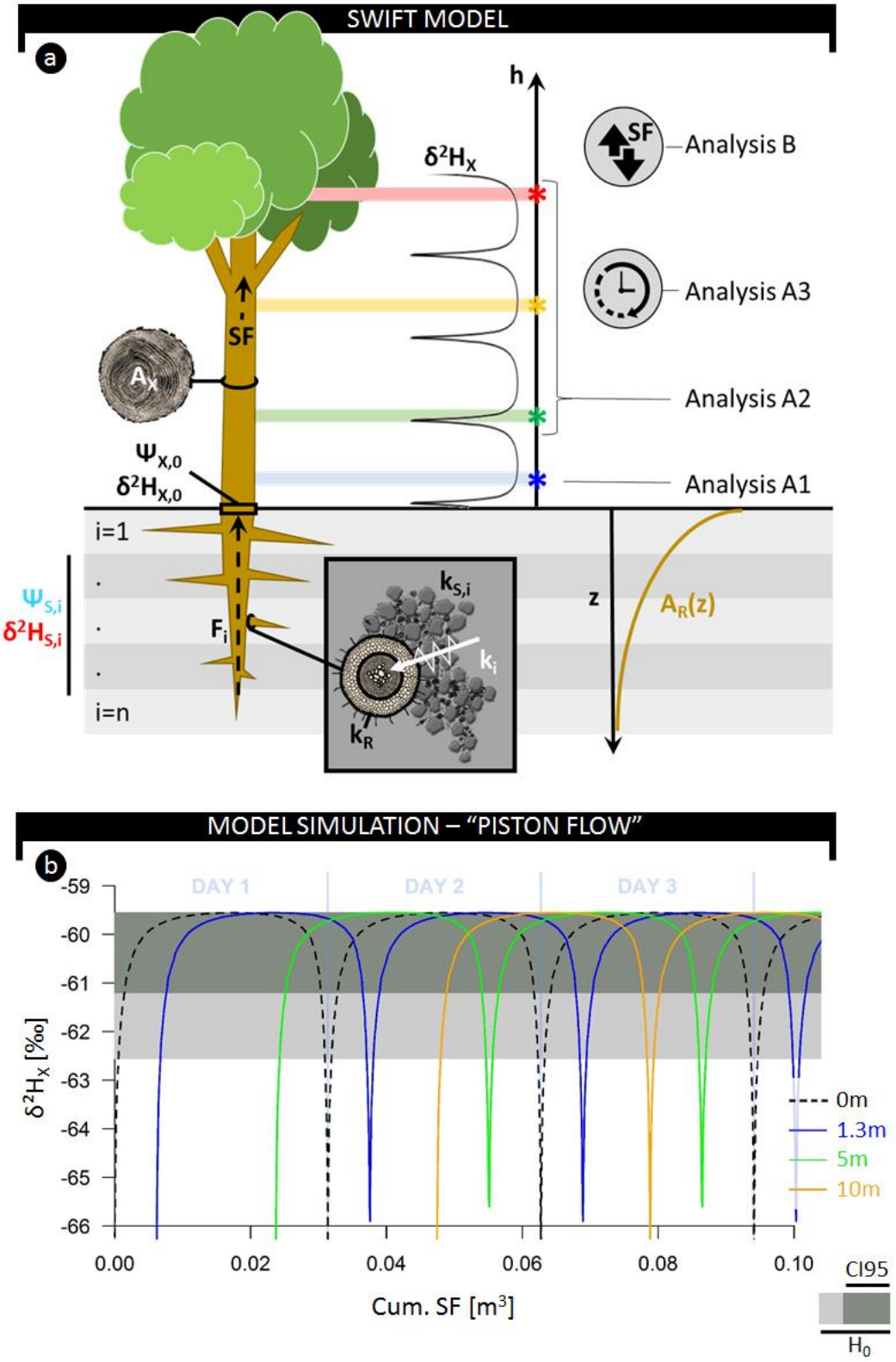


Fig 1. The use of stable water isotopes (δ^2H and $\delta^{18}O$) to assess the depth of root water uptake (RWU) requires a depth gradient in isotopic composition of soil water (δ^2H_S) to be present (**a, line 1**), as only then can the relative contribution of different soil layers to the isotopic composition in a plant's xylem water (δ^2H_X) be derived. These δ^2H_S gradients occur naturally as the result of evaporative soil drying during drought conditions, however, these conditions also result in the formation of a gradient in soil matric potential (Ψ_S), ensuring an increasing Ψ_S with depth (**a, line 2**). RWU and sap flow in plants are passive processes where water flows in the direction of decreasing water potentials. Specifically for RWU, this implies that water influx through the absorptive root area (A_R; **a, line 3**) of a plant's root is facilitated whenever the water potential in the root (Ψ_R) is more negative than the surrounding Ψ_S. As A_R and Ψ_S are generally not uniform with soil depth (z), the relative contribution of a specific soil layer to RWU will depend on (i) the difference between Ψ_S and Ψ_R in that soil layer, and (ii) the relative amount of absorptive root area in that soil layer. Stable water isotopes techniques assume that the δ^2H_X

reflects the contribution of δ^2H_S from all soil layers. However, this does not account for diurnal fluctuations in Ψ_R which are invoked by the diurnal patterns in a plant's transpiratory water demands (**panel b**). Typically, more negative Ψ_R values are observed when water demands are high, i.e. around midday. However, a decrease in Ψ_R will result in higher RWU, and alter the contribution of different soil layers to RWU. Specifically, dryer and shallower soil layers, with more negative Ψ_S, could start contributing to RWU as Ψ_R decreases (**panel c**). For example, in the early morning (situation t_x) when Ψ_R is high, only deeper soil layers where $\Psi_S > \Psi_R$ contribute to overall δ^2H composition of the RWU flux. As Ψ_L and Ψ_R decrease towards midday (situation t_y) more water can be absorbed from shallower soil layers. As the A_R in these shallow soil layers is high, they strongly affect the relative contribution of δ^2H_S entering the plant. Hence, diurnal fluctuations in Ψ_R will result in fluctuating mixtures of δ^2H_S entering the plant. As these δ^2H_S mixtures are transported along the xylem pathway, they produce variance in δ^2H_X, which could complicate RWU assessments via stable water isotope analysis.

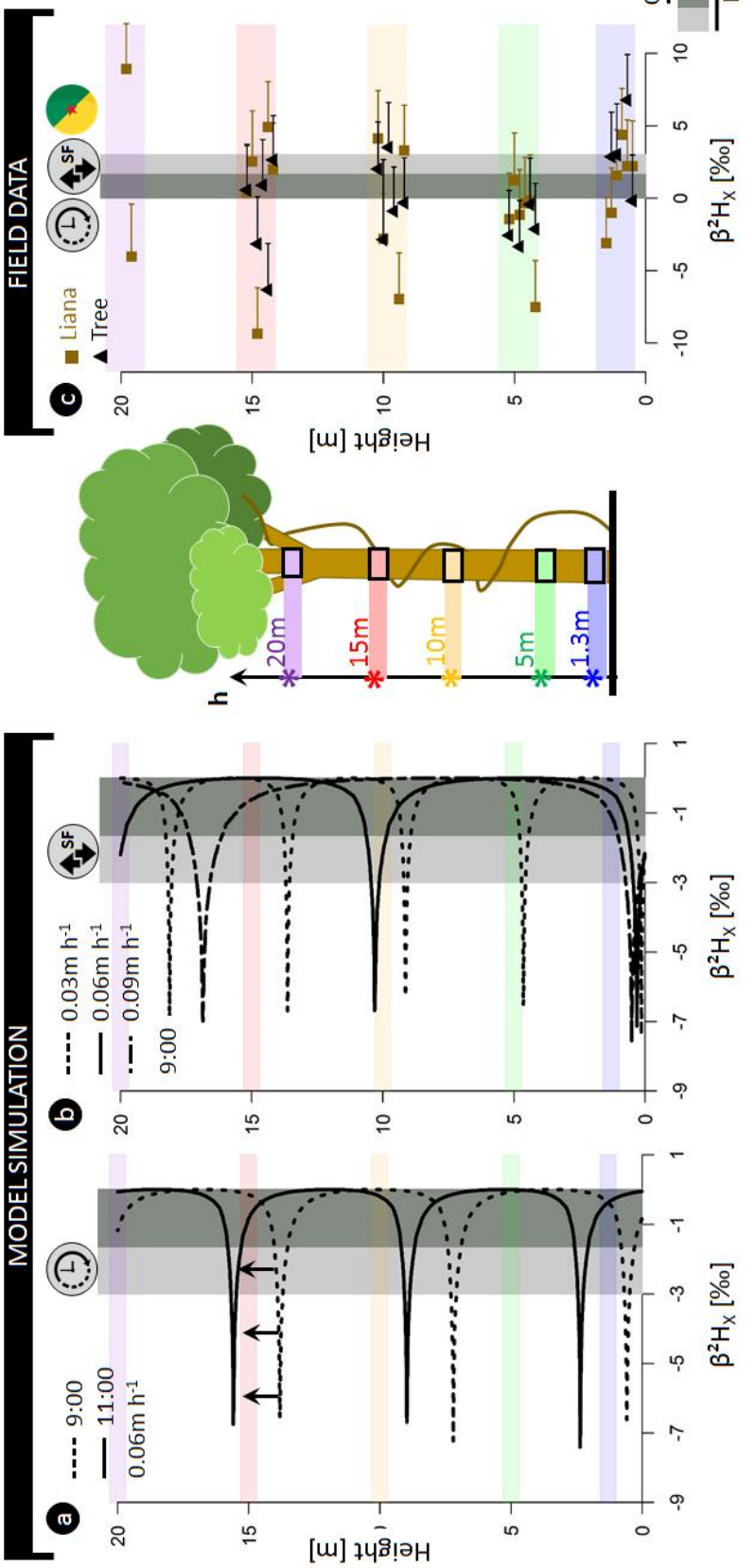

SWIFT MODEL
a
h
δ²H_X
SF
Analysis B
Analysis A3
Analysis A2
Analysis A1
A_X
SF
Ψ_X,0
δ²H_X,0
i=1
i=n
Ψ_S,i
δ²H_S,i
F_i
k_S,i
k_i
k_R
z
A_R(z)
MODEL SIMULATION – "PISTON FLOW"
b
DAY 1
DAY 2
DAY 3
δ²H_X [‰]
-59
-60
-61
-62
-63
-64
-65
-66
0.00
0.02
0.04
0.06
0.08
0.10
Cum. SF [m³]
0m
1.3m
5m
10m
CI95
H_0

891

892

893 **Fig. 2. (a)** Schematic representation of the model and considered analysis detailed in the text.
894 **(b)** Simulated fluctuations in δ^2H composition of plant xylem water as a function of the
895 cumulative sap flow volume measured at various heights: stem base (0 m, black dashed), 1.3 m
896 (blue), 5 m (green) and 10 m (red). The horizontal grey colored envelope delineates the
897 acceptable variance from the stem mean according to the null model (H_0), i.e. assuming no
898 variance along the length of a lignified plant aside from potential extraction error (i.e. 3‰).
899 Herein, the dark grey envelope indicates the confidence interval comprising 95% of potential
900 extraction error (CI95).

901


MODEL SIMULATION
FIELD DATA
a
b
c
h
9:00
11:00
0.06m h-1
0.03m h-1
0.06m h-1
0.09m h-1
9:00
Liana
Tree
SF
Height [m]
β2HX [‰]
CI95
H0
20m
15m
10m
5m
1.3m

902

903

Fig 3. (a) Model outputs for model analysis A3 representing the normalized δ^2H composition of xylem water (β^2H_X) as a function of the tree height simulated for different sampling times (9:00 and 11:00). The modeled tree has an average daily sap flux density of 0.06 m h^{-1} (SF_S; ~ daily true sap flux density SF_V = 0.42 m h^{-1}). **(b)** Model outputs for model analysis B where β^2H_X in relation to stem height is shown at 9:00 a.m., but parameterized with distinct SF_S, i.e. 0.09, 0.06 and 0.03 m h^{-1} (corresponding to SF_V of 0.64, 0.42 and 0.19 m h^{-1}, respectively). The standard parameterization used for both study analysis is detailed in Table S1. **(c)** Field measurements of β^2H_X for six lianas (■) and six trees (▲). Error whiskers are the combination of potential extraction and measurement errors of the isotope analyzer. A species-specific breakdown of the field data is provided in Fig S2. The horizontal grey colored envelope in all panels delineates the acceptable variance from the stem mean according to the null model (H_0), i.e. assuming no variance along the length of a lignified plant aside from potential extraction error (i.e. 3‰). Herein, the dark grey envelope indicates the confidence interval comprising 95% of potential extraction error (CI95).

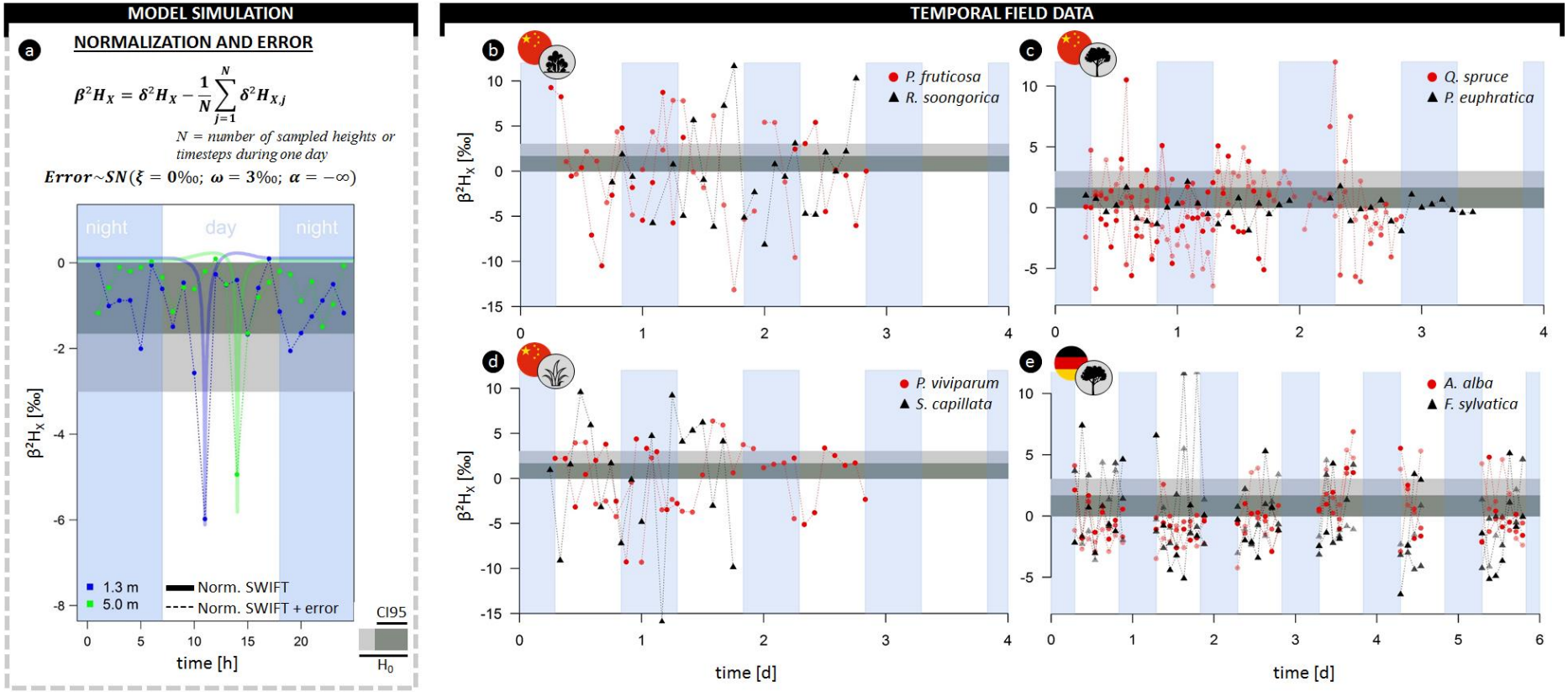

MODEL SIMULATION
a
NORMALIZATION AND ERROR
$\beta^2 H_X = \delta^2 H_X - \frac{1}{N}\sum_{j=1}^{N} \delta^2 H_{X,j}$
N = number of sampled heights or timesteps during one day
$Error \sim SN(\xi = 0‰;\ \omega = 3‰;\ \alpha = -\infty)$
night
day
night
$\beta^2 H_X$ [‰]
time [h]
1.3 m
5.0 m
Norm. SWIFT
Norm. SWIFT + error
CI95
H_0
TEMPORAL FIELD DATA
b
P. fruticosa
R. soongorica
c
Q. spruce
P. euphratica
d
P. viviparum
S. capillata
e
A. alba
F. sylvatica
time [d]

Fig 4. (a) Illustrative example of model simulations transformed in normalized δ^2H composition of xylem water (β^2H_X) at 1.3 (blue) and 5m (green) sampling height, with the formula provided. Thicker lines indicate model simulations without error, line connected dots indicate a scenario of hourly sampling with consideration of extraction error (i.e. a negative skew-normal distribution; ξ = 0‰, the scale ω = 3‰, and shape α = -∞). **(b-e)** High temporal field measurements of β^2H_X of (b) two shrubs, (c) two trees, and (d) two herb species sampled in the Heihe River Basin (northwestern China); and (e) two tree species sampled in the "Freiamt" field site in south-west Germany. The horizontal grey colored envelope in all panels delineates the acceptable variance from the stem mean according to the null model (H_0), i.e. assuming no variance along the length of a lignified plant aside from potential extraction error (i.e. 3‰). Herein, the dark grey envelope indicates the confidence interval comprising 95% of potential extraction error (CI95). A breakdown of the field data on species and individual level is provided in the supplementary figures (Fig S3-S4-S5-S6)

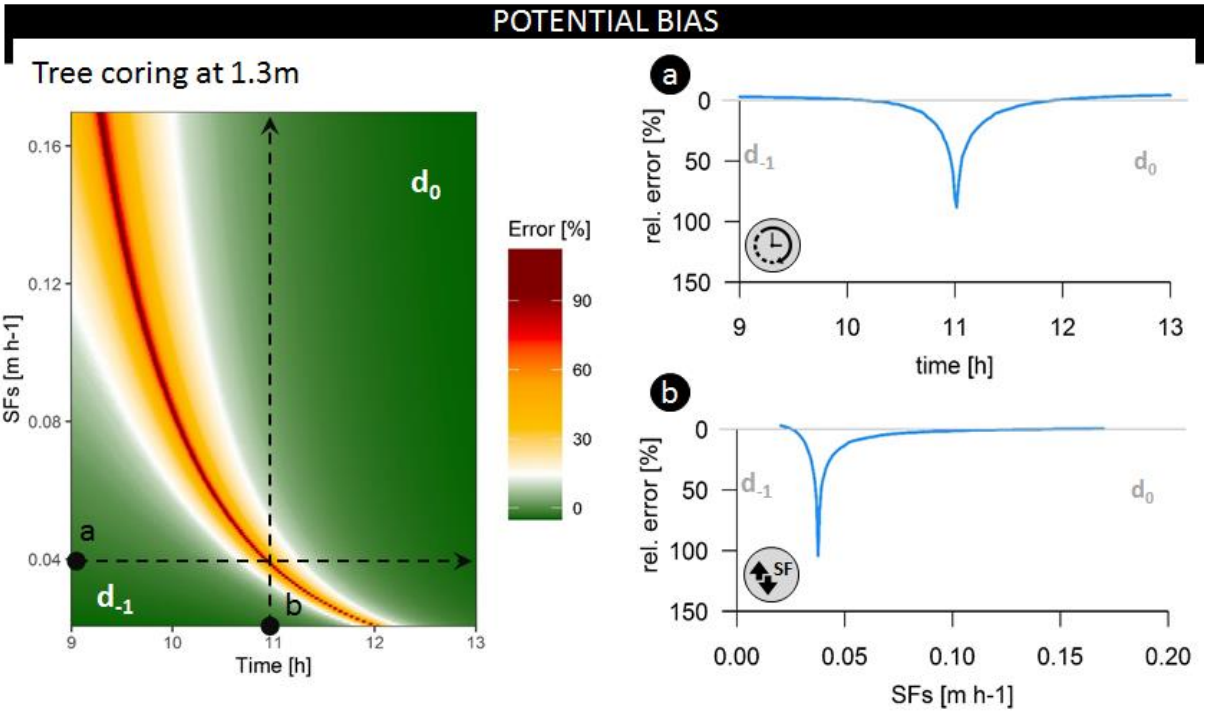
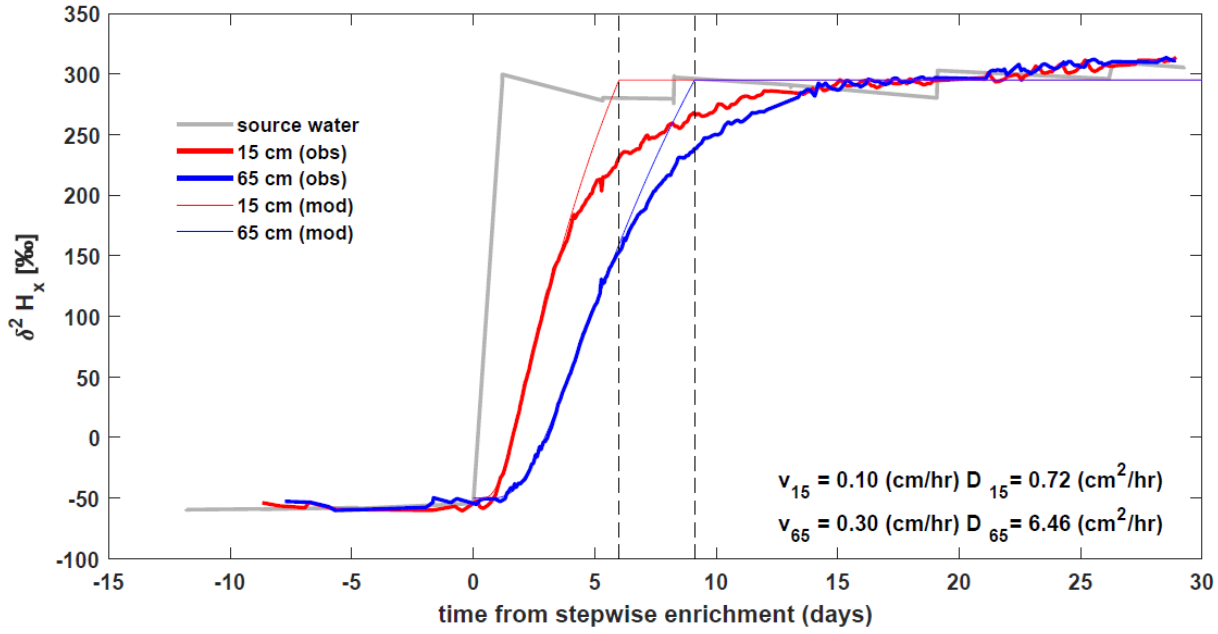


Fig 5. Relative error on the inferred average root water uptake depth (i.e. bias between the average daily and the instantaneous derived average RWU depth) at coring height of 1.3m, throughout the common sampling period (9:00 until 13:00) and over a range of potential SF_S (in m h^{-1}) – corresponding to SF_V range of 0.15–1.25 m h^{-1}. Both dotted lines describe test scenarios evaluated in the breakup panels. The dynamics in relative error when sampling **(a)** over different time steps, restricting sap flux density at 0.04 m h^{-1} (i.e. SF_V = 0.28 m h^{-1}), or **(b)** over different SF_S-values when restricting sampling time at 11 am. d_{-1} and d_0 indicate whether the derived average RWU depth error corresponds to the previous or current day of measurement.



946

947 **Fig. 6.** Basic model validation, comparing continuous *in situ* δ^2H_X measurements of a stepwise

948 ^2H enrichment experiment (Marshall *et al.*, 2020) with analytical solutions of advection-

949 diffusion equation, at heights 0.15m (—) and 0.65m (—) on a pine tree (*Pinus pinea L*). The

950 source water of the intact-root, isotopic enrichment greenhouse experiment, is presented in

951 grey. Model parameters, velocity, and diffusion were fitted by visual inspection independently

952 for the two heights to match the initial increase in isotope signature (values reported in the

953 bottom right)

Supporting Information

Article title: Causes and consequences of pronounced variation in the isotope composition of plant xylem water

Authors: Hannes P.T. De Deurwaerder, Marco D. Visser, Matteo Detto, Pascal Boeckx, Félicien Meunier, Kathrin Kuehnhammer, Ruth-Kristina Magh, John D. Marshall, Lixin Wang, Liangju Zhao, Hans Verbeeck

Method A:

Detailed description data collection French Guiana

We used data for six canopy trees and six canopy lianas sampled on two subsequent dry days (24-25 August 2017) at the Laussat Conservation Area in Northwestern French Guiana. The sampling site (05°28.604'N-053°34.250'W) lies approximately 20 km inland at an elevation of 30 m a.s.l. This lowland rainforest site has an average yearly precipitation of 2500 mm yr^{-1} (Baraloto *et al.*, 2011). Average and maximum daily temperatures of respectively 30°C and 36°C were measured during the sampling period. Sampled individuals are located in the white sands forest habitat (Baraloto *et al.*, 2011), on a white sandy ultisol with a typically high percentage of sand.

Individuals (Table A1) were selected based on the assessment of climbable tree, intactness of leafy canopy vegetation and close vicinity with one another to optimize similarity in meteorological and edaphic characteristics. Liana diameters were measured at 1.3 m from the last rooting point (Gerwing *et al.*, 2006), tree diameters were measured at 1.3 m (Table A1). Liana and tree sampling allowed highly contrasted sap flux density (Gartner *et al.*, 1990).

Sampling strategy

The stem xylem tissue of individual plants was sampled at different heights (1.3, 5, 10, 15, and 20 m where possible) at the same radial position of the stem, between 9:00 and 15:00 to assure high sap flow. Since upstream δ_{xyl} enrichment due to Péclet effect, in close vicinity to evaporative surfaces has been observed in the literature (Dawson & Ehleringer, 1993; Barnard *et al.*, 2006), sampling was restricted to coring of the main stems. The order of sampling, i.e. ascending versus descending heights, was randomized. Tree stem xylem samples were collected with an increment borer (5 mm diameter), resulting in wooden cylinders from which bark and phloem tissues were removed. Coring was performed within the horizontal plane at the predefined heights, oblique to the center of the stem to maximize xylem and minimize heartwood sampling, and slowly to avoid heating the drill head and fractionation. Taking one sample generally took between 5 and 10 minutes. Since coring lianas was not possible, we collected cross-sections of the lianas after removing the bark and phloem tissue with a knife. Soil samples were collected at different depths (0.05, 0.15, 0.30, 0.45, 0.60, 0.90, 1.20, and 1.80m) within close vicinity to the sampled individuals using a soil auger. All materials were thoroughly cleaned between sampling using a dry cloth to avoid cross-contamination. Upon collection, all samples were placed in pre-weighed glass collection vials, using tweezers, to reduce contamination of the sample. Glass vials were immediately sealed with a cap and placed in a cooling box, to avoid water loss during transportation.

Sample processing

Sample processing was performed as in De Deurwaerder *et al.* (2018). Specifically, all fresh samples were weighed, transported in a cooler, and frozen before cryogenic vacuum distillation (CVD). Water was extracted from the samples via CVD (4 h at 105°C). Water recovery rates were calculated from the fresh weight, weight after extraction, and oven-dry weight (48 h at 105°C). Samples were removed from the analysis whenever weight loss resulting from the extraction process was below 98% (after Araguás-Araguás *et al.*, 1998). Nearly all soil samples fell below this benchmark and were therefore excluded from further analysis (Fig S1). The isotope composition of the water in the samples was measured by a Wavelength-Scanned-Cavity Ring-Down Spectrometer (WS-CRDS, L2120-i, Picarro, California, USA) coupled with

a vaporizing module (A0211 High Precision Vaporizer) through a micro combustion module to avoid organic contamination (Martin-Gomez *et al.*, 2015; Evaristo *et al.*, 2016). Post-processing of raw δ-readings into calibrated δ-values was performed using SICalib (version 2.16; Gröning, 2011) and internal laboratory references , i.e. Lab1 (δ^2H: 7.74±0.4‰; δ^{18}O: 5.73±0.06‰,), Lab3 (δ^2H: -146.98±0.4‰; δ^{18}O: -20.01±0.06‰,) and quality assurance samples (δ^2H: -48.68±0.4‰; δ^{18}O: -7.36±0.06‰). Calibrated δ-values are expressed on the international V-SMOW scale.

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Table A1. Sampled liana and tree individuals, provided with their species, respective diameter at breast height (DBH, in cm) and their δ^2H and $\delta^{18}O$ ranges (in ‰, VSMOW) measured per individual.

Code	Growth form	DBH [cm]	Family	Species name	δ^2H_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

Method B:

Exploring the effect of diffusion on xylem transport of isotopes

The current version of the model assumes a negligible impact of diffusion on the variance in the isotopic composition of the xylem water in the stem. Here, the validity of this assumption is discussed in more detail. We will use analytical and numerical solutions of the advection-diffusion equation to simulate the transport of isotope within the xylem, followed by a short discussion.

Theory

One-dimensional solute flux (J) of a solute concentration (C) through a pipe can be expressed as the sum of the advection and diffusion processes:

$$J = uC + q \quad (1)$$

where u is the fluid flow velocity and q the diffusion flux.

The one-directional diffusion flux along the direction x can be expressed by Fick's law:

$$q = -D\frac{\partial C}{\partial x} \quad (2)$$

where D ($m^2\ s^{-1}$) is the diffusion constant. The mass conservation can be written:

$$\frac{\partial C}{\partial t} = -\frac{\partial J}{\partial x} \quad (3)$$

The diffusion equation

Assuming no flow ($u = 0$) and inserting (2) into (3) we obtain:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \quad (4)$$

Solutions of (4) for an instantaneous point source can be given in the form

$$C(x,t) = \frac{M}{\sqrt{4\pi Dt}} exp\left(-\frac{x^2}{4Dt}\right) \quad (5)$$

where M is the mass of solute injected uniformly across the cross-section of the pipe at $x = 0$. Using the superimposition principle, we can also derive the solution for the one-dimensional stagnant case (an initial step function concentration without advection) as

$$C(x,t) = \frac{C_0}{2} erfc\left(\frac{x}{\sqrt{4\pi Dt}}\right) \quad (6)$$

where C_0 is the initial concentration at $x < 0$ and erfc is the complementary error function.

Advection-diffusion equation

In the case of flow with velocity, (4) is modified as:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} + u\frac{\partial C}{\partial x} \quad (7)$$

The solution for constant concentration at $x = 0$ with initial zero concentration on a semi-infinite domain, i.e.

$$\begin{cases} C(x,0) = 0, & x > 0 \\ C(0,t) = C_0, & t > 0 \end{cases} \quad (8)$$

is given by (Ogata & Banks, 1961):

$$C(x,t) = \frac{C_0}{2}\left(erfc\left(\frac{x-ut}{\sqrt{4\pi Dt}}\right) + exp\left(\frac{xu}{D}\right) erfc\left(\frac{x+ut}{\sqrt{4\pi Dt}}\right)\right) \quad (9)$$

This solution can describe the dynamic of a solute concentration along the xylem under constant velocity, with a fixed concentration at the inlet point.

Numerical solutions

Solutions for problems with different boundary conditions and variable velocity are not available. In order to investigate the case with periodic concentrations at the inlet of the pipe and periodic velocity we used numerical solutions of the advection-diffusion equation

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} + u_0 f(t)\frac{\partial C}{\partial x} \quad (10)$$

where $f(t)$ is a periodic function. We used the wrapped normal distribution defined as

$$f(t) = \sum_{i=-100}^{i=100} \exp\left[\frac{\left(\frac{2\pi t}{24} - \pi - 2\pi k\right)^2}{2\sigma^2}\right] \quad (11)$$

The boundary conditions at the inlet and outlet are defined as

$$\begin{cases} C = (C_{max} + C_{min})g(t) + C_{min} & x = 0, t > 0 \\ \frac{\partial C}{\partial t} = 0 & x = H, t > 0 \end{cases} \quad (12)$$

where $g(t)$ is another periodic function defined as

$$g(t) = \sum_{i=-100}^{i=100} \exp\left[\frac{\left|\frac{2\pi t}{24} - \pi - 2\pi k\right|^3}{2\sigma^3}\right] \quad (13)$$

The third power in (13) was chosen to match the diurnal cycle of the isotopic concentration at the tree base obtained by SWIFT. The equation was solved using the function pdepe implemented in Matlab (R2019a), explicitly designed to solve initial-boundary value problems for parabolic-elliptic partial differential equations in 1-D (Skeel & Berzins, 1990).

Unfortunately, numerical solutions of the advection-diffusion equation suffer numerical oscillation for values of the Péclet number greater than one (Zienkiewicz *et al.*, 2000), so results are presented for values of diffusivity 50, 100, 200 and 400 cm^2 hr^{-1}. These values are much larger than the diffusivity of heavy water and they will produce stronger smoothing.

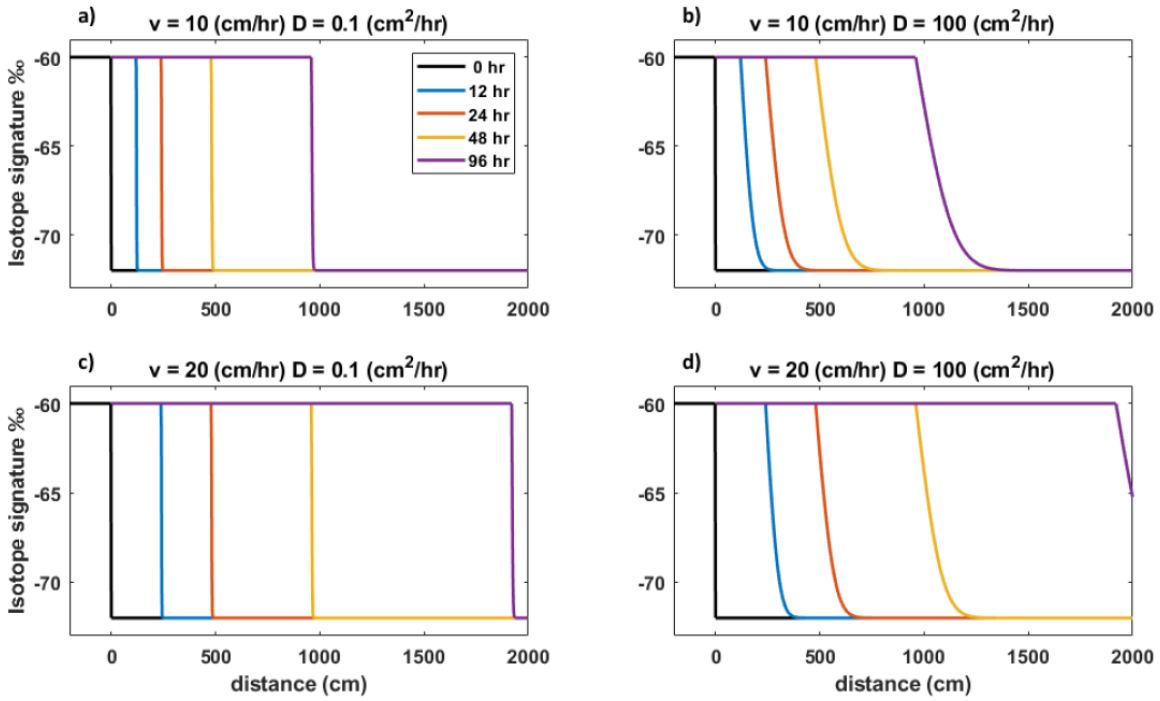

Fig B1: Analytical solutions of advection-diffusion equation on a semi-infinite 1-D domain (Eq. (9)) with 12 ‰ step-change in isotope signature for different values of flow velocity and diffusivity. The plots show the impact of diffusion on the isotopic composition of xylem water. Colored lines show the solution at different time intervals: 0, 12, 24, 48, and 96 hr. Note that the values of diffusivity are much higher than these reported for heavy water (e.g. D=0.1 cm^2 h^{-1}; Meng et al., 2018)

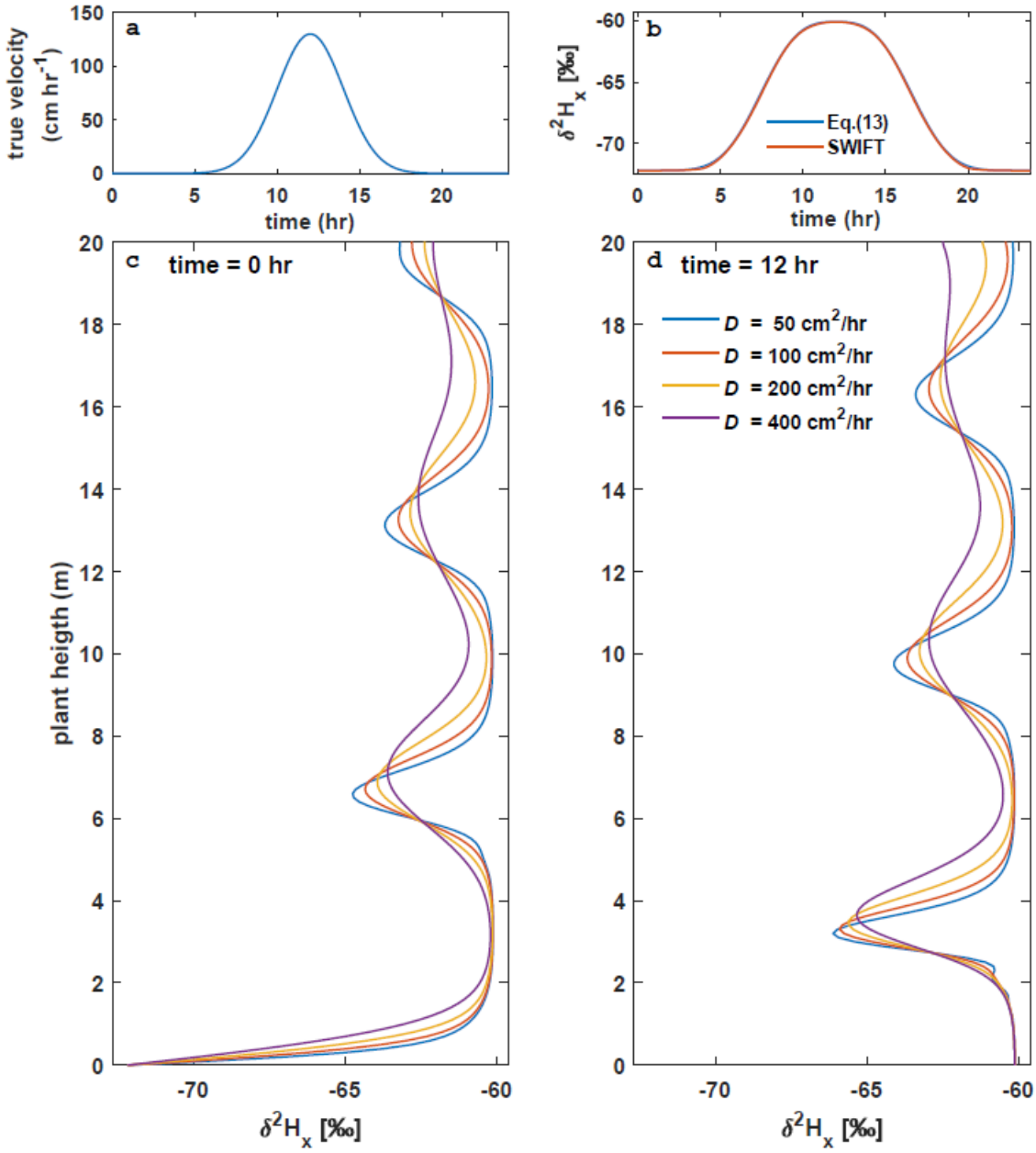

Fig B2: Numerical solutions of advection-diffusion equation on a finite 1-D domain (Eq. (10-13)) with 12 ‰ step-change in isotope signature for different values of diffusivity along the length of the xylem. The periodic forcing used in the simulations are shown in panel a and b. Panels c and d show the solutions for two different time of the day. Colored lines show the solution at different diffusivity (see legend in d). Note that the values of diffusivity are much higher than these reported for heavy water (e.g. D=0.1 $cm^2\ h^{-1}$; Meng et al., 2018).

Results and Discussion

The diffusivity of ^2H in water depends on temperature: at 20 ^oC is D = 6.87 10^{-2} cm^2 hr^{-1}, at 40 ^oC is D = 1.37 10^{-1} cm^2 hr^{-1} (Meng et al., 2018). Another process that can cause substantial mixing is the random movement of particles in the xylem network. Within each vessel, the flow is laminar, but in vessels with a larger diameter, velocity is higher than in vessels with a smaller diameter. According to the Hagen–Poiseuille law, the flow is proportional to the fourth power of diameter (hence, the velocity is proportional to diameter square). Therefore, the variable velocity experienced by the particles in the xylem network can generate substantial random motion in the transport of a solute in a similar manner of diffusion in a porous media.

Molecular diffusivity results in a relatively negligible impact of diffusion on the variance in ^2H when high sap flux densities are considered, as shown in Fig B1. For example, for diffusivity of 0.1 cm^2 hr^{-1}, after 96 hours, diffusion results in smearing in a range ± 10cm (Fig. B1a). The case with a flow velocity of 25 cm hr^{-1}, comparable to the velocity of sap in xylem, shows that the transport of the solute is minimally affected by diffusion (Fig B1 a and c). In order to appreciate the effect of diffusion, the diffusivity needs to increase three orders of magnitude (Fig B1 b and d). However, because homogenization increases with time, the impact of diffusion on δ^2H dynamics can be non-negligible for very low sap flux velocities.

Numerical solutions with the periodic forcing (Fig B2 a and b), show that for high values of diffusivity there could be a substantial smoothing in the peak (Fig B2 c and d). The smoothing progress along the path-length of the flow. However, note that a very high value of diffusivity (>400 cm^2 hr^{-1}) is required for complete homogenization above 10 m.

For the general application to isotope transport in xylem with variable input concentrations and variable sap flow velocity, diffusion can cause a smoothing of the peak and a consequent increase in the width of the δ^2H_X-baseline drop. Therefore, the probability of sampling a non-representative section within this δ^2H_X-baseline might increase, which means that neglecting diffusion could lead towards a conservative assessment of the bias in RWU estimates. However, the minimal reduction of the peak in δ^2H_X over time might lead to reducing the variability in time and space compared to the case with no diffusion. In conclusion, while diffusion does affect both the absolute range of δ^2H_X variance and the width of the δ^2H_X-baseline drop (i.e. increased probability of extracting biased samples), the impact is small in the lower part of the tree and over the timeframe and sap flow flux considered in this study. Hence, for this study, diffusion will not result in the complete homogenization of the δ^2H_X along the length of the studied trees, consistent with empirical datasets (Fig 3c, Fig S2.).

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Method C:

A detailed description of the performed transport dynamics and sensitivity analyses.

Transport dynamics

The intact-root greenhouse experiment of Marshall *et al.* (2020) allows assessment if other processes besides molecular diffusivity might contribute to isotope transport through the plant, especially when very low sap flow velocities are considered. Specifically, the experiment follows the impact of a stepwise ^{2}H enrichment of the source water, i.e. from δ^2H=-59.28 ± 0.24 ‰ to δ^2H=290.57 ± 3.08‰ (see Fig 6), on the δ^2H_X dynamics in a pine tree (*Pinus pinea L.)*. The tree was placed in a large pot, with the root system fully submerged in aerated water (using mini-pumps) and subjected to artificial light conditions (12h light, 12h dark, light transition at 7:00 o'clock). δ^2H_X was monitored continuously and *in situ* at two sampling heights, 0.15 cm, and 0.65 cm, respectively, using a novel borehole technique. Concomitant, sap flow velocity was measured using a sap flow sensor (heat pulse velocity sensor, Edaphic Scientific, Australia), installed at 0.85m height, and perpendicular to the upper borehole. For specific details of this experiment, we refer to Marshall *et al.* (2020).

In this setup, roots are submerged in a uniform isotopic solution, so the SWIFT model parameterization of soil and root is not necessary. The isotopic composition of the source water will, therefore, almost instantly reflect the δ^2H at the stem base. The impact of diffusion could not be considered negligible as sap flow velocities are very low (daily mean SF_V = 0.97 ± 0.39 cm h^{-1}) and the experiment lasted out 38 days before equilibrium was reached between the δ2HX of the source water and the δ2HX in both boreholes. For simulating the isotopic dynamics, we used an analytical solution of the advection-diffusion, as described in supplementary methods B, coupled to the SWIFT model. Model parameters, velocity, and diffusion were fitted by visual inspection independently for the two heights to match the initial increase in isotope signature.
Note that the studied tree shows strong tapering (diam. at 0.15cm = 9.9cm; diam. at 0.65cm = 8.0cm), causing an acceleration of the sap flow along the pathway length as a same volume of water is propelled through a diminishing cross-area. This is also reflected in the allocated velocity parameters.

Sensitivity analyses

We first assessed model sensitivity to (bio)physical variables by modifying model parameters of soil type, sap flow, and root properties as compared to the standard parameterization (given in Table S1). The following sensitivity analyses were considered:

Soil type: The soil moisture content overall soil layers ($\theta_{S,i,t}$) can be deduced from the considered Meißner et al. (2012) $\Psi_{S,i,t}$ profile (see Fig. S8 and Table S1) using the Clapp & Hornberger (1978) equation:

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

Where θ_{sat}, Ψ_{sat} and b are soil-type specific empirical constants that correspond to sandy loam soil textures in the standard model parameterization (Clapp & Hornberger, 1978). The derived soil moisture profile ($\theta_{S,i,t}$), in turn, then provides a basis to study the impact of other soil textures. A new soil texture specific $\Psi_{S,i,t}$ profile can then be deduced by using θ_{sat}, Ψ_{sat} and b values corresponding to different soil texture types (values from Table 2 of Clapp & Hornberger (1978)). This enabled us to study $\Psi_{S,i,t}$ profiles for four distinct soil types, i.e. (i) sand, (ii) loam, (iii) sandy clay and (iv) clay soils, in relation with the original silt loam $\Psi_{S,i,t}$ profile.

Volume of water uptake: We varied the total diurnal volume of water taken up by the tree. New SF_t values are scaled using algorithms from the literature that provide an estimate of the daily sap flow volume of a tree based on its DBH (Andrade *et al.*, 2005; Cristiano *et al.*, 2015).

Root conductivity: We varied the root membrane permeability (k_R) to match multiple species-specific values found in the literature (Sands *et al.*, 1982; Rüdinger *et al.*, 1994; Steudle & Meshcheryakov, 1996; Leuschner *et al.*, 2004).

The second set of sensitivity analyses test the impact of root hydraulics, sap flux density, and sampling strategies on the sampled δ^2H_X. We obtained 1000 samples per parameter from corresponding distributions and ranges (given in Table S2) with a Latin hypercube approach (McKay *et al.*, 1979; McKay, 1988). This is a stratified sampling procedure for Monte Carlo simulation that can efficiently explore multi-dimensional parameter space. In brief, Latin Hypercube sampling partitions the input distributions into a predefined number of intervals (here 1000) with equal probability. Subsequently, a single sample per interval is extracted in an effort to evenly distribute sampling effort across all input values and hence reduce the number of samples needed to accurately represent the parameter space.

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Figures and tables

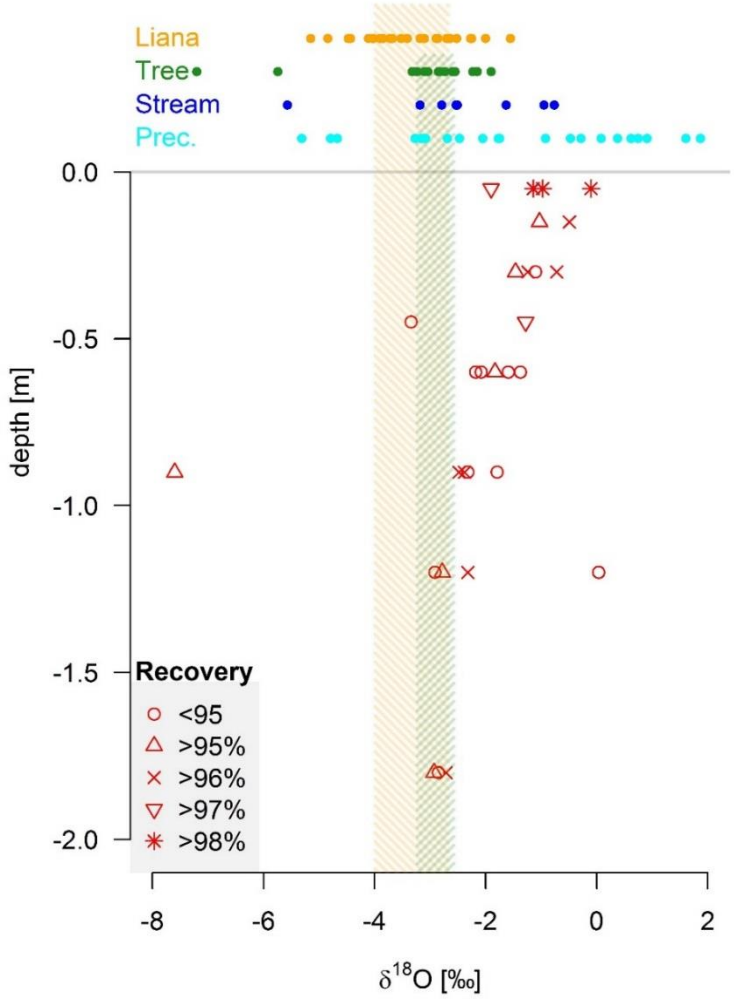


Fig. S1. Oxygen isotope composition ($\delta^{18}O$, in ‰ V-SMOW) of bulk soil water sampled at different depths (red), xylem water of lianas (orange) and trees (green), and from bulk stream (blue) and bulk precipitation water (cyan) in Laussat, French Guiana. Different soil $\delta^{18}O$ composition symbols indicate the extraction recovery rates, where 98% presents the generally pursued benchmark. Shaded areas show the Q25-Q75 intervals for lianas and trees in orange and green respectively.

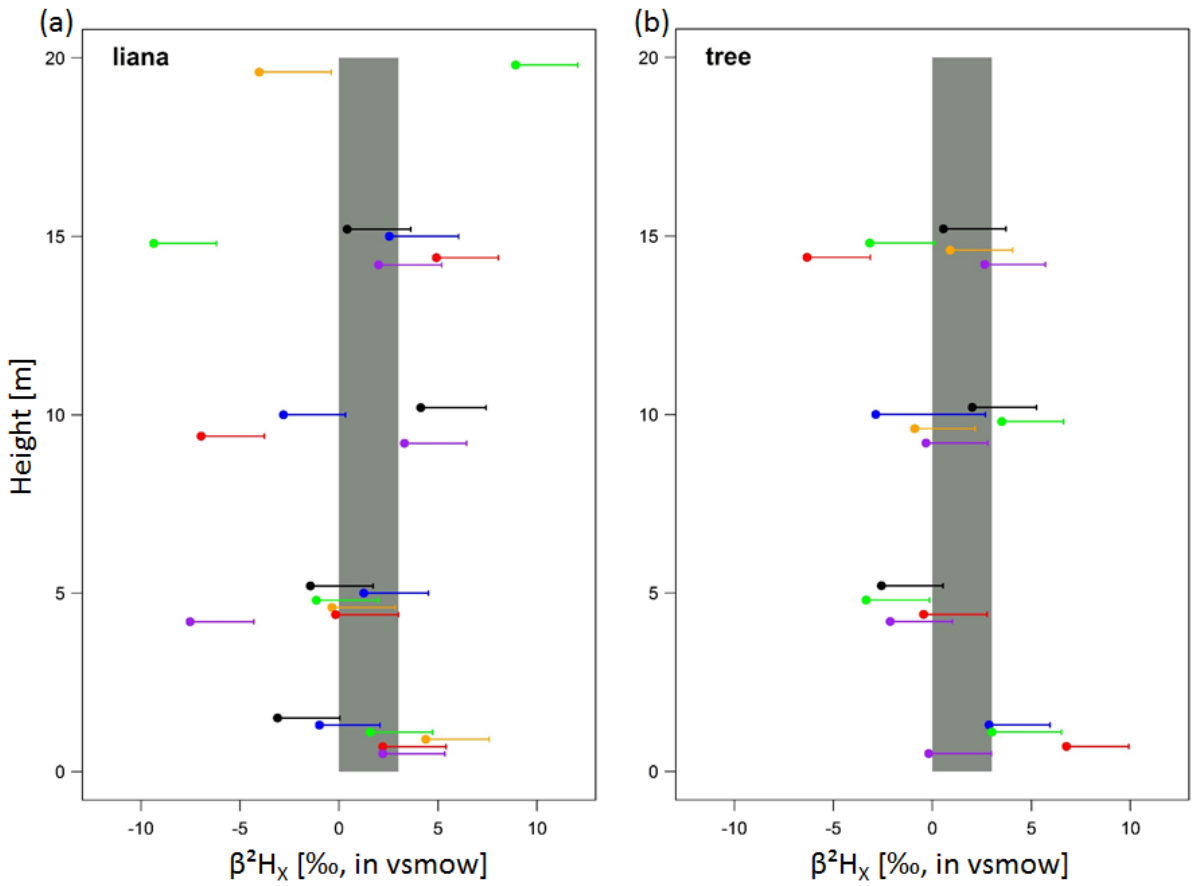


Fig. S2. Field measurements of normalized intra-individual δ^2H_X (β^2H_X) for six lianas (panel a) and six trees (panel b). Individuals are provided in different colors; liana species: ■ *Coccoloba sp.*, ■ sp.2, ■ sp.3, ■ *cf. rotundifolium Rich.*, ■ *Maripa cf violacea*, ■ *Maripa sp.*; tree species: ■ *Coussapoa sp.*, ■ *Vouacapoua americana*, ■ *Erisma nitidum*, ■ *Micropholis guyanensis*, ■ *Tapirira guyanensis*, ■ *Albizia pedicellaris*. Error whiskers are the combination of potential extraction and measurement errors of the isotope analyzer. The former presents a positive skew-normal distribution $SN_{empirical}(\xi = 0‰, \omega = 3‰, \alpha = +\infty)$. 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.

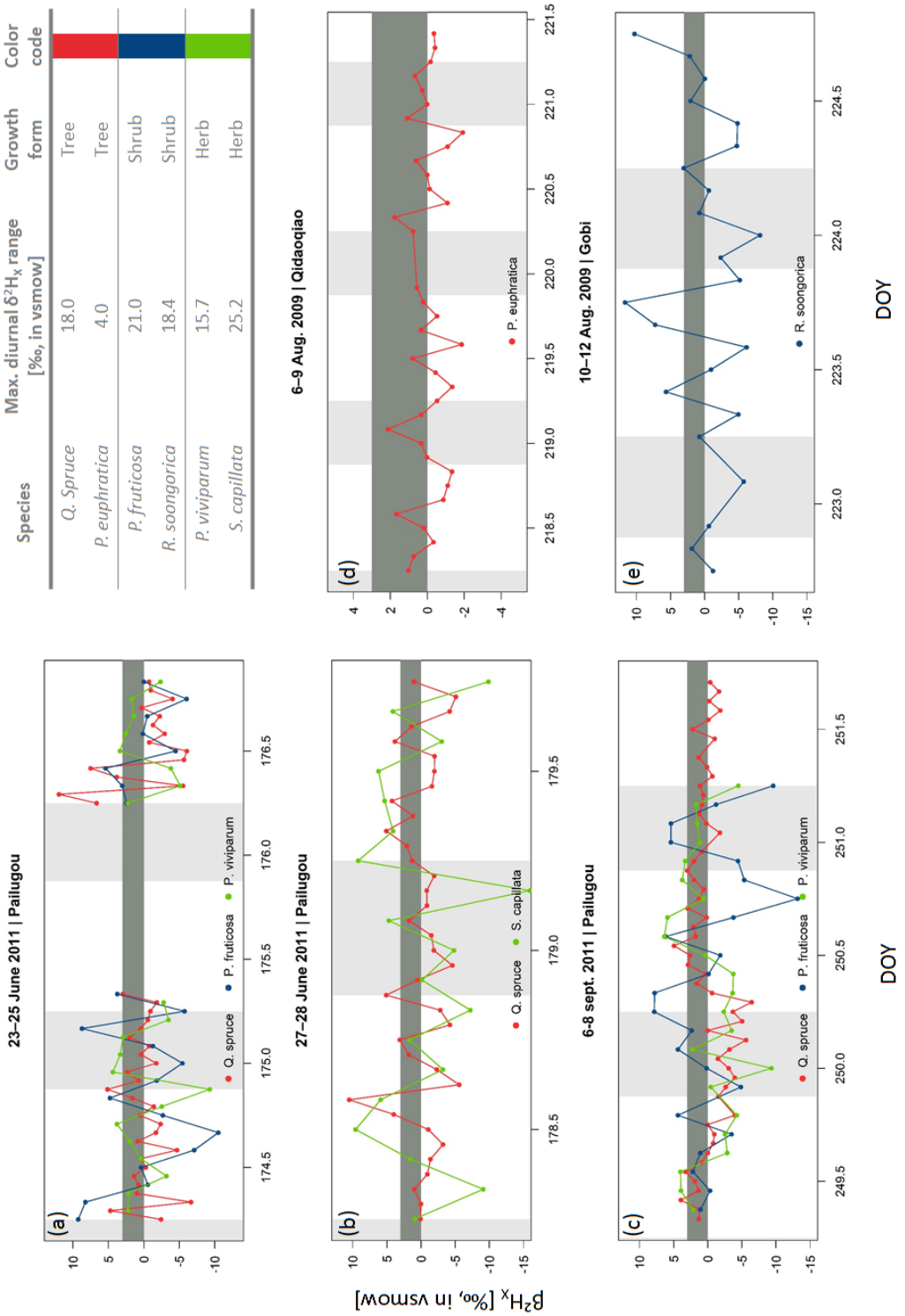


Fig. S3. High temporal field measurements of normalized δ^2H composition of xylem water (β^2H_X) of two trees (red, stem samples), two shrubs (blue, stem samples) and two herbs (green, root samples) species sampled in the Heihe River Basin (northwestern China) shown for the respective measurement periods. Timing and location of sampling are provided in the panel titles. Horizontal dark grey colored 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. Light grey vertical envelopes mark the nighttime periods. The table provides the maximum measured diurnal δ^2H_X range per species.

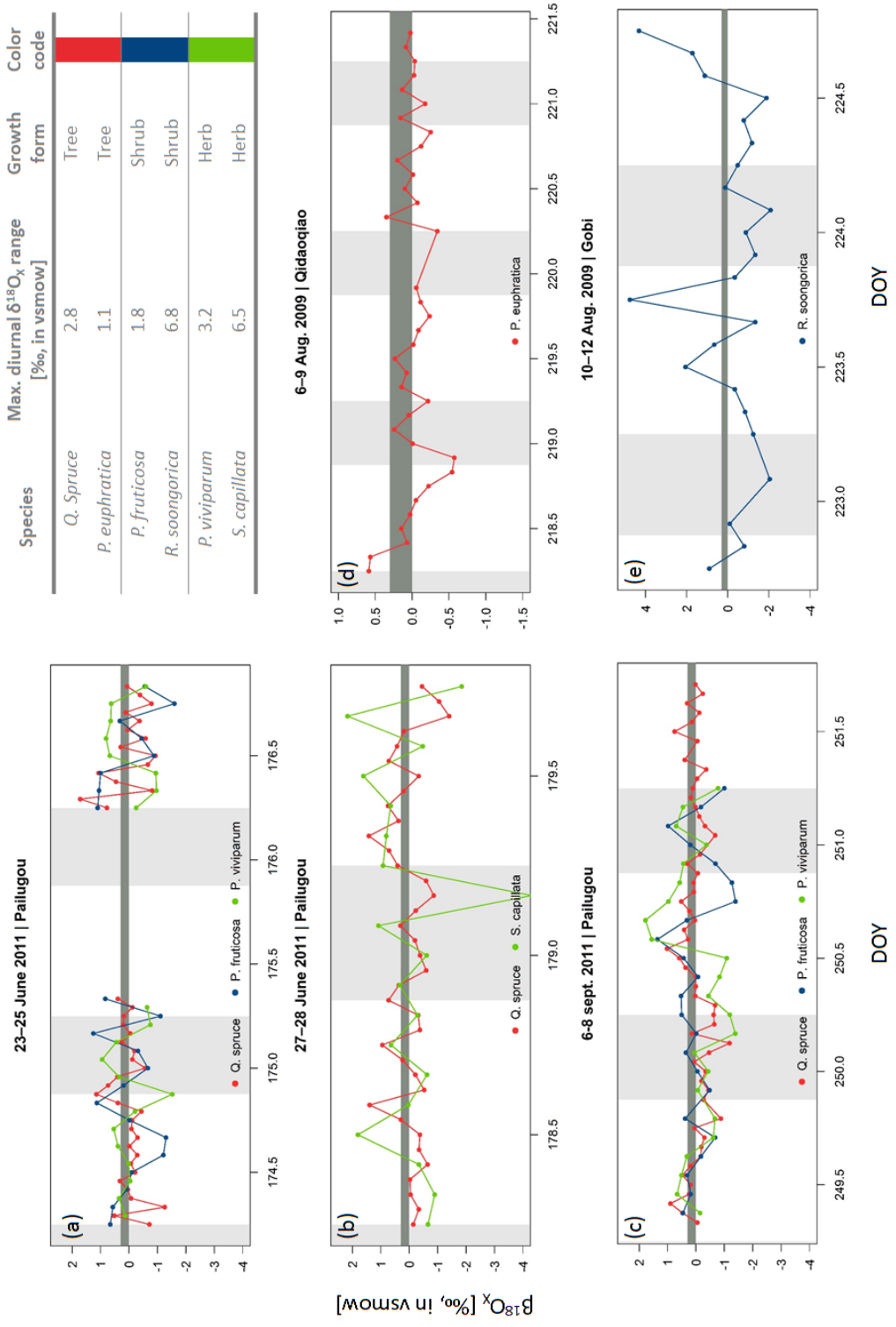


Fig. S4. High temporal field measurements of normalized $\delta^{18}O$ composition of xylem water ($\beta^{18}O_X$) of two trees (red, stem samples), two shrubs (blue, stem samples) and two herbs (green, root samples) in the Heihe River Basin (northwestern China) shown for the respective measurement period. Timing and location of sampling are provided in the panel title. Horizontal dark grey colored envelope delineates the acceptable variance from the stem mean (i.e. 0.3‰) according to the standard assumption of no variance along the length of a lignified plant. Light grey vertical envelopes mark the nighttime periods. The table provides the maximum measured diurnal $\delta^{18}O_X$ range per species.

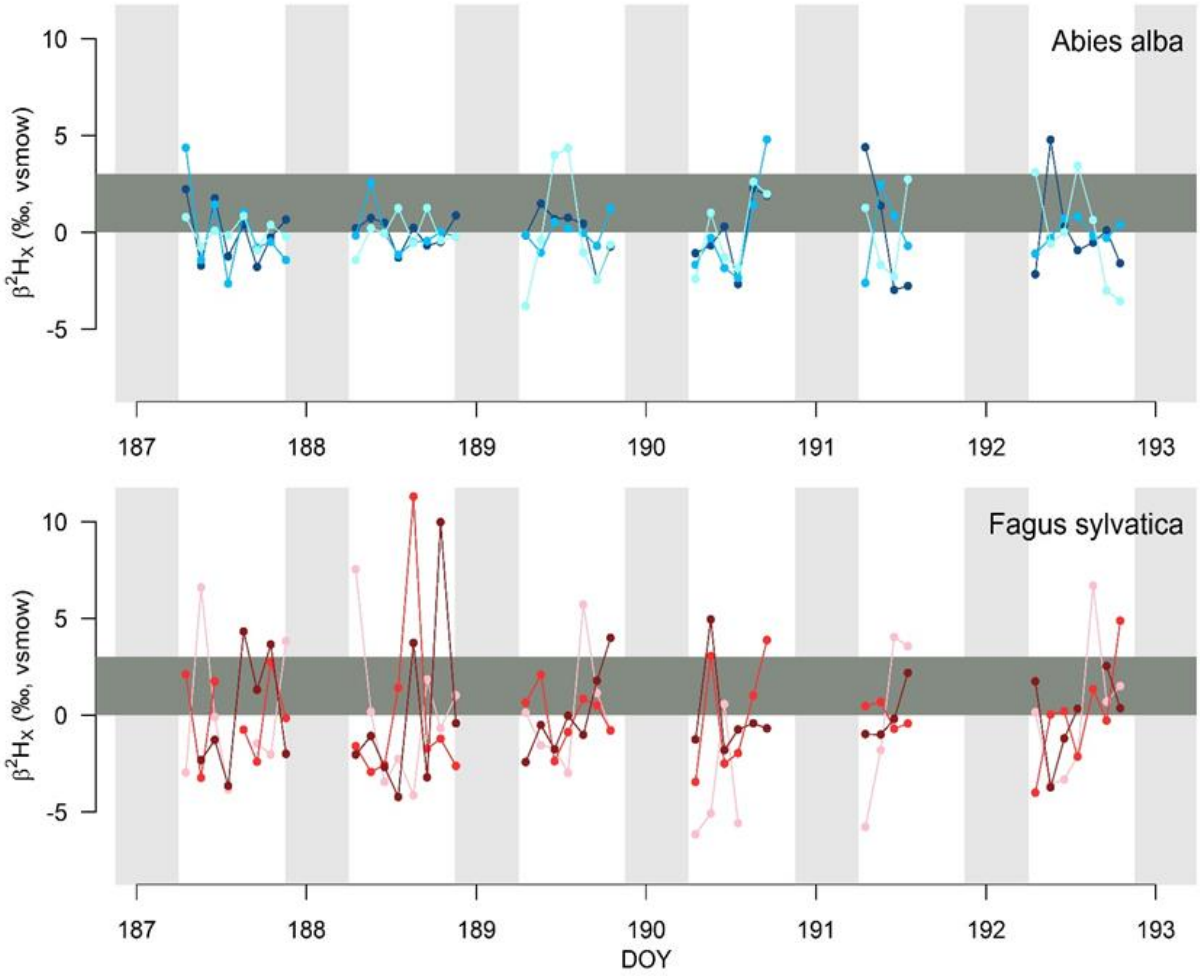


Fig. S5. High temporal field measurements of normalized δ^2H composition of xylem water (β^2H_X) of three *Abies alba* individuals (blue, branch samples) and three *Fagus sylvatica* individuals (red, branch samples) sampled during a drought period in July 2017 in the "Freiamt" field site in south-west Germany. Horizontal dark grey colored 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. Light grey vertical envelopes mark the nighttime periods.

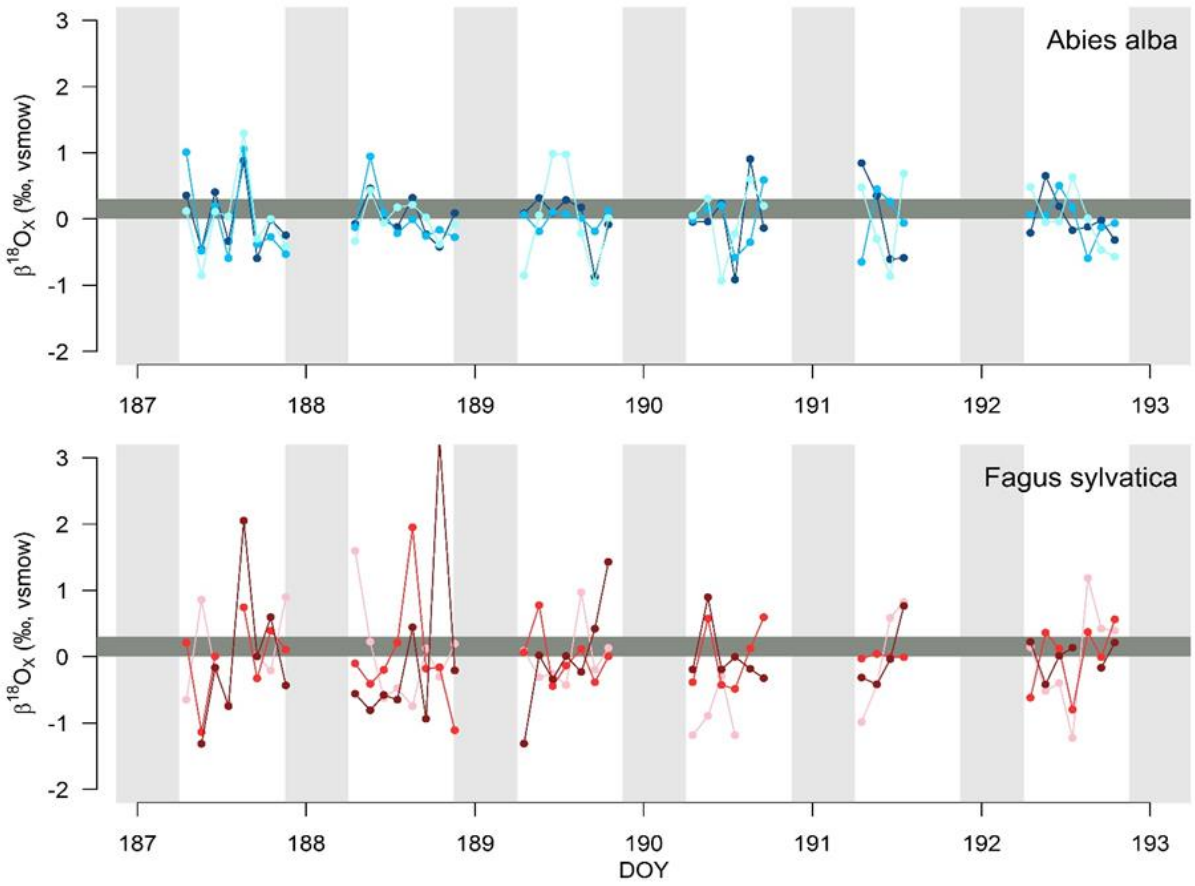
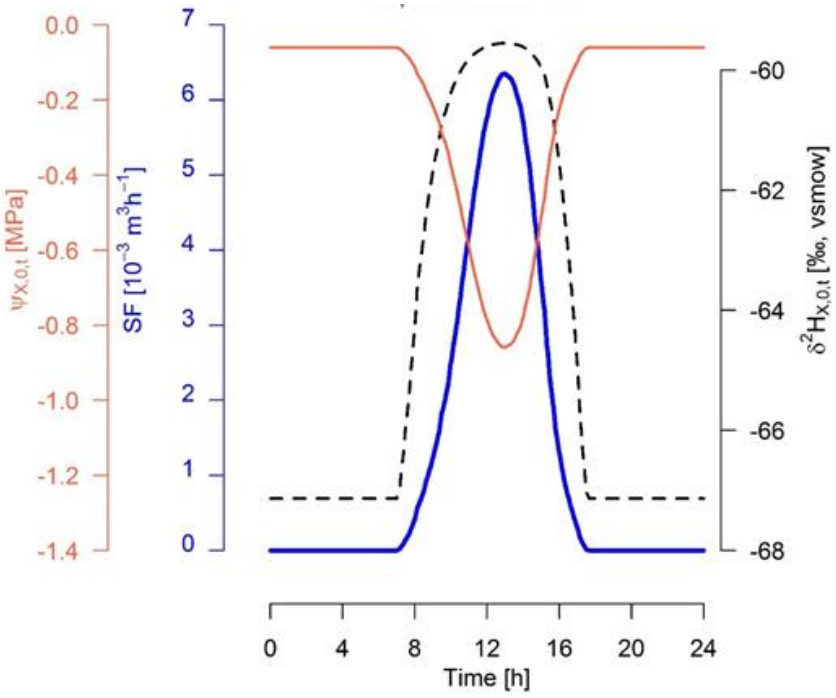


Fig. S6. High temporal field measurements of normalized $\delta^{18}O$ composition of xylem water ($\beta^{18}O_X$) of three *Abies alba* individuals (blue, branch samples) and three *Fagus sylvatica* individuals (red, branch samples) sampled during a drought period in July 2017 in the "Freiamt" field site in south-west Germany. Horizontal dark grey colored envelope delineates the acceptable variance from the stem mean (i.e. 0.3‰) according to the standard assumption of no variance along the length of a lignified plant. Light grey vertical envelopes mark the nighttime periods.



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391

392 **Fig S7:** Sap flow rate (*SF*, blue line), δ^2H composition of xylem water at stem base ($\delta^2 H_{X,0,t}$
393 black dashed line) and water potential at stem base ($\Psi_{X,0,t}$, red line) shown for a single day.
394

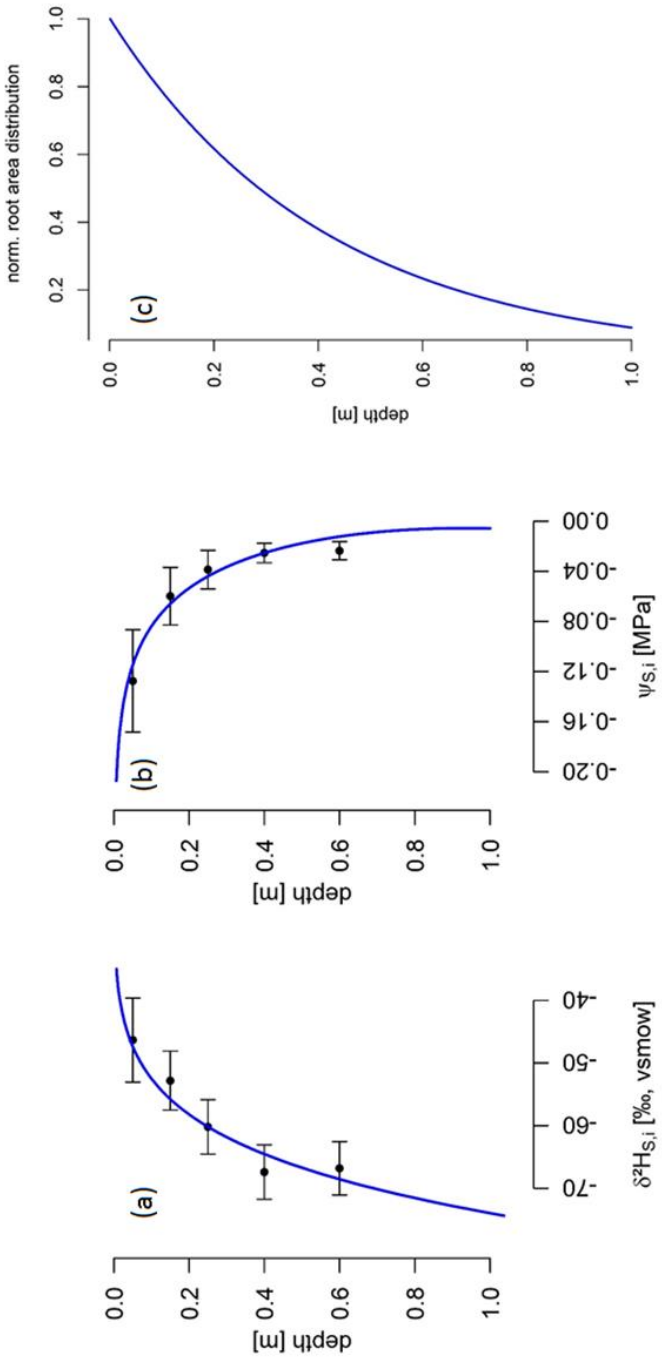
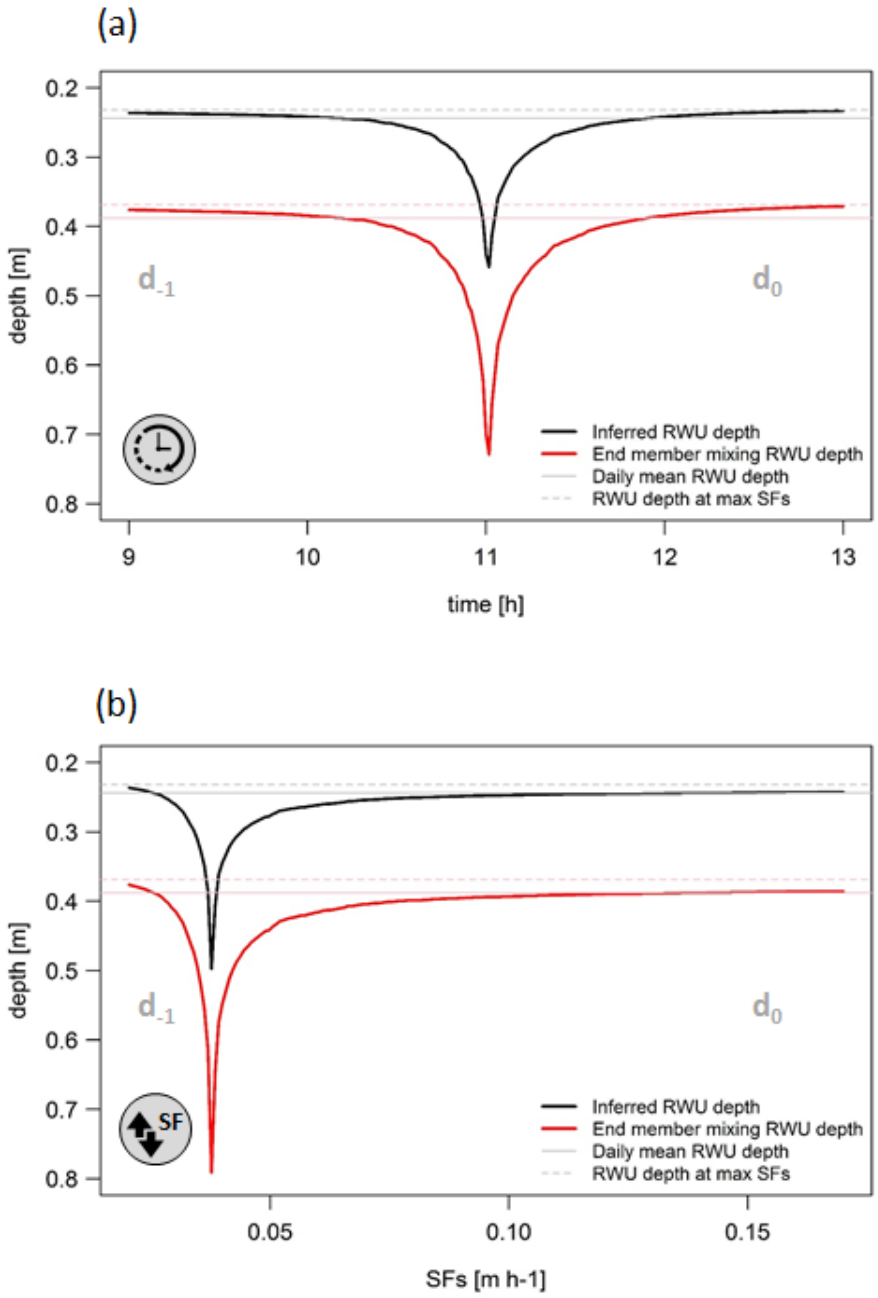


Fig. S8. (a) Soil depth profile of the deuterium isotope composition of soil water ($\delta^2H_{S,i}$), data from Meißner et al. (2012). **(b)** Soil water potential ($\Psi_{S,i}$) over the soil depth, data from Meißner et al. (2012). **(c)** The relative absorptive root area distribution with soil depths adapted from Jackson et al. (1995) and normalized to the topsoil. All equations and corresponding parameters for the fitted curves can be found in Table S1.



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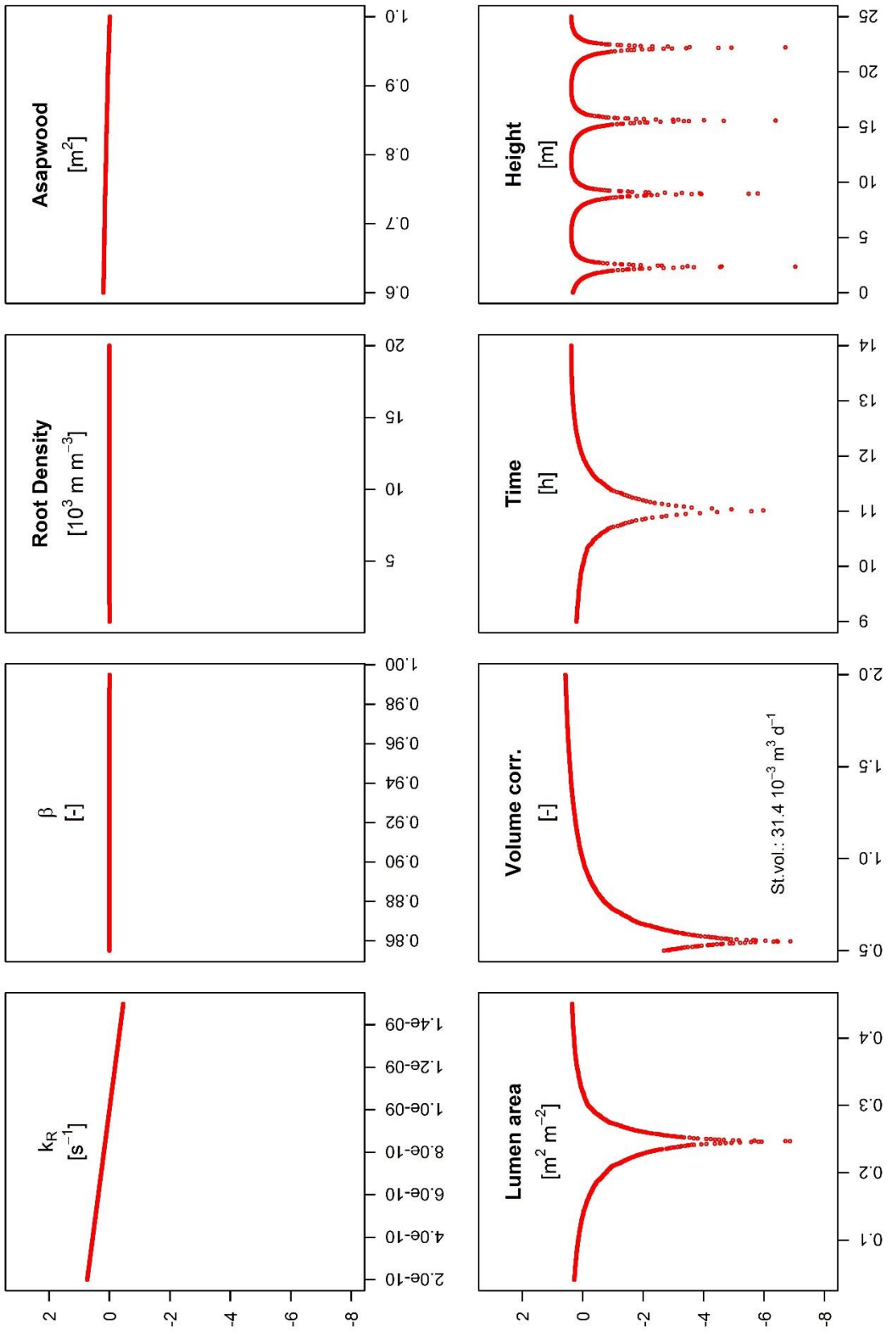
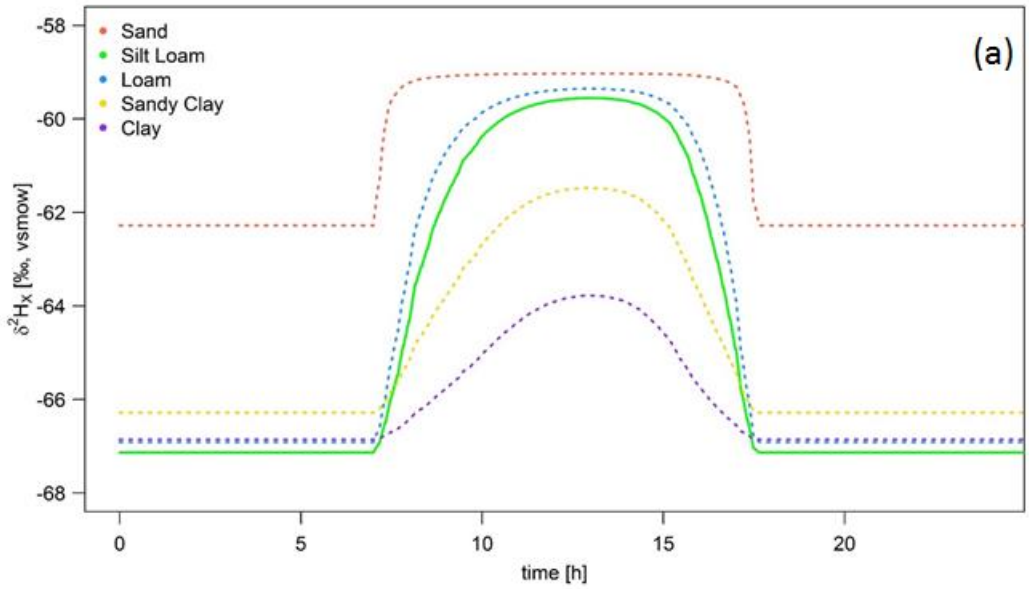
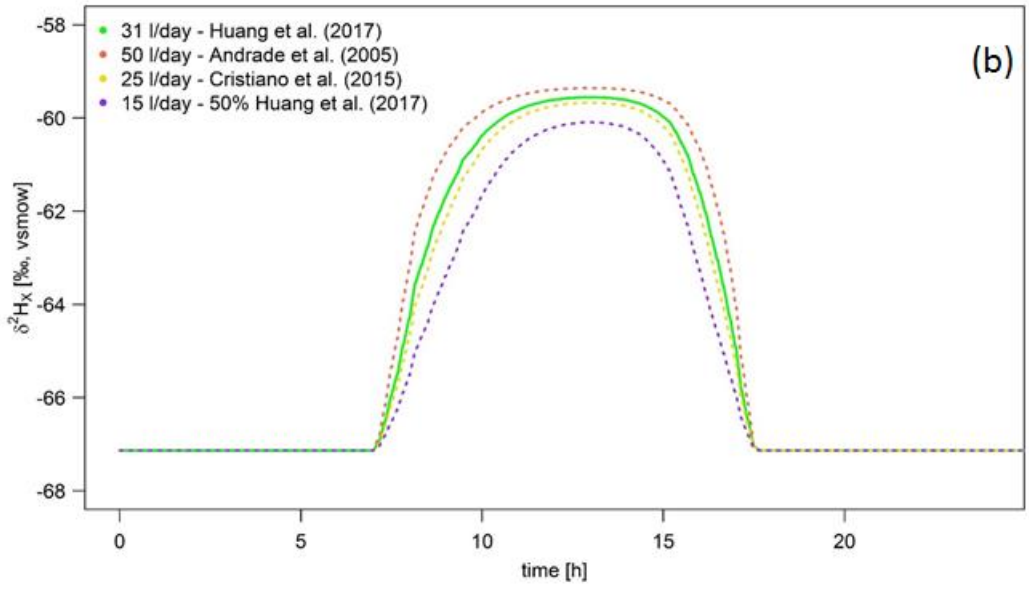
402 **Fig. S9.** Differences between the root water uptake (RWU) depth derived from using either the
403 direct inference (black line) or the end member mixing (red line) approach. **Panel a:** The
404 derived RWU depth for a tree sampled at standard tree coring height (i.e. 1.30 m) having a sap
405 flux density (SF_S) of 0.04 m h^{-1} (i.e. SF_V = 0.28 m h^{-1}), over the common sampling period (9:00
406 until 13:00). **Panel b:** The derived RWU depth considering a tree sampled at standard tree
407 coring height (1.30 m) at 11:00, but which differs in SF_S. The grey and pink solid lines represent
408 daily mean RWU depth while the grey and pink dashed lines represent the RWU depth at peak
409 sap flow activity, respectively, for the direct inference and end-member mixing model
410 approach. d_{-1} and d_0 indicate whether the derived RWU depth error corresponds to the previous
411 or current day of measurement.

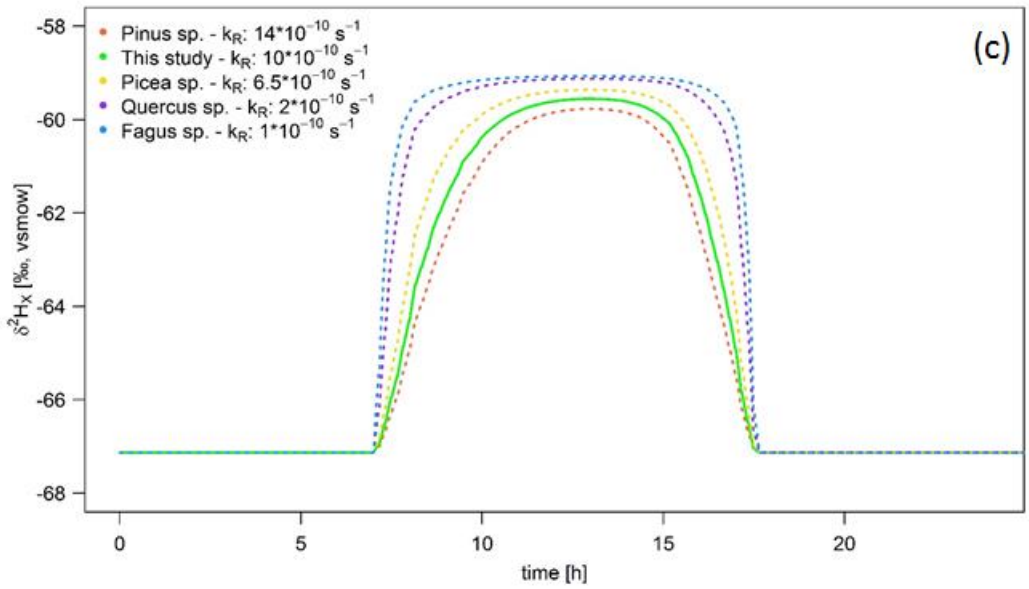

δ²Hx Bias [‰, in VSMOW]
Asapwood [m²]
Root Density [10³ m m⁻³]
β [-]
kR [s⁻¹]
Height [m]
Time [h]
Volume corr. [-]
St.vol.: 31.4 10⁻³ m³ d⁻¹
Lumen area [m² m⁻²]

412

Fig. S10. Sensitivity analysis where all parameters are varied one-at-the-time as compared to the standard parameterization (see Table S1). For each studied variable, 1000 model runs were performed, studying the resulting δ^2H_X bias in comparison with the standard run. Each time, the studied parameter value was assigned randomly from a defined probability distribution or range using a Latin Hypercube scheme (see Table S2). The effective root radial conductivity (k_R, in s^{-1}), the β (-), and root density (in 10^3 m m^3) together form an informative proxy for the soil to root resistance. The lumen fraction (in m^2 m^{-2}), sapwood area (*Asapwood*, in m²), and the total diurnal transported sap flow volume, i.e. net root water uptake (Volume corr., factor of standard run volume), provide an informative proxy for the sap flux density. (see Table S1). Time (in h) and height (in m) respectively represent the timing of sampling and the height of sample collection.


(a)
Sand
Silt Loam
Loam
Sandy Clay
Clay
-58
-60
-62
-64
-66
-68
δ²H_X [‰, vsmow]
0
5
10
15
20
time [h]


(b)
31 l/day - Huang et al. (2017)
50 l/day - Andrade et al. (2005)
25 l/day - Cristiano et al. (2015)
15 l/day - 50% Huang et al. (2017)
-58
-60
-62
-64
-66
-68
δ²H_X [‰, vsmow]
0
5
10
15
20
time [h]


(c)
Pinus sp. - k_R: 14*10^-10 s^-1
This study - k_R: 10*10^-10 s^-1
Picea sp. - k_R: 6.5*10^-10 s^-1
Quercus sp. - k_R: 2*10^-10 s^-1
Fagus sp. - k_R: 1*10^-10 s^-1
-58
-60
-62
-64
-66
-68
δ²H_X [‰, vsmow]
0
5
10
15
20
time [h]

424

Fig. S11. Model sensitivity to (bio)physical parameters. The standard model run is shown by the solid green line in all panels. **Panel a:** fixed soil moisture and depth profile in the isotope composition of soil water ($\delta^2 H_{S,i}$), but with different soil types influencing the soil conductivity and soil water potential gradient in the soil ($\Psi_{S,i,t}$). Parameterization for each soil type is derived from Clapp & Hornberger (1978). **Panel b:** Impact of altering volumes of water taken up by the plant. **Panel c:** Effect of altering values of the effective root radial conductivity (k_R) values. Values are species-specific and are derived from the literature (Sands *et al.*, 1982; Rüdinger *et al.*, 1994; Steudle & Meshcheryakov, 1996; Leuschner *et al.*, 2004). In each panel all other parameters follow the standard plant parameterization (Table S1).

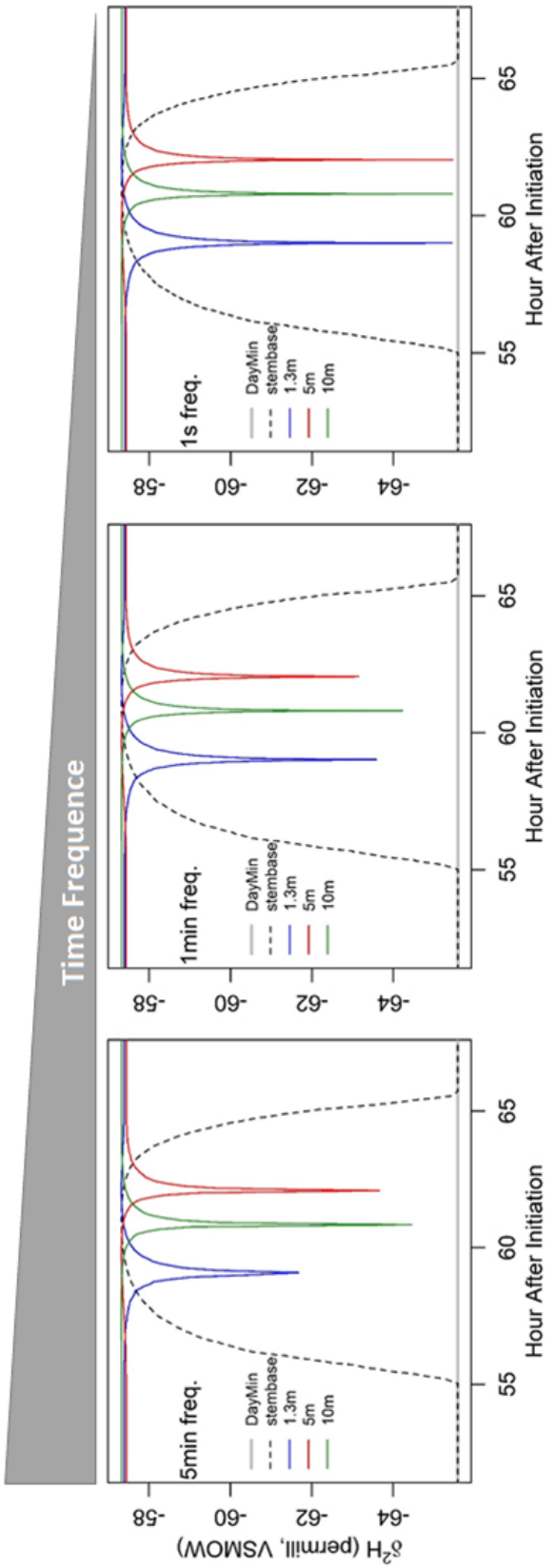


Fig. S12. Model simulations performed with varying temporal resolutions, i.e. 5min, 1min, and 1sec.

Table S1. An overview of the model standard parameterization of the present model, including sap flow, with corresponding references to literature.

Abbr.	Parameter	Unit	Value	Source
A_{Rtot}	The plants' total absorptive root area	m^2	$e^{0.88 \cdot \ln\left(\pi \cdot \left[\frac{DBH \cdot 10^2}{2}\right]^2\right) - 2}$	Čermák *et al.* (2006) $A_{Rtot} = 23.825\ m^2$
$A_{R,i}$	The absorptive root area distribution over soil layer *i*	m^2	$A_{Rtot} \cdot \beta^{100 \cdot z_i} \cdot (1 - \beta^{100 \cdot \Delta z})$	A_{Rtot} multiplied by the integrated root distribution of each soil layer adapted from Jackson *et al.* (1996)
			β =0.976	Huang *et al.* (2017)
$A_{SAPWOOD}$	Sapwood area	m^2	$\frac{1.582 \cdot [DBH \cdot 10^2]^{1.764}}{10^4}$	Meinzer *et al.* (2001)
Ax	Total lumen area	m^2	$LF \cdot A_{SAPWOOD}$	
B_i	The overall root length density per unit of soil, not necessarily limited to the studied plant.	$m\ m^{-3}$	$R_0 \cdot \beta^{100 \cdot z_i} \cdot \ln(\beta)$	Adapted from Huang *et al.* (2017) R_0= -438 688 β =0.976
DBH	Diameter at breast height	m	0.213	Huang *et al.* (2017)
$\delta^2 H_{S,i}$	Deuterium isotope composition of soil water of the sampled soil layers	in ‰, VSMOW	$a + (z_i + b)^c$	Adapted from Meißner *et al.* (2012) …a: -73.98008 …b=0.001 …c=0.148735;
Δz	The thickness of each soil layer	m	0.001	
f_t	Temporal resolution	s^{-1}	1/60	
k_R	The effective root radial conductivity	s^{-1}	10^{-9}	Huang *et al.* (2017)

Table S1 (continuation)

Abbr.	Parameter	Unit	Value	Source
$K_{S,i}$	The soil hydraulic conductivity defined per soil depth	m s^{-1}	$K_{s,max} \cdot \left(\frac{\Psi_{sat}}{\Psi_{S,i,t}}\right)^{2+\frac{3}{b}}$	Huang *et al.* (2017)
			$K_{s,max}$= 7.2·10^{-6} m s^{-1}	Clapp & Hornberger (1978) [Table 2, silt loam soil]
			Ψ_{sat}= -0.786 m H_2O	Clapp & Hornberger (1978) [Table 2, silt loam soil]
			b = 5.30	Clapp & Hornberger (1978) [Table 2, silt loam soil]
LF	Lumen fraction per unit sapwood area	m^2 m^{-2}	0.136	Zanne *et al.* (2010) [Table 2]
SF_t	Instantaneous sap flow at time t	m^3 s^{-1}		Adapted from Huang *et al.* (2017) [derived from scenario 6, day 11]
$\Psi_{S,i,t}$	Water potential at a specific soil layer depth *i* and time *t*	m H_2O	$(a + b \cdot log(z_i) - c \cdot z_i^2) \cdot CT$	Adapted from Meißner *et al.* (2012) a: 19.8455·10^{-3} b: 44.8909·10^{-3} c: 25.5594·10^{-3} CT: 101.97 (i.e. conversion factor between MPa and m H_2O)

z_i the soil depth of the i^{th} soil layer (in m)

Table S2. An overview of the defined distribution and ranges used for the sensitivity analysis whose results are displayed in Fig S10.

Model Variable	Description	Unit	Distribution	Specification
Variables that provide an informative proxy for the soil to root resistance				
k_R	The effective root radial conductivity	s^{-1}	Uniform	St.=$10 \cdot 10^{-10}$, min = $2 \cdot 10^{-10}$, max = $15 \cdot 10^{-10}$
Root density	Integral of B_i for entire soil depth by changing R0 (see Table S1)	m	Uniform	St.= 4000, min = 1000, max = 20000
β	Factor defining root length density profile (see Table S1)	[-]	Uniform	St.= 0.976, min = 0.855, max = 0.995
Variables that provide an informative proxy for the sap flow velocity of a plant				
$A_{SAPWOOD}$	Sapwood area	m^2	Uniform	St.= 0.979, min = 0.6, max = 1
Lumen Fraction	Lumen fraction	$m^2\ m^{-2}$	Uniform	St.=0.136, min = 0.0411, max = 0.451
Volume corr.	Correcting factor of the daily total transported sap flow volume which in the standard run corresponds to 31.4 10^{-3} m^3	[-]	Uniform	St.= 1, min = 0.5, max = 2.0
Variables related to the sample collection protocol				
Height	Height of sampling	m	Uniform	St. = 1.3, min = 0, max = 25
Time	Timing of sampling	h	Uniform	St. = 12, min = 9; max = 14

With: *St.* parameter value of the standard run, *min* and *max* the minimum and maximum assigned value

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