

## ***Interactive comment on “Disentangling temporal and population variability in plant root water uptake from stable isotopic analysis: a labeling study” by Valentin Couvreur et al.***

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Authors responses to Referee#3

RC| This manuscript compares two alternative modeling strategies for deriving the sink term (root water uptake) in a controlled ecotron experiment. Strategy 1 uses a simplified root water uptake model which however incorporates the main features of the three dimensional soil water flow, including hydraulic redistribution. The unknown model parameters are calibrated based on isotope data in the tiller and leaf water potentials. Strategy 2 derives root water uptake based on isotope data using Bayesian inference.

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The authors find that the results between the two strategies diverge. They show that Bayesian inference yields unphysical fluxes. Based on the model results they conclude that spatial variation (“swarm-like”) in tiller isotopic signal is misinterpreted as a strongly fluctuating time series, whereas it actually reflects the different rooting depths of plant individuals. Additionally, they argue that both the root water uptake model and the soil moisture time series suggest hydraulic lift, which cannot be captured by the Bayesian inference based on isotope data alone. Therefore, they conclude that the results obtained based on Bayesian inference could be due to an artifact.

This is a valuable contribution illustrating how sampling choice may affect the interpretation of isotope data. Especially the application of a straightforward process model for comparison with the Bayesian inference together with the dense measurements are extremely helpful to explain the shortcomings of deriving uptake profiles based on isotope data alone. The case is well argued and the methods are sound. I feel the manuscript has potential to making an impact and will find strong interest in the readership of HESS. The paper is mostly well structured, although I have some concerns with the Abstract and Methods section, as well as with some formulations (see below).

AC| Dear reviewer, we thank you for the detailed list of specific comments, for which we hope you will find our answers satisfactory.

RC| I have two general concerns, and a number of editorial remarks (below). The investigated case is a particular one, e.g. with a strong labelling pulse added below the rooting zone. This needs to be explicitly stated and the manuscript should discuss in which other situations such a strong influence of spatial variation is to be expected (and where it is not a concern).

AC| To address this general concern, we propose to add a section 3.3 entitled “Progresses and Challenges in soil water isotopic labeling for RWU determination” to the discussion with the following text:

“What can we draw from our findings about the use of isotopic labeling pulses for

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RWU analysis? Often in the field, the vertical dynamics of both soil water oxygen and hydrogen isotopic compositions are not strong enough (or show convolutions leading to issues of identifiability) for partitioning RWU among different contributing soil water sources. As a consequence, we unfortunately cannot make use of the natural variability in isotopic abundances for deciphering soil-root transfer processes (Beyer et al., 2018; Burgess et al., 2000). To address this limitation of the isotopic methodology, labeling pulses have been applied locally at different depths in the soil profile (e.g., Beyer et al., 2016) or at the soil upper/lower boundaries under both lab and field conditions by mimicking rain events (e.g., Piayda et al., 2017) and/or rise of the groundwater table (Meunier et al., 2017).

After labeling, we are faced with two problems: (i) the labeling pulse might enhance RWU at the labeling location if the volume of added water significantly changes the value of soil water content. It therefore poses the question of the meaningfulness of the derived RWU profiles, and this independently from the model used (i.e., physically-based soil-root model or statistical multi-source mixing model). In other words: are we observing a natural RWU behavior of the plant individual or population or are we seeing the influence of the labeling pulse? Certainly a way to move forward is environmental observatories such as ecotron and field lysimeters (e.g., Groh et al., 2018; Benettin et al., 2018) that provide means to better constrain hydraulic boundary conditions and reduced their isotopic heterogeneity. They allow for a mechanistically and holistic understanding of soil-root processes from stable isotopic analysis.

Another topic of concern is (ii) the difficulty to properly observe in situ (1) the propagation of the labeling pulse in the soil after application and (2) the temporal dynamics of the plant RWU isotopic composition. Beyer and Dubbert (2019) presented a comprehensive review on recent isotopic techniques for non-destructive, online, and continuous determination of soil and plant water isotopic compositions (Rothfuss et al., 2013; Quade et al., 2019; Volkmann et al., 2016) as alternatives of the widely used combination of destructive sampling and offline isotopic analysis following cryogenic vacuum

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extraction (Orlowski et al., 2016) or liquid-vapor direct equilibration (Wassenaar et al., 2008). These techniques have the potential for a paradigm change in isotopic studies on RWU processes to the condition that, e.g., isotopic effects during sample collection are fully understood.

The present study highlights the need not to “trust” our isotope data alone and always complement them by information on environmental factors as well as on soil and plant water status to go beyond the simple application of statistical models. This is especially the case in the framework of labeling studies where strong soil water isotopic gradients may induce strong dynamics of the RWU isotopic composition from a low variability of rooting depths.”

RC| The study suffers from lack of opportunity for validation: The heterogenous rooting depths cannot be measured in situ and therefore it remains a hypothesis. This is ok. But it requires diligent consideration of other assumptions of the model that may have had a similar effect on the model result. How about the inherent assumptions of a big leaf? Could individual differences in leaf development incur similar results? Those considerations need to enter more than now into the discussion. I propose adding a section dealing with the effect of inherent model assumptions.

AC| We totally agree with the referee. Our analysis shows that the tiller water isotopic signature is very sensitive to rooting depth in that kind of labelling experiment, generating heterogeneity in the aforementioned signatures of the population which in many cases could be confused for temporal variability of tiller water isotopic signature and root water uptake depth. We think this is an important *in silico* result and clarified in the discussion that its experimental validation would necessitate to estimate the variability of rooting depth in situ, which is currently not possible. Future studies using transparent soils, as in Downie et al. (2012) might take us one step closer to a validation, though distinguishing roots from different plants, for instance with fluorescent roots in mutant lines, would be another challenge.

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The assumption that all plants transpire at the same rate (“big leaf”) pointed out by the referee was not discussed in the manuscript, though it would be an interesting piece of discussion. Non-uniform patterns of transpiration within the plant population would affect two of our simulated variables: the isotopic signature of the tiller water and the leaf water potential.

- In our analysis, we have shown that large temporal fluctuations of transpiration (Figure 3 panel b) barely affect temporal fluctuations of the isotopic signature of tiller water (continuous grey lines Figure 5 panel a). Hence, we expect that the spatial heterogeneity of transpiration, likely smaller than its temporal heterogeneity, would have an even smaller impact on tiller water isotopic signature heterogeneity. Given the low sensitivity of tiller water isotopic signature to transpiration rate, and the lack of data on transpiration rate spatial heterogeneity, we think it is not worth developing additional simulations to study the effect of this factor in this manuscript. However, we added a paragraph on it in the discussion.

- Unlike the tiller water isotopic signature, leaf water potential turned out to be very sensitive to transpiration rate in our simulations (see temporal fluctuations of grey lines in Figure 5 panel c) and not very sensitive to root distribution (see small variations of leaf water potential across individuals in Figure 5 panel c). This high sensitivity of leaf water potential to transpiration suggests that in this setup the hydraulic conductance of the soil-root system limits shoot water supply more than the distribution of roots Sulis et al. (2019). A consequence of the high sensitivity of leaf water potential to transpiration rate is that if transpiration rate was spatially heterogeneous, substantial deviations of the measured leaf water potential would have been observed, relative to the simulated “baseline” leaf water potential (i.e. leaf water potential in case of uniform transpiration rate across the plant population). Simulated baseline leaf water potentials (for uniform transpiration rates) are shown as grey lines in Figure 5 panel c, and measured leaf water potentials as a green line in the same panel. We were positively surprised to find out that the simulated baseline leaf water potentials fit the measured temporal fluctu-

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ations of leaf water potential quite well under the assumption of uniform transpiration rate, despite the high sensitivity of leaf water potential to transpiration rate. This result reinforces the idea that transpiration rate was likely not spatially heterogeneous among the plant population. In consequence, we think that transpiration rate was rather uniform among the plant population (so that the “big leaf” approach was justified), and therefore, the tiller water isotopic signature, whose sensitivity to transpiration rate is already very low, was likely not affected by transpiration rate heterogeneity. This piece of discussion will be added in the revised manuscript.

RC| Detailed comments:

RC| Abstract: Line 18-19: This sentence sounds vague, e.g. “semi-controlled” and “such variables”, please formulate more specifically.

AC| We will list in the few sentences after the monitored variables in question. We will remove “semi-controlled” (indeed a vague term) and “such variables” from the text.

RC| Line 23-24: “results underlined the discrepancy.” At this point unclear what is meant

AC| We will put emphasis on “temporal disconnection” instead of “discrepancy”.

RC| Line 29-30: The sentence starting with “The physical model.. “is difficult to understand, please reformulate

AC| We will split the sentence in two and name two examples of variables instead of referring to them by “the former” and “the latter”.

RC| Line 33: “local increase...” this results is not stated earlier and at this position confusing.

AC| We will not refer to “the” local increase – which indeed was not mentioned earlier – anymore.

RC| Lines 35-62 List of variables. Some variables are missing, please complete. Also

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I propose erasing all the repetitions of “units of”.

AC| Done. We will add the missing two variables, namely the soil hydraulic conductivity parameter ( $\lambda$ , dimensionless) and the soil relative water content (Sej). In addition, repetitions of “in units of” will be removed. Instead, we will add headers to the table (i.e., “Name”, “Symbol”, and “Units”).

RC| Later in the paper, it will be useful to express volumetric water content as vol-% and I propose adding it here.

AC| Please see our answer to your specific comment regarding L329ff below.

RC| Introduction

RC| Line 75-76: The description of the “mean value of...weighted by” is confusing, please rephrase

AC| Consider it done!

RC| Material and Methods

RC| Line 114: Please mention that CS616 is a time domain sensor (TDR). Also, reflectometer is a correct, but awkward term for soil moisture sensor. I propose using the latter, just to avoid confusion.

AC| Consider it done!

RC| Line 123: The description of the soil is confusing. Is District Cambisol a typo for “Dystric Cambisol”. Otherwise, I am not aware what a District Cambisol would refer to, please explain. Besides, Cambisol refers to a soil in situ and after specific pedogenesis which is completely removed in your experiment. Maybe say “The soil originates from a xx Cambisol”. Also, does the bulk density refer to the original soil, and is it required to be mentioned?

AC| There is no typo here. But we agree that it was not properly formulated, and that

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the substrate which we filled the rhizotron with could not possibly be referred to as a “cambisol”. Therefore we will write that the soil substrate originates from the Lp horizon of an agricultural field part of the Observatory of Environment Research (ORE), INRA Lusignan, France (0°60W, 46°250N) which is classified as District Cambisol (particle size distribution: sand 15%, silt 65%, clay 20%).

RC| Line 125: Add “layer” before “by”

AC| We would propose the following reformulation: “450 kg of soil was filled in the rhizotron by 0.10 m increment a. . .”

RC| Line 128: sols-PST55 sensors are missing above, where installation depths were mentioned. Add there. Please add where they are installed. Line 130: “between its position and measured soil water content” unclear, please rephrase

AC| The retention curves were determined in situ in the same type of macro-rhizotron during another experiment (at the same soil bulk density) of which the results were published by Meunier et al. (2017a). In order to clarify this, we will not mention the type of sensors used – which was indeed misleading – and write: “The closed-form soil water retention curve van Genuchten (1980) was derived in a previous study by Meunier et al. (2017a) from synchronous measurements of soil water content and matric potential from saturated to residual water content (see Appendix B for its hydraulic parameters).”

RC| Lines 132-136: Please shortly state: Were the plants watered? What was the lower boundary condition?

AC| During a period of 165 day following seeding, the tall fescue cover was exclusively watered from the reservoir with local water in order to (i) keep the soil bottom layer (< -1.3 m) close to water saturation, and to (ii) not to disrupt the natural soil water  $\delta^{18}O$  profile. Done. We will add this information in the revised manuscript.

RC| Line 139: Do you mean “sides” instead of “slides”

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AC| Yes, exactly, many thanks for finding this typo!

RC| Line 144: “three plants were sampled” - does it mean the entire plant or some leaves?

AC| We sampled the entire plant. We will insert “whole” after “three”.

RC| Line 148-149 I believe you mean “from the atmosphere surrounding the rhizotron”. Also, I am assuming the latter means the ambient air in the lab? Would be good to specify.

AC| Thank you for these propositions!

RC| Line 162: “60 tall festucae root systems ..” Why 60 plants?

AC| This choice was arbitrary. We estimated that there were about 1500 plants per square meter in the rhizotron, so that there would be 300 plants on total in the experiment. Running simulations for 300 plants would have required a lot of computational resources. That is why we focused on a subset of 60 representative plants, that met our computational capabilities for the inverse modelling scheme. Each “representative plant” was called a “class” of plants that is included in the simulations under the form of a “big root” and “big leaf”, with root lengths and transpiration rate corresponding to 5 plants for each class. This is an important point, which we will clarify in the revised manuscript.

RC| Eq. (1) Personally, I do not find this equation obvious. Please motivate the origin. Are there any other assumptions involved besides the big root one?

AC| This equation is indeed not obvious. It was derived by Meunier et al. (2017a) in its Appendix C. The reference was indeed missing. We will add it to the revised manuscript “, as derived by Meunier et al. [2017],”.

RC| Line 173-174: “dimension of the domain...” I do not understand this statement

AC| The horizontal domain of simulation typically has two dimensions (X and Y), but

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it some cases, the studied problem has an essentially radial dimension between bulk soil and root surface. We will simplify the text as follows: “with B (dimensionless) a geometrical factor simplifying the horizontal dimensions into radial domains between the bulk soil and root surfaces”.

RC| Line 176-177: Sentence starting with “The averaged distance ..” seems wrong. Maybe erase the last words? AC| The referee is correct. The reported sentence referred to , not to the average distance between roots. We will correct the text as follows: “It can be deduced from the observed root length density (. . .)”.

Eq (4): Se is not part of the List of variables, plus the S stated there refers to the sink term not saturation. Please use a different abbreviation.

AC| Relative water content (Sej) is both introduced in Eq (4) is defined in Eq. (5). The water “sink term” is introduced in Eq. (9).

RC| Line 188: Could not the measured root length density profiles be used?

AC| Not here because root lengths, and thus root system conductances, are class-specific. The bulk root length densities may not account for the fact that each class has its own root length and RLD at each depth.

RC| Line 191: “were derived” unclear how this was derived? Also, where was  $k_{axial}$  in Eq.(7) taken from? Please explain. Ok, I learn later this was calibrated. Maybe mention this here.

AC| We apologize for the confusion, and will include this clarification at this point in the revised manuscript: “were calculated as equivalent “big root” specific axial conductance per root system class ( $k_{axial}$ ,  $m^4 MPa^{-1} d^{-1}$ , to be optimized by inverse modelling) as”

RC| Line 191: “root system class” Unclear what is meant with “class”.

AC| See reply to comment on simulated 60 root systems.

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RC| Line 194: standard sink distribution is not a standard term and requires a bit more explanation to be convincing.

AC| The term was defined in the following sentence. We moved it ahead for clarity.

RC| Line 195-196: “potential difference between soil and leaf”: You are dealing with a soil profile and a leaf canopy. Thus, where in the soil and leaf are you referring to. Please also translate to what this means for your experimental setup either here or in the discussion.

AC| That is a good point. The leaf is a “big leaf”, and the soil water potential used in the definition of  $K_{soil-root}$  is the SSF-averaged bulk soil water potential. We will clarify it in the text: “The variable SSF is the relative distribution of water uptake in each soil layer under vertically homogeneous soil water potential conditions (Couvreur et al., 2012), and  $K_{soil-root}$  represents the water flow per unit water potential difference between the SSF-averaged bulk soil water potential and the “big leaf” (assuming a negligible stem hydraulic resistance)”.

RC| Line 196: “assuming negligible stem conductance”: Does this imply that all conductance / resistance happens in the root system? Is this a reasonable assumption?

AC| As far as grass is concerned we think so. The main hydraulic resistances between the bulk soil and the leaf insertion (where leaf water potential is measured) being the drying soil and the root radial resistance (Stedule and Peterson, 1998). Reference will be added in the text.

RC| Line 202: “class” unclear

AC| See previous reply about the 60 root systems.

RC| Line 205: “where axial conductances” this comes too late, please move up.

AC| It was in the right place, but we will rewrite the sentence for clarification as “where  $K_{(soil-root)}$  was assumed to control the compensatory RWU which arise from a het-

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erogeneously distributed soil water potential, due to large axial conductances (Couvreur et al., 2012)”.

RC| Line 218: I propose moving the inverse modeling procedure out the appendix and add it to the main text. It is important information.

AC| We think that the Material and methods are very dense already, and since the inverse modelling method is a state-of-the-art method in modelling, we think it would be better to leave its detailed description in the appendix.

RC| Line 226: Not sure what is meant with “ten identified potential water sources” .. “10 distinct soil layers” Can you be more specific?

AC| These water sources were defined to originate from 10 distinct soil layers (0.00-0.03, 0.03-0.07, 0.07-0.15, 0.15-0.30, 0.30-0.60, 0.60-0.90, 0.90-1.20, 1.20-1.32, 1.32-1.37, and 1.37-1.44 m). This information be added in the revised version, thank you.

RC| Results and discussion:

RC| Lines 335-252: Small issue: Please add some paragraphs in this section.

AC| Thank you for the tip! We will split the text into three paragraphs which refer to soil water content (§1), soil water oxygen isotopic composition (§2), and root length density (§3) profiles.

RC| Line 281: Do you mean “and” instead of “et”

AC| Consider it done, thank you!

RC| Line 304: With “all the population” do you mean all individuals?

AC| Indeed. We will replace it by “all individuals” for clarification.

RC| Line 311-313: Sentence is difficult to understand, please rephrase.

AC| We will rephrase as follows: “As no correlation could be expected between the drivers, the maximum rooting depth of the sample plants and canopy transpiration rate,

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our analysis explains the absence of correlation between  $\delta_{\text{tiller}}$  and  $\psi_{\text{leaf}}$  or transpiration rate”.

RC| Lines 329ff: Since there is repeatedly reference to increasing by xx% this may be strongly confusing. Better use vol-% to be on the safe side.

AC| We would like to keep using the information of dimension for soil volumetric water content ( $\theta$ ) rather than using a relative unit. We need this information for, e.g., explaining how we calculate  $\theta$  from the soil gravimetric water content ( $\theta_{\text{grav}}$ , in kg kg<sup>-1</sup>). We converted the vol% to m<sup>3</sup> m<sup>-3</sup> back in section 3.2.2. Thank you for pointing out these inconstancies!

RC| Line 340 Lambda is not in the list of variables.

AC|  $\lambda$  will be added to the list of variables, thank you.

RC| Also, this information is very compact, and difficult to understand. Please elaborate.

AC| The following clarification will be added: “The optimal value of  $k_{\text{sat}}$  was quite high (Table 1) but reportedly very correlated to  $\lambda$  (i.e. soil unsaturated hydraulic conductivity is proportional to  $k_{\text{sat}}$ , but also to  $\text{Se}\lambda$ ; van Genuchten, 1980), so that the low value of the latter compensated the high value of the former, thus they should be considered as effective rather than physical parameters”.

RC| Lines 345ff: I strongly recommend bringing Fig E into the main text. It is discussed and seems therefore sufficiently important. Also, because this is one of the two alternative water uptake profiles which comparison is the main motivation of the manuscript.

AC| The authors agree. Figure E will be part of the main document and become “Figure 7”.

RC| Line 360: Replace “first” with “top”

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AC| Done. Thank you.

RC| Line 367: Not sure what is meant with “broadcasted”

AC| We will not use the term “broadcasted” anymore and propose to write instead: “This case study highlights (i) the potential limitations of water isotopic labeling techniques for studying RWU: the soil water isotopic artificial gradients induced from water addition result in an improvement in RWU profiles determination to the condition that they are properly characterized spatially and temporally.”

RC| Figure 2: I was confused about the positive water potentials. Since they were named  $\psi$  I was instinctively assuming to see matric potential, but plotted are the water potentials. I propose renaming to  $h$ . If you want to stick to  $\psi_{\text{soil}}$  because of  $\psi_{\text{leaf}}$  (although I seriously think it would not be an issue), please obviously state the reference elevation to avoid this type of confusion.

AC| Figure 2 shows the profiles of the log transformation of soil water matric potential  $\psi_{\text{soil}}$  (i.e., not the soil hydraulic head, which you term  $h$ ).  $\psi_{\text{soil}}$  is always negative, therefore for the log transformation we must take “ $-\psi_{\text{soil}}$ ”. We do not mean by that that  $\psi_{\text{soil}}$  was negative during the experiment.

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