Additional comment to the reviewers and Editor Prof. Markus Weiler

Please note that we have decided to opt for another more “catchy” title: “The rollercoaster and the swarm: disentangling plant water isotopic composition variabilities in response to soil water labelling”

For the co-authors,

Valentin Couvreur and Youri Rothfuss

Anonymous Referee #1

Couvreur and colleagues present an interesting isotopic labelling experiment and innovative simulations of the processes in the soil-roots interactions. Their study is addressing current research gaps and will thus be of interest to the readership of HESS. The manuscript is well prepared and the figures are mostly informative. I provide two general recommendations and several minor technical comments below. I recommend publication after addressing these comments.

> Dear reviewer, we thank you for your general comments as well as technical corrections of our manuscript! You can find our answers below:

General aspects:

The "rollercoaster hypothesis" and the "swarm pattern hypothesis" both focus on the variation of $\delta^{18}O$ in tiller across plants and/or over time, respectively. However, the studied system is likely to be more complex due to heterogeneity of the water flow/capillary rise. Do you see a chance to improve the modelling results when moving from a uniform flow/capillary rise to some kind of dual-permeability approach accounting for potential subsurface isotopic heterogeneity?

This is an excellent comment. Other sources of variability may indeed have affected the variability of measured $\delta^{18}O_{tiller}$ and ψ_{leaf}, such as:

- The lateral heterogeneity of soil water isotopic composition (as mentioned by the referee). The idea is that water in micropores is less mobile than water in meso- and macropores, so that it is likely that, in the lower half of the profile, the capillary rise of labelled water affected the signature of water in meso- and macropores more than in micropores. If roots have more access to meso- and macropore water, then the water absorbed by roots would be isotopically enriched, as compared to the "bulk soil water" characterized experimentally. The importance of this possible bias depends on soil texture and heterogeneity (e.g. existence of more isolated "pockets" of soil or compact clusters), as well as on the speed of water mixing between mobile and immobile water fractions. Including this process in the modelling would necessitate sufficient observations to estimate the aforementioned properties, and ideally some quantification of the lateral heterogeneity of soil water isotopic composition at the micro-scale. We think it would be an excellent idea for a future study, but including it in the model in this study would involve extrapolating simulations beyond what we can justify with the measured dataset;
- The lateral heterogeneity of bulk soil water potential and soil water content (or the observational errors) may have slightly affected our estimation of soil water potential, and in turn our predictions of root water uptake distribution. The experiment was designed to maximize vertical gradients and minimize lateral bulk soil water potential gradients by wetting soil from the bottom and letting it drain, so we consider that any lateral heterogeneity must be small. However, in the revised version of the MS we tested the impact on our results of deviations of soil water potential, that could be due to observational errors in soil water content measurements with the gravitational method, and found no qualitative change;
- The lateral heterogeneity of soil hydraulic properties and root distribution may also have participated to the generation of lateral soil water potential heterogeneities, particularly in undisturbed soils. If one had access to data on lateral heterogeneity of soil properties and rooting density, it would be possible to simulate 3-D soil-root water flow with a tool such as R-SWMS (Javaux et al., 2008), using a randomization technique for soil properties distribution as in Kuhlmann et al. (2012), in order to obtain estimations of the relative importance of this type of heterogeneity on $\delta^{18}O_{tiller}$ and ψ_{leaf} variability. However, in this experiment we consider that the substrate and rooting heterogeneity were minimized by the sieving of the soil, and thus focused on the vertical profiling in measurements and modelling.

Overall, our treatment of the soil media in this experiment (sieving, irrigating from the bottom) makes it different from soils in natural systems, which are most likely more heterogeneous laterally. This method allowed us to study specifically the impact of the vertical component of soil water isotopic signature on tiller water isotopic signature. It also justified the use of a simplistic 1-D model adapted to the vertically resolved measurements. This was clarified

in our revisions, and the perspective of comparing bulk soil water isotopic signature to the signature of “mobile water” in meso- and macropores was discussed.
A new section “3.2.3 Other sources of variability and observational error” was added to discuss these points (L388-422):

> Our treatment of the soil media in this experiment (sieving, irrigation from the bottom) makes it laterally more homogeneous than natural soils. This method allowed us to study specifically the impact of the vertical gradients of δ_{soil} on δ_{tiller}. It also justified the use of a simplistic 1-D model adapted to the vertically resolved measurements. If lateral heterogeneity of soil water content remained and was accounted for, our predictions of root water uptake distribution, δ_{tiller} and ψ_{leaf} would be altered. Observational errors in the gravimetric soil water content measurement (turned into soil water potential using the soil water retention curve) would as well alter these predictions. In order to quantify the sensitivity of our simulated results to such heterogeneity or observational error, we varied the soil water content input by ± 0.02 $m^3 m^{-3}$ at three critical depths (-0.9, -1.1 and -1.3 m, before interpolation), at the last observation time, during which measurements and simulations suggested that hydraulic lift occurred. Our results were mostly sensitive to soil water content alterations at -0.9 m, and barely differed in response to alterations at -1.1 and -1.3 m, though the conclusions were not affected qualitatively. No statistically significant difference between predicted and observed δ_{tiller} distributions for the overall dataset could be found when pooling 3 simulated δ_{tiller} randomly at each observation time (predicted and observed δ_{tiller} distributions were closest to differ when soil water content was reduced by 0.02 $m^3 m^{-3}$ at 0.9 m depth; $P>0.01$ in 76 cases out of 100 repeated drawings). Measured and simulated ψ_{leaf} remained very correlated in all cases (from $R^2=0.69$ to 0.74 when adding or removing 0.02 $m^3 m^{-3}$ at 0.9 m depth, respectively). Furthermore, when adding or removing 0.02 $m^3 m^{-3}$ at 0.9 m depth, cumulative water exudation at -0.9 m varied between 0.0019 and 0.0035 $m^3 m^{-3}$, uptake at -1.1 m varied between 0.0080 and 0.0108 $m^3 m^{-3}$, and the simulated change of δ_{soil} ranged between 0.28 and 0.40 ‰, respectively.
> Lateral heterogeneity of soil water isotopic composition may as well occur at the microscopic scale. As water in micropores is less mobile than water in meso- and macropores (Alletto et al., 2006), it is likely that, in the lower half of the profile, the capillary rise of labelled water affected the signature of water in meso- and macropores more than in micropores. If roots have more access to meso- and macropore water, then the water absorbed by roots would be isotopically enriched, as compared to the “bulk soil water” characterized experimentally. The importance of this possible bias depends on soil texture and heterogeneity (e.g. existence of more isolated “pockets” of soil or compact clusters), as well as on the speed of water mixing between mobile and immobile water fractions (Gazis and Feng, 2004). Including this process in the modelling would necessitate sufficient observations to estimate the aforementioned properties, and ideally some quantification of the lateral heterogeneity of soil water isotopic composition at the micro-scale.
> The lateral heterogeneity of soil hydraulic properties and root distribution may also have participated to the generation of lateral soil water potential heterogeneities, particularly in undisturbed soils. If one had access to data on lateral heterogeneity of soil properties and rooting density, it would be possible to simulate 3-D soil-root water flow with a tool such as R-SWMS (Javaux et al., 2008), using a randomization technique for soil properties distribution as in Kuhlmann et al. (2012), in order to obtain estimations of the relative importance of this type of heterogeneity on δ_{tiller} and ψ_{leaf} variability.”

I was missing a discussion of the uncertainties regarding for example soil moisture estimates and the impact of such uncertainties for the interpretation regarding potential processes (i.e., hydraulic lift).
We agree that this should be added (see second bullet point in our reply to the previous comment and first paragraph of the revision in the reply to the previous comment).

I further think that the implications of their interesting findings (i.e., no match between the ensemble of various simulations and the observations; Fig. 5) for both field studies labelled or with natural isotope compositions and the modelling of the soil-root interactions could be made clearer. This way, the manuscript might have a higher impact and could provide recommendations to overcome limitations in observation techniques and modelling approaches.
We have now removed the regression lines in Figure 4 for which the p-value of the linear model was higher than 0.01, hoping that it clarifies the absence of significant linear correlation between given hydraulic (e.g., Transpiration flux *T*) and isotopic variables (e.g., oxygen stable isotopic composition of tiller water, δ_{tiller}). We provide in a separate discussion section 3.3 "Progresses and Challenges in soil water isotopic labeling for RWU determination" recommendations to overcome the aforementioned limitations.

I appreciate that the authors will upload the data of the study. Are they further intending to make the model code available?
We are indeed, as it may be useful to the scientific community working on such data. We will upload it as soon as the MS is accepted.

Technical comments:

L 77: monotonic gradient? Consider sinusoidal variability across the depth, which would cause issues of identifiability
You are right! It reads (now L83-84):

> "…the soil water isotopic composition depth gradient is strong and monotonic (thus avoiding issues of identifiability)"

L 80: Not only GW, also due to increasing dispersion with depth – even if the GW table is several meters deep
The impact of dispersion was added in the revised text (now L86-87):

> "…(due to the isotopic influence of the groundwater table and increasing dispersion with depth)"

L 100: This paragraph is kept quite general after a very informative introduction. I suggest to be more specific and especially pose hypothesis or specific research questions.
Indeed, the objectives were not clearly stated in our initial submission. We now write (L107-113):

> "Building on the work of Meunier et al. (2017a), the objective of the present study is to (i) model in a physically-based manner (i.e., by accounting for soil and plant environmental factors) the temporal dynamics of the isotopic composition of RWU of a population of *Festuca arundinacae* cv Soni (tall fescue) during a semi-controlled experiment following an isotopic labeling pulse of deep soil water, (ii) investigate the implication of the model-to-data fit quality in terms of meaningfulness of the isotopic information to reconstruct RWU profiles, and finally (iii) confront the simulated root water uptake profiles with estimations obtained on basis of isotopic information alone (i.e., provided by a Bayesian mixing model)."

L 117: Since you provide the variable and unit for soil moisture, you probably should also add that to matric potential.
Done. It now reads (L127):

> "…volumetric content (θ, in $m^3\ m^{-3}$) and matric potential (ψ_{soil}, in MPa)."

L 140: replace "isotopic" with "δ18O"
Done. It now reads (L154-155):

> "That same day at 17:00, the reservoir's water was labelled and its $\delta^{18}O$ measured at +470 ‰."

L 140: How was the sampling done? Soil corer? How much soil was sampled?
We now add (L155-157):

> "Soil was sampled before (DaS 166 - 15:45) and after labeling on DaS 167 - 07:00, DaS 167 - 17:00 and DaS 168 - 05:00 using a 2 cm diameter auger through the transparent polycarbonate side of the rhizotron…"

L 149: provide info about temperature, applied vacuum and time of extraction

It now reads (L168-172):

> "Water from plant (i.e., tillers and leaves) and soil samples were extracted by vacuum distillation for 14 to 16 hours depending on the sample mass (e.g., ranging between 18 to 28 g for soil) at temperatures of 60 and 90°C, respectively. The residual water vapor pressure at the end of each successful extraction procedure invariably reached 10^{-1} mbar. The oxygen isotopic compositions of tiller, leaf, and soil water (i.e., δ_{tiller}, δ_{leaf}, and δ_{soil}) together with…"

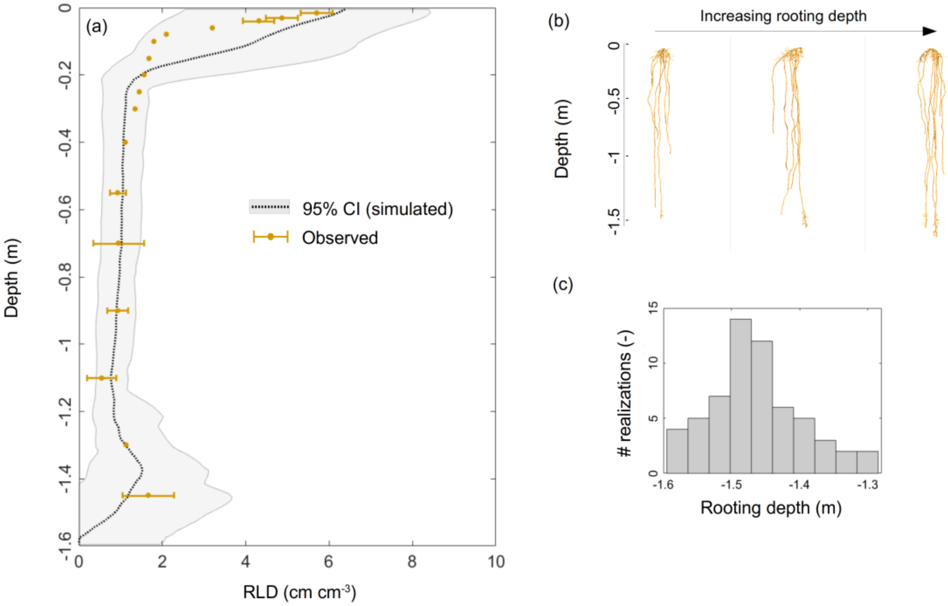
L158: Not sure what "(95 m root (g root)−−1)." Means

We removed "root" from the mention of the dimension for clarifications, so now it now reads (L180-181):

> "…specifically for tall fescue (95 m g^{-1})".

Figure 1: The circles connecting the bottom of the profile of Figure 1a and the histogram of 1c are more confusing than helping. I suggest to get rid of them. The same would apply for the arrow connecting to 1b.

Done. We updated Fig. 1:



L 172: All variables should be explained here. For example Lpr is explained in L 216

That is right. We added the meaning of all terms below equation 1 (L195-196):

> "with r_{root} (m) the root radius, $l_{\mathrm{root},i,j}$ (m) the root length of plants of group i in soil layer j, L_{pr} (m MPa^{-1} d^{-1}) the root radial hydraulic conductivity, $k_{\mathrm{soil},j}$ (m^2 MPa^{-1} d^{-1}) the soil hydraulic conductivity in layer j, and B_j (dimensionless) …"

L 181: The variable "n" should be briefly explained as one of the MVG parameters. Also, consider adding n and Sej to the list of variables.

We agree with the referee and made the suggested changes in the revised version of the MS (see list of variables and L205):

> “m (dimensionless) and λ (dimensionless) are soil hydraulic parameters (with m = 1 – 2/n)”

L 209: Please define conditions for exudation. I believe it is for Sj<0, but not sure.
The referee is correct. This was clarified in the revised version of the MS (L246-247):

> “… the depth at which the transition between nighttime water uptake and exudation (Si,j<0, i.e. release of water from root to soil) takes place”

L 239: I do not see how the soil moisture varied notably at 1.3 m depth. What do you mean here? How comes that you refer to 12:00 and 20:00 on DaS 167, while that is not shown in Figure 2a?
Thank you, this was corrected and now reads (L266-267):

> “Soil moisture remained unchanged in the top 25 cm during the sampling period (θ = 0.08 ±0.00 m^3 m^{-3}) as well as at –1.30 m from DaS 166 - 15:45 to DaS 168 - 05:00 (θ = 0.33 ±0.01 m^3 m^{-3}).”

L 243: Again, you refer to a time (7:30), which is not shown in the Figure and you should refer to it as soil labelled” and not “soil” to be consistent with Figure 2.
Indeed! This was also corrected (now L271):

> “δ_{soil} reached a value of 36.9 ‰ at –1.50 m on DaS 167 - 17:00.”

L 244: “lead us to assume” or “leads to the assumption”
Thank you. We now write (L272):

> “…and the observed surface θ values lead us to assume…”

L 262: It is unclear which of the correlations are describe a significant relationship. I suggest to only draw the regression lines for significant relationships in Figure 4.
Thank you for this suggestion: we removed the regression lines for which the p-value of the linear model was lower than 0.01 and now indicate in the caption of Figure 4:

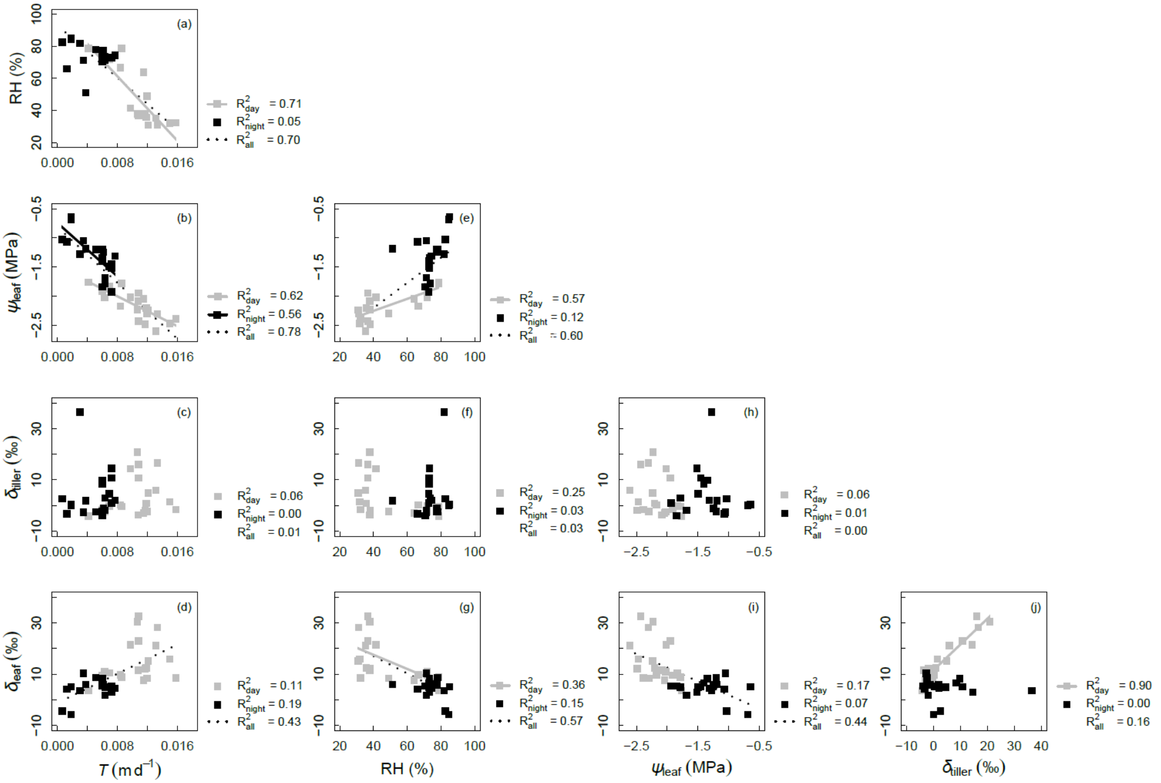


Figure 4. Correlations between measured variables: oxygen isotopic compositions of xylem and leaf waters (δ_{tiller} and δ_{leaf}, in ‰), transpiration rate (T, in m d^{-1}), relative humidity (RH, %), and leaf water potential (ψ_{leaf}, in MPa). Coefficient of determinations (R^2) are reported for all data, and separately for 'day' data (gray symbols) and 'night' data (black symbols) (see Appendix C for definition of 'day' and 'night' experimental periods). Regression lines are drawn for linear models with p-value < 0.01.

L 281: replace "et" with "and"
Thank you ☺! Done.

L 298: Unclear what is meant with "over all dataset". I believe you mean the 60 different root system classes. Please be more specific.
That is right. We clarified the sentence as follows (L336-337):

> "The predicted versus observed δtiller distributions including all plant groups and observation times differed noticeably but not significantly (…)".

L 315: It seems to me that in-situ measurements would overcome these limitations. One could sample in parallel several plants and thus, observe the temporal dynamics at individual plant level.
We could not agree more! We now mention these new methodological developments in a dedicated new subsection 3.3 "Progresses and Challenges in soil water isotopic labeling for RWU determination"

L 319: What is the expected accuracy of your volumetric soil moisture measurements. Given that you derived this from gravimetric water content and a bulk density, which was assumed to be constant in the repacked soil. However, relatively small differences in bulk density of just a few g cm−3will affect the estimates of the volumetric water content. It would be good to account for such uncertainties in this discussion.

We now add information on the validity of our assumption that the bulk density was constant in the soil profile L160-162:

> "The hypothesis of a constant value for ρ_b across the reconstructed soil profile was further validated from the quality of the linear fit (coefficient of determination $R^2 = 1.0$) between the θ values measured by the sensors at the six available depths and (–0.05, –0.10, –0.30, –0.60, – 1.05 and –1.30 m) and those computed from θ_{grav}."

Yet, the impact of observational errors were investigated as a sensitivity analysis in the revised MS, and did not yield any qualitative change in our results (see new section 3.2.3).

L 325: What do you mean with "significantly higher"? Did you apply a statistical test? I believe that you mean that the difference is higher than the measurement uncertainty.
Yes, the p-value is 1.4e-04. It was clarified in the revised version of the MS (L366).

Figure 6b: The title says "standard error", but the caption says "standard deviation". Which one is it? Please correct.
It is standard deviation, thank you for spotting this typo. We have updated Figure 6b accordingly.

L 360: the upper half of the soil profile
Done (now L438)

L 367: "water addition is localized and not broadcasted in the soil" is unclear. What do you mean with "broadcasted"?
We don't use the term "broadcasted" anymore and write instead (L491-493):

> "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."

L 370: "simple"? In addition to the usual struggle of assessing meaningful MVG parameters to describe the soil water transport, also like for example Lpr and Kaxial are needed, which are not easily derived, but its estimation adds to the uncertainty of the uptake depths.
We meant "simple soil-root model", relative to (i) complex soil-root models, which include more parameters (e.g. profile of root hydraulic properties changing with root segment age, etc.), and (ii) absent soil-root models, in the typical Bayesian approach. We clarified that more measurements are needed than with no soil-root model. Extra measurements could be limited if appropriate assumptions on the model parameters can be done (e.g. using soil pedotransfer functions, root hydraulic properties reported in the literature, etc.). The revised text now reads (L495-498):

> "… calls for the use of simple soil-root models (though requiring additional water status measurements, as compared to the traditional Bayesian approach, and making simplifying assumptions in the description of the soil-plant system more explicit) for inversing isotopic data and gain insights into the RWU process."

Referee #2: Dr. Matthias Beyer

The manuscript hess-2019-543 ‘Disentangling temporal and population variability in plant root water uptake from stable isotopic analysis: a labeling study’ by Couvreur et al. present a lab-/field- and model-based study of root water uptake during an artificial tracer experiment, where the soil is wetted from below (as opposed to often, via irrigation). They support their isotope analysis by hydraulic measures in order to provide a holistic understanding of RWU.

The authors address the urgent and contemporary need for increasing the reliability of RWU models and improve the understanding root water uptake patterns. It has been often proposed to combine hydraulic, water isotope and other information in order to do so, the presented study is in my opinion a holistic and promising approach. The rollercoaster vs. swarm hypothesis is also a good idea, though (as the authors state themselves) it should be validated further. It is also great that both data from an experiment and modeling are provided, rather than only one of the two. This manuscript is well prepared, and the topic is highly relevant. The figures are suitable and well-explained.

I highly recommend this manuscript for HESS, though I have a number of comments/questions that might help to improve this manuscript further. In brief, a few general comments, which are all rather minor:
Dear Dr. Beyer, we thank you for the time you spent in carefully revising our manuscript! We hope that we have sufficiently addressed the issues you raised in our revised version.

- The discussion on hydraulic redistribution should be strengthened. Do the authors see a clear sign or not? I think strengthening this part would be of utmost interest for many people from the ecohydrological community.
This is indeed an important part of the discussion. In the revised manuscript, we clarified in section 3.2.2 that all measurable signs of hydraulic redistribution are positive (local increase of soil water content, local enrichment of water isotopic signature) and converge with independent simulated results (water exuded at the same time and location, at a rate compatible with measurements) to yield a robust “yes we think that hydraulic lift was happening at that time at that depth” (L377-379):

> “Therefore, all relevant measurements (local increase of soil water content, local enrichment of water isotopic signature) and simulation results (S<0, i.e. local water release from roots) clearly converge to the conclusion that hydraulic lift occurred in the vicinity of -0.9 m depth in the early morning of DaS 168.”

Furthermore, in the new section 3.2.3, we show that these results are not sensitive to an observational error of +/- 0.02 $m^3 m^{-3}$ in the soil water content measured with the gravitational method.

- When reading the results and discussion section, I realized that there are very small differences discussed in the manuscript (e.g. 0,41 per mill, 1 per mil, etc....). I think it is necessary to think about uncertainties in that respect and really decide which of the differences are likely ‘true’ differences or simply within the variability/uncertainty.
We now discuss in further detail (please see our answers to your specific comments) the problematic of meaningfulness of our isotope data, i.e., whether these differences of isotopic compositions are the result of given processes or the mere translation of e.g., soil lateral heterogeneity. Importantly, the simulated isotopic enrichment below 0.5 ‰ remains despite the consideration of soil water content measurement errors of +/- 0.02 $m^3 m^{-3}$ (see new section 3.2.3). In the revised manuscript we also clarify with ANOVA analysis that the locally increased δ_{soil} at 0.9 m depth significantly differs from δ_{soil} at previous times (L365-366):

> “… (–6.2 ‰, a value significantly higher than –7.1 ‰ ± 0.1 ‰ at earlier times based on ANOVA analysis, P<0.01)…”

- I find the discussion of the physical experiment slightly too weak compared to the results drawn from the modeling (I also indicated this in the detailed comments below).

We hope that we strengthened the discussion of the experimental experiment (see answer to your specific comment to L247-249)

- The authors use δtiller, etc. without providing the water isotope (e.g.δtiller18O). I think this is important to clarify (it was only 18O used, correct?) starting with the symbol description. Why was only oxygen-18 used? (and not 2H in addition?)

For clarification, we now systematically add "oxygen" before "isotopic composition" throughout the manuscript as well as in the "List of variables with symbols and units" (Page 2). We also now write (L116):

> "Data on soil and plant oxygen stable isotopic signature and hydraulic status were monitored for 34 hours".

We only measured the water $\delta^{18}O$ our IRMS ("Isoprep 18 - Optima, Fison, Great-Britain" L172-173) and not water $\delta^{2}H$ and $\delta^{18}O$ simultaneously with, e.g., a laser spectrometer for two reasons: (1) to the contrary of laser spectrometers, IRMS are not affected by the presence of volatile organic substances which should be present in the distillated water from soil and plant samples. (2) The added information on $\delta^{2}H$ profiles should not be discriminating for determination of RWU profiles as $\delta^{2}H$ remains constant in the lower half of the soil profile (mostly contributing to RWU) which is influenced by labeling.

- Will the model be made publicly available? It would be very interesting to apply the model with other datasets (e.g. some in situ datasets of joint soil and plant water isotopes)

We are indeed willing to make the code open source, as it may be useful to the scientific community working on such data. We will upload it as soon as the MS is accepted.

I wish the authors good luck and look forward to the final publication.
Greetings and best wishes, Matthias Beyer

Detailed comments:

- Abstract is very well written

Thank you ☺

- L.78/79: depends on how deep the groundwater table is. In thick unsaturated zones, often mixing of old water is also a reason. Further, over short time periods a seasonal pattern might persist in the soil

We agree! If soil water (and eventually groundwater) is replenished by rain events of which the isotopic compositions is highly dynamic in time, it can generally lead to issues of identifiability. This is why we added (L83-84):

> "(i) the soil water isotopic composition depth gradient is strong *and monotonic (thus avoiding issues of identifiability)*"

- L.140: in oxygen-18 I guess? Could the authors please add this information?

Done. It now reads (L154-155):

> "…the reservoir's water was labelled and its $\delta^{18}O$ measured at +470 ‰."

- L.149: Can the authors please add specifics on the extraction? (Extraction temperature and time for soil and plant samples, how was complete extraction assessed?)The community has been asking in many occasions to provide more transparency of extraction procedures; hence it would be appreciable to add this information.

This information is added L168-170:

> "Water from plant (i.e., tillers and leaves) and soil samples were extracted by vacuum distillation (applied vacuum: 10–3 mbar) at temperatures of 60 and 90°C, respectively,…"

In addition, complete extraction was assessed…

> "based on the comparison of sample weight loss during distillation and mass of collected distillated water" (L308-309)

- L.152/153: the loss of mass would also include evaporation; was this neglected (please clarify) [I see that this is mentioned later in the text, but perhaps better to clarify here]

Yes, thank you. It has been clarified (L174):

> "transpiration (m d^{-1})" is now replaced by "evapotranspiration rate (in m d^{-1})"

- L.163: literature- Chapter 2.4: The explanation and equations make sense to me, but for a detailed evaluation and/or comments on the equations a true modeler might be considered (e.g. M. Cuntz, Wingate/Ogee group)

This is true! We have already received a comprehensive review from referee #1 on the modeling aspects of our work which we hope to have properly addressed in our answer.

- Chapter 3.1.1: Figure 2 is mentioned first in the text, then Figure 1....hence, those might be switched Results/Discussion

We make reference to Fig. 1 at L187-188 (under section "2.4 Modeling of RWU and δ_{tiller}"), thus before citing Fig. 2 (section 3.1.1).

- L.243-247 and Fig. 2: There were two soil moisture profiles measured, but only one is shown in Fig.2 (or is that averaged over the two?) I am not sure if that justification that no evaporation was present is sufficient, as the moisture profiles oscillate greatly and over one or two days the effect of evaporation might be minimal (which on the other hand supports the assumption ET=T). Still, evaporation is probably occurring (though at a low rate).

There were 1 profile taken per sampling time, thus four profiles are shown in Figure 2: DaS 166 - 15:45 (orange line), DaS 167 - 07:00 (blue), DaS 167 - 15:45 (red), and DaS 168 - 05:00 (black)

We agree with the reviewer that evaporation could have been partly the reason of the observed differences in water content at the soil surface across sampling times, the other reason being the lateral heterogeneity. We can only make the assumption that evapotranspiration = transpiration, assumption that we carefully mention, based also on the very negative values of soil water potential in shallow layers.

- L.247-249: Yes, but these three options should be discussed by the authors

We now strengthen the discussion at this point and added (L275-285):

> "The differences in soil water oxygen isotopic profile observed at the four different sampling dates were therefore either due to lateral heterogeneity (e.g., upper soil layers), to the soil capillary rise of labelled water from the reservoir (deep soil layers), or to the hydraulic redistribution of water through roots (to the condition that the isotopic composition of the redistributed water differs from that of the soil water at the release location). We note an isotopic enrichment of 1.0 ‰ of soil water observed on DaS 168 - 05:00 at –0.9 m with respect to the mean δsoil value across previous sampling dates. This could partly be due to, e.g., upward preferential flow of labelled water from the bottom soil layers and therefore be the sign of the lateral heterogeneity of the soil. Another reason for this would be hydraulic redistribution of labelled water by the roots. It was however not possible to evaluate the relative importance of these three processes (lateral heterogeneity, capillary rise/preferential flow, and hydraulic redistribution) in the setting of the soil water isotopic profile since the physically-based soil-root model presented in section 2.4 does not account for soil liquid and vapor flow. This was also not the primary intent of the present study."

- L. 250: minimal minimum instead maximal maximum

Thank you. Done.

- L. 252: delete level

Done. We replaced "level" by "value".

- 3.1.2: Again, if results/discussion is mixed here, those differences and diurnal patterns should be discussed and explained here

We agree that section 3.1.2 (as well as 3.1.1) stays rather descriptive. It is the case because we choose to discuss both soil and plant isotopic data in section 3.1.3 by cross-comparing them with soil and plant hydraulic data.

- L.269: 'Rayleigh distillation corrections' – this is not explained in the methods. Could the authors provide details on these corrections and/or provide a citation?

We now add two references to these corrections and how they should be applied:

> "Galewsky, J., Steen-Larsen, H. C., Field, R. D., Worden, J., Risi, C., and Schneider, M.: Stable isotopes in atmospheric water vapor and applications to the hydrologic cycle, Rev. Geophys., 54, 809-865, doi:10.1002/2015rg000512, 2016."
>
> "Dansgaard, W.: Stable Isotopes in Precipitation, Tellus, 16, 436-468, doi:10.1111/j.2153-3490.1964.tb00181.x, 1964."

- 3.1.3: Well-written and explained

Thank you ☺

- L.298: yes, but still: 3 per mill is notable for 18O...

Indeed, we agree with this comment. We clarified that a difference of 2.9 ‰ between simulated and measured mean δ_{tiller} is notable, though relatively small compared to the datasets standard deviations (8.4 ‰) and to the isotopic ratio of the labelled water (470 ‰; non-labelled soil water isotopic ratio between -7.4 ‰ and 1.3 ‰). Statistically we could not systematically conclude that simulated and measured δ_{tiller} differed. By drawing randomly simulated δ_{tiller} in 3 plants at each time step (as in the measurements), comparing the overall distributions of measured and simulated pooled δ_{tiller} with an ANOVA analysis, and repeating the random drawings for all 40 observation times 100 times, measured and simulated δ_{tiller} distributions were not statistically different in 92% of drawings (P>0.01). We reformulated the sentence as (now L336-339):

> "The predicted versus observed δ_{tiller} distributions including all plant groups and observation times differed noticeably but not significantly (6.6 ± 8.4 ‰ and 3.7 ± 8.4 ‰, respectively) when pooling 3 simulated δ_{tiller} randomly at each observation time, as in measurements (p-value>0.01 in 92 cases out of 100 repeated drawings)"

- L.325: I am not sure if an 0,9 per mil increase is significant...were replicates taken for each soil depth? What is the std of those (-often this can be in that range already)....if no replicates were taken, this might be well within the uncertainty rather than a true increase

The observed δ_{soil} at the first three observation times are -7.17 ‰, -7.00 ‰, and -7.21 ‰. We confirm that it differs from -6.2 ‰ with an ANOVA analysis (P<0.01). This was clarified in the revised version of the manuscript (L365-366):

"…(–6.2 ‰, a value significantly higher than –7.1 ‰ ± 0.1 ‰ at earlier times based on ANOVA analysis, P<0.01)…"

- L.327 depths instead heights

Done. Thank you!

- L.345 model instead models

Done.

- L. 360 upper half instead first half

Done. It now reads (L438):

> "In the upper half of the profile…"

- L.363-365: But couldn't this be implemented to the Bayesian approach via the construction of priors?

Thank you, this is a very keen remark. It is true that we decided to go for (Appendix E, L733-734):

> “flat Dirichlet a priori rRWU distribution (i.e., rRWUJ=1/10)”

We were missing an explanation on why we did not implement the construction of priors. This is not done (L442-448):

> “Note that the outcome of the statistical model may significantly depend on the definition of the a priori relative RWU profile. In the present study, we set it to follow a “flat” distribution (i.e., rRWUJ = 1/10, see Appendix E), in other word, each layer was initially defined to contribute equally to RWU. To the contrary of other studies (e.g., Mahindawansha et al., 2018), where the a priori rRWU profile was empirically constructed on basis of soil water content and root length density profiles, we decided not to further arbitrarily constrain the Bayesian model for the sake of comparison with the physically-based soil-root model.”

Figure 2:
- it’s 18O data shown, could this be added to the title (instead of only delta)...OK it’s in the figure description, still...

We hope mention of “oxygen” in the title and now repeatedly throughout the manuscript has clarified this.

- why is matric potential ‘calculated’ shown if it was measured?

ψ_{soil} was calculated on basis of θ data, and not directly measured. We now clarify this confusion by moving the mention of soil matric potential to section 2.2 (L141-142):

> “It was used to compute the soil water matric potential (ψsoil, in MPa) on basis of volumetric water content data during the present experiment.”

In addition, we added “Measured” in Fig. 2’s caption:

> “Measured soil volumetric water content (θ, panel a), oxygen isotopic composition (δ_{soil}, panel b), and calculated soil matric potential (ψ_{soil}, panel c) profiles during the sampling period”

- Not sure if the inset graphic for the water content is helping the figure

Done. The inset was removed from Fig. 2 in the revised version.

Anonymous Referee #3

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.

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).

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

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).

To address this general concern, we added a section 3.3 entitled "Progresses and Challenges in soil water isotopic labeling for RWU determination" (L450-476):

> "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., 2017a; Kühnhammer et al., 2019].
> 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 worlds: 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 mechanistic 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 [e.g., Rothfuss et al., 2013; Quade et al., 2019; Volkmann et al., 2016a] as alternatives of the widely used combination of destructive sampling and offline isotopic analysis following cryogenic vacuum extraction [Orlowski et al., 2016b] 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."

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.

We totally agree with the referee. Our analysis shows that the tiller water isotopic signature is very sensitive to rooting depth in this 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), and continuous determination of soil and plant water isotopic composition (e.g., Rothfuss et al., 2013; Quade et al., 2019; Volkmann et al., 2016) might take us one step closer to a validation. This is now discussed in the new section 3.3.

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.
- 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 d). 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 fluctuations 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 was added in the revised manuscript (L423-431):

> "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 d). This suggests that in this setup the hydraulic conductance of the soil-root system limited shoot water supply more than the distribution of roots, as in Sulis et al. (2019). Simulated baseline (i.e. for uniform transpiration rates) leaf water potentials are shown as grey lines in Figure 5 panel c, and measured leaf water potentials as a green line in the same panel. The fact that they match well despite the high sensitivity of leaf water potential to transpiration rate, reinforces the idea that transpiration rate was likely not spatially heterogeneous among the plant population. Therefore, the tiller water isotopic signature, whose sensitivity to transpiration rate is already very low, was likely not affected by transpiration rate heterogeneity."

Detailed comments:

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

We list in the few sentences after (now L21-24) the monitored variables in question. We removed "semi-controlled" (indeed a vague term) and "such variables" from the text. It now reads (L20-21):

> "In this study, a population of tall fescue (Festuca arundinacae cv Soni) was grown in a macro-rhizotron and monitored for a 34-hours long period following the oxygen stable isotopic (^{18}O) labeling of deep soil water"

Line 23-24: "results underlined the discrepancy.." At this point unclear what is meant

We now put emphasis on "temporal disconnection" instead of "discrepancy" (L24-26):

> "While there were strong correlations between hydraulic variables as well as between isotopic variables, the experimental results underlined the partial disconnection between temporal dynamics of hydraulic and isotopic variables."

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

The sentence was clarified in the revised version of the manuscript (L31-33):

> "The physical model thus explained the discrepancy between isotopic and hydraulic observations: the variability captured by δ_{tiller} was spatial and may not correlate with the temporal dynamics of ψ_{leaf}."

Line 33: "local increase..." this results is not stated earlier and at this position confusing.

The "local increase" was not mentioned earlier, indeed. We write now instead (L36-37):

> "It further supported that concomitant increases of soil water content and isotopic composition observed overnight above the soil region influenced by the labeling were due to hydraulic lift."

Lines 35-62 List of variables. Some variables are missing, please complete. Also I propose erasing all the repetitions of "units of". Later in the paper, it will be useful to express volumetric water content as vol-% and I propose adding it here.

Done. We added the missing two variables, namely the soil hydraulic conductivity parameter (λ, dimensionless) and the soil relative water content (Se_j). In addition, repetitions of “in units of” were discarded from the list of variables. Instead, we added L39 headers to the table “Name”, “Symbol”, and “Units”.

Introduction

Line 75-76: The description of the “mean value of...weighted by” is confusing, please rephrase
Done. The sentence now reads (L81-82):

> “equals the sum of the product between the soil water isotopic composition and relative contribution to RWU across plant water sources.”

Material and Methods

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.
Done. It now reads (L124):

> “The rhizotron was equipped with two sets of CS616 time domain reflectometer (TDR) profiles”

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?
There is no typo here. But we agree that it was not properly formulated, and that the substrate which we filled the rhizotron with could not possibly be referred to as a “cambisol”. Therefore we write now (L134):

> “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%).”

Line 125: Add “layer” before “by”
We propose the following reformulation (L137-138):

> “450 kg of soil was filled in the rhizotron by 0.10 m increment a..”

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
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 do not mention the type of sensors used – which was indeed misleading – of we write (L138-141):

> “The closed-form soil water retention curve of 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).”

Lines 132-136: Please shortly state: Were the plants watered? What was the lower boundary condition?
Done. We added the information (L149-151):

> “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.”

Line 139: Do you mean “sides” instead of “slides”
Yes, exactly, many thanks for finding this typo!

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

We sampled the entire plant. We now insert “whole” after “three” (L163)

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.

Thank you for these propositions! We now write (L166-167):

> “In addition, air water vapor was collected from the ambient atmosphere surrounding rhizotron”

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

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” (“group” in the revised MS) 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 group. This is an important point, which we clarified in the revised manuscript (L184-191):

> “The experimental setup included about 300 tall fescue plants. In order to limit the computational requirement in the inverse modelling loop, we only generated 60 virtual root systems whose rooting depths ranged from –1.30 to –1.60 m depth (based on our own observations and those of the litterature, e.g., Schulze et al., 1996; Fan et al., 2016) with the root architecture simulator CRootBox (Schnepf et al., 2018), so that the simulated RLD matched observations (Fig. 1a). In order to reach the right amount of plants, each root system was replicated 5 times, forming a “group”. Each group was assumed to occupy one sixtieth of the total horizontal area, and considered as a “big root” hydraulic network (5 identical plants per “big root”) with equivalent radial and axial hydraulic conductances (thus neglecting architectural aspects but accounting for each group’s respective root length density profile).”

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?

This equation is indeed not obvious. It was derived by Meunier et al. (2017) in its Appendix C. The reference was indeed missing. We added it to the revised manuscript (L193):

> “, as derived by Meunier et al. (2017),”.

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

The horizontal domain of simulation typically has two dimensions (X and Y), but it some cases, the studied problem has an essentially radial dimension between bulk soil and root surface.

We simplified the text as follows (L196-197):

> “with B_j (dimensionless) a geometrical factor simplifying the horizontal dimensions into radial domains between the bulk soil and root surfaces,…”.

Line 176-177: Sentence starting with “The averaged distance ..” seems wrong. Maybe erase the last words?

The referee is correct. The reported sentence referred to ρ, not to the average distance between roots. We corrected the text as follows (L200-201):

> “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.

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

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

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

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.

We apologize for the confusion, and included this clarification at this point in the revised manuscript (L215-216):

> "were calculated as equivalent "big root" specific axial conductance per root system group (k_{axial}, m^4 MPa^{-1} d^{-1}, to be optimized by inverse modelling) as"

Line 191: "root system class" Unclear what is meant with "class".

See reply to comment on simulated 60 root systems.

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

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

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.

That is a good point. The leaf is a "big leaf", and the soil water potential used in the definition of Ksoil-root is the SSF-averaged bulk soil water potential. We clarified it in the text (L220-223):

> "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 $K_{\mathrm{soil\text{-}root}}$ 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, [Steudle and Peterson])".

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

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 (Steudle and Peterson, 1998). Reference added in the text (L223).

Line 202: "class" unclear

See previous reply about the 60 root systems.

Line 205: "where axial conductances" this comes too late, please move up.

It was in the right place, but we rewrote the sentence for clarification (L231-232):

> "where $K_{\mathrm{soil-root}}$ was assumed to control the compensatory RWU which arise from a heterogeneously distributed soil water potential, due to large axial conductances (Couvreur et al., 2012)".

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

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.

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

Done. We now give the 10 soil layers' upper/lower boundaries L252-255:

> "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)…"

Results and discussion:

Lines 335-252: Small issue: Please add some paragraphs in this section.
Done. We now 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.

Line 281: Do you mean “and” instead of “et”
Done, thank you!

Line 304: With “all the population” do you mean all individuals?
Indeed. We replaced it by “all individuals” for clarification (L343-344).

Line 311-313: Sentence is difficult to understand, please rephrase.
We rephrased as follows (L350-352):

> “As no correlation could be expected between the drivers (the maximum rooting depth of the sample plants and canopy transpiration rate) our analysis explains the absence of correlation between δ_{tiller} and ψ_{leaf} or transpiration rate”.

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

Line 340 Lambda is not in the list of variables.
λ has been added to the list of variables, thank you.

Also, this information is very compact, and difficult to understand. Please elaborate.
The following clarification was added (L384-387):

> “The optimal value of k_{sat} was quite high (Table 1) but reportedly very correlated to λ (i.e. soil unsaturated hydraulic conductivity is proportional to k_{sat}, but also to Se^{λ} (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”.

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.
Done. Figure E is now in the main document under “Figure 7”.

Line 360: Replace “first” with “top”
Done. Thank you.

Line 367: Not sure what is meant with “broadcasted”
We don’t use the term “broadcasted” anymore and write instead (L491-493):

> “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.”

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_soil because of psi_leaf(although I seriously think it would not be an issue), please obviously state the reference elevation to avoid this type of confusion.

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

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~~Disentangling temporal~~The rollercoaster and ~~population variability in~~ the swarm: disentangling plant ~~root~~ water ~~uptake from stable~~ isotopic composition variabilities in response to soil water labelling ~~analysis: a labeling study~~

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Abstract. Isotopic labeling techniques have the potential to minimize the uncertainty of plant root water uptake (RWU) profiles estimated through multi-source (statistical) modeling, by artificially enhancing soil water isotopic gradient. ~~Furthermore~~On the other end of the modelling continuum, physical models can account for hydrodynamic constraints to RWU if simultaneous soil and plant water status data is available.

In this study, a population of tall fescue (*Festuca arundinacae* cv Soni) was grown in a macro-rhizotron ~~setup under semi-controlled conditions to monitor such variables~~ and monitored for a 34-hours long period following the oxygen stable isotopic (^{18}O) labeling of deep soil water. Aboveground variables included tiller and leaf water oxygen isotopic compositions (δ_{tiller} and δ_{leaf}) as well as leaf water potential (ψ_{leaf}), relative humidity, and transpiration rate. Belowground profiles of root length density (RLD), soil water content and isotopic composition were also sampled. While there were strong correlations between hydraulic variables as well as between isotopic variables, the experimental results underlined the ~~discrepancy between variations~~ partial disconnection between temporal dynamics of hydraulic and isotopic variables.

In order to dissect the problem, we reproduced both types of observations with a one-dimensional physical model of water flow in the soil-plant domain, for 60 different realistic RLD profiles. While simulated ψ_{leaf} followed clear temporal variations with little differences across plants as if they were "on board of the same rollercoaster", simulated δ_{tiller} values within the plant population were rather heterogeneous ("swarm-like") with relatively little temporal variation and a strong sensitivity to rooting depth. The physical model thus ~~suggested that~~explained the discrepancy between isotopic and hydraulic observations ~~was~~

~~logical, as~~: the variability captured by ~~the former~~ δ_{tiller} was spatial and may not correlate with the temporal dynamics of ~~the latter~~ ψ_{leaf}.

For comparison purposes, a Bayesian statistical model was also used to simulate RWU. While they predicted relatively similar cumulative RWU profiles, the physical model could differentiate spatial from temporal dynamics of the isotopic signature~~, and~~. It further supported that ~~the local~~ concomitant increases of soil water content ~~and formation of a peak of labelled water~~ and isotopic composition observed overnight above the soil region influenced by the labeling were due to hydraulic lift.

List of variables with symbols and units

Name	Symbol	Units
Leaf water potential/head:	ψ_{leaf}	~~in units of~~ MPa
Soil water potential/head:	ψ_{soil}	~~in units of~~ MPa
Water volumetric mass:	ρ_w	~~in units of~~ kg m^{-3}
Soil apparent density:	ρ_b	~~in units of~~ kg m^{-3}
Soil gravimetric water content:	θ_{grav}	~~in units of~~ kg kg^{-1}
Soil volumetric water content:	θ	~~in units of~~ m^3 m^{-3}
Intensity of water uptake (sink term):	S	~~in units of~~ d^{-1}
Transpiration rate per unit soil area:	T	~~in units of~~ m d^{-1}
Air relative humidity	RH	~~in units of~~ %
Soil horizontal area:	A_{soil}	~~in units of~~ m^2
Soil layer depth (for each layer):	z	~~in units of~~ m
Soil layer thickness (for each layer):	ΔZ	m ~~in units of~~ ~~m~~
Root length (for each soil layer):	l_{root}	~~in units of~~ m
Relative Root Water Uptake	rRWU	dimensionless
Best run	br	dimensionless
Root Length Density:	RLD	~~in units of~~ m m^{-3}
Soil water oxygen isotopic composition:	δ_{soil}	~~in units of~~ ‰
Tiller water oxygen isotopic composition:	δ_{tiller}	~~in units of~~ ‰
Leaf water oxygen isotopic composition:	δ_{leaf}	~~in units of~~ ‰
Soil-root system conductance:	$K_{soil\text{-}root}$	~~in units of~~ m^3 MPa^{-1} s^{-1}
Soil-root radial conductance:	K_{radial}	~~in units of~~ m^3 MPa^{-1} s^{-1}
Root radial conductivity:	L_{pr}	~~in units of~~ m MPa^{-1} s^{-1}
Root axial conductance:	K_{axial}	~~in units of~~ m^3 MPa^{-1} s^{-1}
Equivalent root axial conductivity:	k_{axial}	~~in units of~~ m^4 MPa^{-1} s^{-1}
Soil hydraulic conductivity:	k_{soil}	~~in units of~~ m^2 MPa^{-1} s^{-1}
Saturated soil hydraulic conductivity:	k_{sat}	~~in units of~~ m^2 MPa^{-1} s^{-1}
Soil hydraulic conductivity parameter	λ	dimensionless
Soil relative water content	Se_j	dimensionless

1 Introduction

~~Since the seminal work of Washburn and Smith (1934)~~Since the seminal work of Washburn and Smith [1934] where it was first reported that willow trees did not fractionate hydrogen stable isotopes in a hydroponic water solution during root water uptake (RWU), water stable isotopologues ($^{1}H^{2}H^{16}O$ and $^{1}H_{2}{}^{18}O$) have been used as indicators for plant water sources in soils. ~~In their review, Rothfuss and Javaux (2017)~~In their review, Rothfuss and Javaux [2017] reported in the period 2015-2016 about no less than 40 publications in which RWU was retrieved from stable isotopic measurements. Novel measuring techniques (e.g., cavity ring-down spectroscopy – CRDS and off-axis integrated cavity output spectroscopy – ICOS) providing ways for fast and cost-effective water stable isotopic analyses certainly enable and emulate current research in that field. Water stable isotopologues are no longer powerful tracers waiting for technological developments ~~(Yakir and Sternberg, 2000)~~[Yakir and Sternberg, 2000] but are on the verge to be used to their full potential for addressing eco-hydrological research questions and identify processes in the soil-plant-atmosphere continuum ~~(Werner et al., 2012; Dubbert and Werner, 2019; Sprenger et al., 2016).~~[Werner et al., 2012; Dubbert and Werner, 2019; Sprenger et al., 2016].

The isotopic determination of RWU profiles is based on the principle that the isotopic composition of xylem water at the outlet of the root system (i.e., in the first aerial and non-transpiring node of the plant) equals the ~~mean value~~sum of the product between the soil water isotopic composition ~~across contributing sources, weighted by their~~and relative contribution to RWU across plant water sources. Results come only with reasonable precision when (i) the soil water isotopic composition depth gradient is strong and monotonic (thus avoiding issues of identifiability) and (ii) the temporal dynamics of RWU and soil water isotopic composition is relatively low. Condition (i) is fulfilled mostly at the surface of the soil, while soil water isotopic composition gradients become usually lower or null with increasing depth (due to the isotopic influence of the groundwater table~~). Condition (ii) is often neglected but is required due to the instantaneous nature of the sap flow samples. As illustrated by Oerter and Bowen (2019),~~ and increasing dispersion with depth). As illustrated by Oerter and Bowen [2019], the lateral variability of the soil water isotopic composition profiles can become significant in the field and could have great implications on the representability and meaningfulness of isotopic-derived estimate of RWU profiles. Condition (ii) is often neglected but is required due to the instantaneous nature of the sap flow samples.

To overcome these limitations, labeling pulses have been increasingly used in recent works to artificially alter the natural isotopic gradients ~~(e.g., Beyer et al., 2016; Beyer et al., 2018; Grossiord et al., 2014; Jesch et al., 2018; Volkmann et al., 2016).~~[e.g., Beyer et al., 2016; Beyer et al., 2018; Grossiord et al., 2014; Jesch et al., 2018; Volkmann et al., 2016b]. However, a precise characterization of the artificial spatial (i.e., lateral and vertical) and temporal distributions of the soil water isotopic composition (driven by e.g., soil isotopic water flow) is crucial. The punctual assessments of the isotopic composition profiles following destructive sampling in the field and subsequent extraction of water in the laboratory might neither be spatially nor temporally representative and can lead to erroneous estimates of RWU profiles ~~(Orlowski et al., 2018; Orlowski et al., 2016).~~[Orlowski et al., 2018; Orlowski et al., 2016a].

The vast majority of isotopic studies use statistical (e.g., Bayesian) modeling to retrieve RWU profile solely from the isotopic composition of water extracted in the soil and the shoot ~~(Rothfuss and Javaux, 2017).~~[Rothfuss and Javaux, 2017]. However, when data on soil and plant water status is available, hydraulic modeling tools can also be used to connect different data types in a process-based manner and estimate root water uptake profiles ~~(Passot et al., 2019).~~[Passot et al., 2019]. Some of the most simplistic models use 1-D relative root distribution and plant-scale hydraulic parameters ~~(Sulis et al., 2019),~~[Sulis et al., 2019], while the most complex rely on root architectures and root segment permeabilities ~~(Meunier et al., 2017c).~~[Meunier et al., 2017c]. Only a handful of studies coupled isotopic measurements in plant tissues and soil material with models describing RWU in a mechanistic manner. For instance, ~~Meunier et al. (2017a)~~Meunier et al. [2017a] could both locate and quantify the volume of redistributed water by *Lolium multiflorum* by labeling of the soil with ^{18}O enriched water under controlled conditions.

~~Building on the work of Meunier et al. (2017a), the objective of the present study is to investigate on the plant and environmental factors that affect the tiller xylem water isotopic composition in a population of *Festuca arundinacae* cv Soni. (tall fescue), in order to unveil pitfalls and opportunities in methods used to predict RWU profiles from stable isotopic analyses following a labeling pulse.~~

Building on the work of Meunier et al. [2017a], the objective of the present study is to (i) model in a physically-based manner (i.e., by accounting for soil and plant and environmental factors) the temporal dynamics of the isotopic composition of RWU of a population of *Festuca arundinacae* cv Soni. (tall fescue) during a semi-controlled experiment following an isotopic labeling of deep soil water, (ii) investigate the implication of the model-to-data fit quality in terms of meaningfulness of the isotopic information to reconstruct RWU profiles, and finally (iii) confront the simulated root water uptake profiles with estimations obtained on basis of isotopic information alone (i.e., provided by a Bayesian mixing model).

2 Material and methods

Our experiment consisted in supplying labeled water from the bottom to a macro-rhizotron in which tall fescue was grown. Data on soil and plant oxygen stable isotopic signature and hydraulic status were monitored for 34 hours. In the following, the oxygen isotopic composition of water will be expressed in per mil (‰) on the "delta" (δ^{18}O) scale with respect to the international water standard V-SMOW ~~(Gonfiantini, 1978).~~[Gonfiantini, 1978].

2.1 Rhizotron experimental setup

The macro-rhizotron (dimensions: 1.6 m x 1.0 m x 0.2 m, see picture in Appendix A) was placed inside a glasshouse (INRA Lusignan, France), where it was continuously weighed (KE1500, Mettler-Toledo, resolution: 20 g) to monitor water effluxes (i.e., bare soil evaporation or evapotranspiration). Underneath the soil compartment and in contact with it, a water reservoir (height: 0.1 m) filled with gravel acted as water table and allowed the supply of water to the rhizotron. The rhizotron was equipped with two sets of CS616 ~~water content reflectrometer~~ time domain reflectometer (TDR) profiles (Campbell Scientific, USA) with 30 cm long probe rods positioned at six depths (–0.05, –0.10, –0.30, –0.60, – 1.05 and –1.30 m) and one profile of tensiometers (SMS 2000, SDEC-France) located at four depths (–0.05, –0.10, –0.30, and –0.60 m) in order to monitor the ~~evolutions~~evolution of soil water volumetric content (θ, in m^3 m^{-3}) and matric potential (ψ_{soil}, in MPa). Finally, relative humidity (RH, %) was recorded above the vegetation with one humidity and temperature probe (HMP45D, Vaisala, Finland). The transparent polycarbonate sides (front and back) allowed the daily observations of root maximal depth. The experimental setup allowed precisely controlling the amount and δ^{18}O of soil input water. Another important feature was the soil depth (i.e., 1.60 m) which minimized the influence of the water table on superficial layers water content and δ^{18}O.

2.2 Soil properties and installation

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~~%) with a dry bulk density of ρ_b = 1420 kg m^{-3}.~~%). Prior installation in the rhizotron, the substrate was sieved at 2 mm and dried out in an air oven at 110 °C during 48 h to remove most of the residual water. 450 kg of soil ~~were installed~~ was filled in the rhizotron by ~~layer of~~ 0.10 m increment and compacted in order to reach ~~the above-mentioned~~ a dry bulk density value of ρ_b = 1420 kg m^{-3}. The closed-form soil~~dry bulk density. Soil~~ water retention ~~data~~ curve of van Genuchten [1980] was derived in a previous study by Meunier et al. [2017a] from synchronous measurements of soil water content ~~(CS616 soil water reflectometers, Campbell Scientific, USA) and~~ and matric potential ~~(Sols – PST55 micro-psychrometers, WESCOR, and SMS 2000 tensiometers, SDEC)~~ from saturated to residual water content~~. The closed-form water retention curve (van Genuchten, 1980) was then parametrized by minimizing the root mean square difference between its position and measured~~ (see Appendix B for its hydraulic parameters). It was used to compute the soil water ~~content~~ matric potential (ψ_{soil}, in MPa) on basis of volumetric water content data during the present experiment.~~couples (Appendix B).~~

2.3 Experimental protocol

After installation, the soil was gradually flooded with local water (δ^{18}O = –6.8 ‰) from the bottom reservoir up to the top of the profile for a period of three days in order to reduce as much as possible the initial lateral and vertical heterogeneities in water content and δ^{18}O. The tall fescue (*Festuca arundinaceae* cv Soni) was sown at a seeding density of 3.6 g m^{-2} (which corresponds for the rhizotron surface area of 0.2 m^2 to roughly 300 plants) when soil water content reached 0.25 m^3 m^{-3} (corresponding to pF 2.3) at –0.05 m, as measured by the soil water sensors, and emerged 12 days later. 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 δ^{18}O profile.

166 days after seeding (DaS 166) the following conditions were fulfilled: (i) there was a strong soil water content gradient between the soil deep [–1.5 m, –1.0 m] and superficial [–0.3 m, 0 m] layers, (ii) the tall fescue roots had reached a depth of –1.5 m (observed through polycarbonate transparent ~~slides~~sides). That same day at 17:00, the reservoir's water was labelled and its ~~isotopic composition~~δ^{18}O measured at +470 ‰. Soil was sampled ~~on four occasions from the surface down to –1.3 m:~~

~~before labeling~~before (DaS 166 - 15:45) and after labeling on DaS 167 - 07:00, DaS 167 - 17:00 and DaS 168 - 05:00 using a 2 cm diameter auger through the transparent polycarbonate side of the rhizotron on four occasions from the surface down to –1.3 m for the determination of soil gravimetric water content (θ_{grav}, in kg kg^{-1}) and oxygen stable isotopic composition (δ_{soil}, in ‰). Gravimetric water content was then converted to volumetric water content ($\theta = \theta_{grav}*\rho_b/\rho_w$, in m^3 m^{-3}, where ρ_b is the bulk soil density and ρ_w is the water density). The hypothesis of a constant value for ρ_b across the reconstructed soil profile was further validated from the quality of the linear fit (coefficient of determination $R^2 = 1.0$) between the θ values measured by the sensors at the six available depths and (–0.05, –0.10, –0.30, –0.60, – 1.05 and –1.30 m) and those computed from θ_{grav}.

On 40 occasions during a 34-hour long period three whole plants were sampled from the vegetation (i.e., 120 plants were sampled in total from the cover). Each plant's ~~tillers~~tiller and leaves were pooled into two separate vials. Dead material as well as the oldest living leaf around each tiller were removed in order not to contaminate tiller samples with transpiring material ~~(Durand et al., 2007). In addition, air water vapor was collected from the atmosphere of the surrounding of the rhizotron.~~[Durand et al., 2007]. In addition, air water vapor was collected from the ambient atmosphere surrounding the rhizotron. The air was run at a flow rate of 1.5 l min-1 through two glass cold traps in series immersed in a mixture of dry ice and pure ethanol at - 80°C. Water from plant (i.e., tillers~~, –~~ and leaves~~,~~) and soil samples ~~were~~ was extracted by vacuum distillation ~~and their~~ for 14 to 16 hours depending on the sample mass (e.g., ranging between 18 to 28 g for soil) at temperatures of 60 and 90°C, respectively. The residual water vapor pressure at the end of each successful extraction procedure invariably reached 10^{-1} mbar. The oxygen isotopic compositions of tiller, leaf, and soil water (i.e., δ_{tiller}, δ_{leaf}, and δ_{soil}) together with that of atmospheric water vapor (δ_{atm}) were measured with an IRMS (Isoprep 18 - Optima, Fison, Great-Britain, precision accuracy of 0.15 ‰). Finally, leaf water potential (ψ_{leaf}, in MPa) was monitored with a pressure chamber on two leaves per sampled plant, and ~~transpiration (*T*,~~evapotranspiration rate (in m d^{-1}) was derived from the changes in mass of the rhizotron at the same temporal scale as plant sampling.

Root biomass was determined from the horizontal sampling of soil between the polycarbonate sides using a 2 cm diameter auger at –0.02, –0.08, –0.10, –0.40, –0.55, –0.70, –0.90, –1.10, and –1.30 m soil depth. Each depth was sampled once to thrice. Each soil core was washed of soil particles and roots were collected over a 0.2 mm mesh filter, and dried at 60°C for 48 hours. Finally, Root Length Density (RLD, in m root (m soil)$^{-3}$) distribution was determined from the root dry mass using the specific root length determined by ~~Gonzalez-Dugo et al. (2005)~~Gonzalez-Dugo et al. [2005] specifically for tall fescue (95 m ~~root (~~g ~~root) –~~$^{-1}$). The reader is referred to Appendix C for an overview of the type and timing of the different destructive measurements during the intensive sampling period.

2.4 Modeling of RWU and δ_{tiller}

~~60 tall fescue root systems with rooting depths ranging from –1.30 to –1.60 m depth (based on our own observations and those of the litterature, e.g., Schulze et al., 1996; Fan et al., 2016) were modeled to represent the community in the rhizotron. The root systems were generated with the root architecture simulator CRootBox (Schnepf et al., 2018) so that the simulated RLD matched observations (Fig. 1a). Each simulated root system was considered as representative of a "class" among the plant~~

~~population. Each root system class was assumed to occupy one sixtieth of the total horizontal area. To simulate RWU by the 60 classes while limiting the computing time in the inverse modeling scheme, we considered each of them as a “big root” hydraulic network with equivalent radial and axial hydraulic conductances (thus neglecting architectural aspects but accounting for their respective root length density profiles).~~

The experimental setup included about 300 tall fescue plants. In order to limit the computational requirement in the inverse modelling loop, we only generated 60 virtual root systems whose rooting depths ranged from –1.30 to –1.60 m depth [based on our own observations and those of the literature, e.g., Schulze et al., 1996; Fan et al., 2016] with the root architecture simulator CRootBox [Schnepf et al., 2018], so that the simulated RLD matched observations (Fig. 1a). In order to reach the right amount of plants, each root system was replicated 5 times, forming a “group”. Each group was assumed to occupy one sixtieth of the total horizontal area, and considered as a “big root” hydraulic network (5 identical plants per “big root”) with equivalent radial and axial hydraulic conductances (thus neglecting architectural aspects but accounting for each group’s respective root length density profile).

The radial soil-root conductance between the bulk soil and each ~~class’s~~group’s (i) root surfaces in soil layer j ($K_{\mathrm{radial,j}}$, $\mathrm{m^3\ MPa^{-1}\ d^{-1}}$), as derived by Meunier et al. [2017a], was assumed as variable in time (t):

$$\cancel{K_{\mathrm{radial},i,j}}K_{radial,i,j}(t) = \cancel{\frac{2\pi r_{\mathrm{root}}\cdot l_{\mathrm{root},i,j}\cdot B_j\cdot L_{\mathrm{pr}}\cdot k_{\mathrm{soil},j}(t)}{B_j\cdot k_{\mathrm{soil},j}(t)+r_{\mathrm{root}}\cdot L_{\mathrm{pr}}}}\frac{2\pi r_{root}\cdot l_{root,i,j}\cdot B_j\cdot L_{pr}\cdot k_{soil,j}(t)}{B_j\cdot k_{soil,j}(t)+r_{root}\cdot L_{pr}} \tag{1}$$

~~with B (dimensionless) a geometrical factor approximating the dimension of the domain between the bulk soil and root surface as radial, as given by Schroeder et al. (2009):~~

with r_{root} (m) the root radius, $l_{\mathrm{root},i,j}$ (m) the root length of plants of group i in soil layer j, L_{pr} ($\mathrm{m\ MPa^{-1}\ d^{-1}}$) the root radial hydraulic conductivity, $k_{\mathrm{soil},j}$ ($\mathrm{m^2\ MPa^{-1}\ d^{-1}}$) the soil hydraulic conductivity in layer j, and B_j (dimensionless) a geometrical factor simplifying the horizontal dimensions into radial domains between the bulk soil and root surfaces, as given by Schroeder et al. [2009]:

$$B_j = \frac{2(1-\rho_j)(1+\rho_j)}{2\rho_j{}^2 ln\rho_j - \rho_j{}^2 + 1} \tag{2}$$

where ρ (dimensionless) represents the ratio of the distance between roots and the root averaged diameter. ~~The averaged distance between roots~~It can be deduced from the observed root length density (RLD_j, $\mathrm{m\ m^{-3}}$~~) and root radius (r_{root}, m~~):

$$\rho_j = \cancel{\frac{\sqrt{\frac{1}{\pi \mathrm{RLD}_j}}}{r_{\mathrm{root}}}}\frac{\sqrt{\frac{1}{\pi RLD_j}}}{r_{root}} \tag{3}$$

~~In Eq. (1), the soil hydraulic conductivity function of Mualem (1976) and van Genuchten (1980) was used:~~

~~$k_{\mathrm{soil},j}$~~The soil hydraulic conductivity function of Mualem [1976] and van Genuchten [1980] was used:

$$k_{soil,j}(t) = \cancel{k_{\mathrm{sat}}}k_{sat}\cdot Se_j{}^{\lambda}(t)\cancel{\left(1-\left(1-Se_j{}^{\frac{1}{\mathrm{m}}}\right)^{\mathrm{m}}\right)^2}\left(1-\left(1-Se_j{}^{\frac{1}{m}}\right)^{m}\right)^2 \tag{4}$$

where k_{sat} (m^2 MPa^{-1} d^{-1}), ~~),~~ m (dimensionless) and λ (dimensionless) are soil hydraulic parameters (with m = 1 – 2/n) and Se_j, the relative water content (dimensionless), is computed from the saturated (θ_{sat}, m^3 m^{-3}) and residual (θ_{res}, m^3 m^{-3}) water contents as:

$Se_j =$ ~~$\frac{\theta_j - \theta_{res}}{\theta_{sat} - \theta_{res}}$~~ $\frac{\theta_j - \theta_{res}}{\theta_{sat} - \theta_{res}}$ (5)

~~The last term to define in Eq. (1) is the root length in each soil layer (l_{root}, m).~~ Unlike the geometrical parameter B, which defines a domain geometry between the bulk soil and roots of the overall population, the l_{root} term is ~~class~~group specific (i) and uses the simulated root length density profiles over an area corresponding to one sixtieth of the total setup horizontal area:

~~$l_{root,i,j}$~~ $l_{root,i,j} =$ ~~$\frac{\Delta Z_j \cdot A_{soil} \cdot RLD_{i,j}}{60}$~~ $\frac{\Delta Z_j \cdot A_{soil} \cdot RLD_{i,j}}{60}$ (6)

with ΔZ (m) and A_{soil} (m^2) the soil layer thickness and horizontal surface area, respectively.

To finalize the connection between root xylem and shoot, axial conductances per root system ~~class~~group (K_{axial}, m^3 MPa^{-1} d^{-1}) were ~~derived from an~~calculated as equivalent “big root” specific axial conductance per root system ~~class~~group (k_{axial}, m^4 MPa^{-1} d^{-1}, to be optimized by inverse modelling) as:

~~$K_{axial,j} = \frac{k_{axial}}{\Delta Z_j}$~~ $K_{axial,j} = \frac{k_{axial}}{\Delta Z_j}$ (7)

~~At each time step, both the total soil-root system conductance ($K_{soil-root}$, m^3 MPa^{-1} d^{-1}) and the standard sink distribution (SSF, dimensionless, summing up to 1) were calculated from K_{radial} and K_{axial}, using the algorithm of Meunier et al. (2017b). The variable $K_{soil-root}$ represents the water flow per unit water potential difference between the bulk soil and the leaf (assuming a negligible stem hydraulic resistance), and SSF the relative distribution of water uptake in each soil layer under vertically homogeneous soil water potential conditions (Couvreur et al., 2012).~~

~~Adding soil hydraulic conductance to the one-dimensional hydraulic model of Couvreur et al. (2014) yields the following solutions of leaf water potential (ψ_{leaf}, MPa) and water sink terms (S, d^{-1}) whose formulation approaches that of Nimah and Hanks (1973):~~

~~$\psi_{leaf}(t) = -\frac{T(t)}{K_{soil-root}(t)} + \sum SSF_j(t) \cdot \psi_{soil,j}(t)$ (8)~~

At each time step, both the total soil-root system conductance ($K_{soil-root}$, m^3 MPa^{-1} d^{-1}) and the standard sink distribution (*SSF*, dimensionless, summing up to 1), were calculated from K_{radial} and K_{axial}, using the algorithm of Meunier et al. [2017b]. 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 leaf (assuming a negligible stem hydraulic resistance [Steudle and Peterson, 1998]).

Adding soil hydraulic conductance to the one-dimensional hydraulic model of Couvreur et al. [2014] yields the following solutions of leaf water potential (ψ_{leaf}, MPa) and water sink terms (S, d^{-1}) whose formulation approaches that of Nimah and Hanks [1973]:

$$\psi_{leaf}(t) = -\frac{T(t)}{K_{soil-root}(t)} + \sum SSF_j(t) \cdot \psi_{soil,j}(t) \qquad (8)$$

Where one sixtieth of the overall transpiration rate (T, m d^{-1}) is allocated to each ~~class~~group, and $\psi_{soil,j}$ (Mpa) is the soil water potential in soil layer j.

~~$$S_{i,j}(t) = \frac{K_{soil-root,i}(t) \cdot SSF_{i,j}(t) \cdot (\psi_{soil,j}(t) - \psi_{leaf,i}(t))}{A_{soil} \cdot \Delta Z_j} \qquad (9)$$~~

~~where axial conductances were assumed to be large enough for $K_{soil-root}$ to control the compensatory RWU which arise from a heterogeneously distributed soil water potential (Couvreur et al., 2012).~~

$$S_{i,j}(t) = \frac{K_{soil-root,i}(t) \cdot SSF_{i,j}(t) \cdot (\psi_{soil,j}(t) - \psi_{leaf,i}(t))}{A_{soil} \cdot \Delta Z_j} \qquad (9)$$

where $K_{soil-root}$ was assumed to control the compensatory RWU which arise from a heterogeneously distributed soil water potential, due to large axial conductances [Couvreur et al., 2012].

Finally, the tiller water oxygen isotopic composition (δ_{tiller}) was calculated as the average of local soil water oxygen isotopic compositions (δ_{soil}) weighted by the relative distribution of positive water uptakes (i.e., not accounting for δ_{soil} at locations where water is exuded by the root), assuming a perfect mixture of water inside the root system ~~(Meunier et al., 2017a):~~[Meunier et al., 2017a]:

~~$$\delta_{tiller} = \frac{\sum_{S_j>0} S_j \cdot A_{soil} \cdot \Delta Z_j \cdot \delta_{soil}(t)}{\sum_{S_j>0} S_j(t) \cdot A_{soil} \cdot \Delta Z_j}$$~~

$$\delta_{tiller} = \frac{\sum_{S_j>0} S_j \cdot A_{soil} \cdot \Delta Z_j \cdot \delta_{soil}(t)}{\sum_{S_j>0} S_j(t) \cdot A_{soil} \cdot \Delta Z_j} \qquad (10)$$

Like in the experiment, δ_{tiller} from three plants were randomly pooled at each observation time. ~~As the experiment included about 300 plants (value based on seeding density, see section 2.3), and we explicitly simulated 60 individual RLD profiles, we assumed that each of them was representative of a class of 5 identical plants, totaling 300 plants.~~ A hundred pools of 3 plants (possibly including several plants of the same ~~class~~group) were randomly selected in order to obtain the pooled simulated δ_{tiller} by arithmetic averaging.

The unknown parameters of the soil-root hydraulic model, i.e., the root radial conductivity (L_{pr}), the root axial conductance (k_{axial}), the soil saturated hydraulic conductivity (k_{sat}), and the soil tortuosity factor (λ) were finally determined by inverse modeling. For details on the procedure, the reader is referred to Appendix D.

In order to evaluate the robustness of the hydraulic model predictions (parametrized solely based on the reproduction of shoot observations in the inverse modeling scheme) from independent perspectives, we also compared predictions and measurements over 4 quantitative "soil-root domain" criteria: (i) the depth at which the transition between nighttime water uptake and exudation ($S_{i,j}<0$, i.e. release of water from root to soil) takes place, (ii) quantities of exuded water and overnight increase of soil water content, (iii) the enrichment of labelled water at the depth where water content increase is observed overnight, and (iv) the order of magnitude of the optimal root radial conductivity value as compared to literature data in tall fescue.

Finally, and as a comparison point, the Bayesian inference statistical model SIAR ~~(Parnell et al., 2013)~~[Parnell et al., 2013] was used to determine the profiles of water sink terms of ten identified potential water sources. These water sources were defined to originate from 10 distinct soil layers ~~for which corresponding δ_{soil} values were computed (Rothfuss and Javaux, 2017).~~(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) for which corresponding δ_{soil} values were computed [Rothfuss and Javaux, 2017]. SIAR solely bases its estimates from the comparison of δ_{tiller} observations to the isotopic compositions of the soil water sources (δ_{soil}). For this, δ_{tiller} measurements were pooled in twelve groups corresponding to different time periods, selected to best reflect the observed temporal dynamics of δ_{tiller}. The reader is here referred to Appendix E for details on the model parametrization and running procedure.

3 Results and discussion

3.1 Experimental data

3.1.1 Soil profiles

Figure 2a and b show a very stable soil water content profile and a more variable δ_{soil} profile from DaS 166 - 15:45 to DaS 168 - 05:00. Soil was dry at the surface (0.058 m^3 m^{-3} < θ < 0.092 m^3 m^{-3} for layer 0.015 - 0.040 m) whereas closer to saturation at depth –1.30 m (θ = 0.34 m^3 m^{-3} ± 0.012 m^3 m^{-3}, estimated θ_{sat} = 0.40 m^3 m^{-3}, see Appendix A). According to the measured soil matric potentials (Fig. 2c), soil water was virtually unavailable (≤ –1.5 MPa) above –0.5 m depth. Soil moisture remained unchanged in the top 25 cm during the sampling period (θ = 0.08 ±0.00 m^3 m^{-3}) ~~while varied noticeably~~as well as at –1.30 m ~~between 12:00 and 20:00 on~~ from DaS 166 - 15:45 to DaS 168 - 05:00 (θ = ~~167 (~~0.33 ±0.01 m^3 m^{-3}), showing that roots were predominantly extracting water from deep soil layers.

Water in the top soil layers (–0.040 m < z < –0.015 m) was isotopically enriched (–3.2 ‰ < δ_{soil} < 0.3 ‰) as opposed to the deepest layer (δ_{soil} = –7.34 ‰ ± 0.30 ‰ at –1.30 m). Following labeling of the reservoir water on DaS 166 - 17:00, δ_{soil} reached a value of 36.9 ‰ at –1.50 m on DaS 167 - ~~07:30~~17:00. The development of the vegetation on DaS 166-168 (LAI = 5.6) and the observed surface θ values lead us to assume that the rhizotron water losses were due to transpiration flux solely (i.e., evapotranspiration = transpiration). The soil water oxygen isotopic exponential-shaped profiles were the product of fractionating evaporation flux, and to a great extent when the soil was bare or when the tall fescue cover was not fully developed. The differences in soil water oxygen isotopic profile observed at the four different sampling dates were therefore either due to lateral heterogeneity (e.g., upper soil layers), to the soil capillary rise of labelled water from the reservoir (deep soil layers), or to the hydraulic redistribution of water through roots (to the condition that the isotopic composition of the redistributed water differs from that of the soil water at the release location). We noted an isotopic enrichment of 1.0 ‰ of soil water observed on DaS 168 - 05:00 at –0.9 m with respect to the mean δ_{soil} value across previous sampling dates. This could partly be due to, e.g., upward preferential flow of labelled water from the bottom soil layers and therefore be the sign of the lateral heterogeneity of the soil. Another reason for this would be hydraulic redistribution of labelled water by the roots. It was however not possible to evaluate the relative importance of these three processes (lateral heterogeneity, capillary rise/preferential flow, and hydraulic redistribution) in the setting of the soil water isotopic profile since the physically-based soil-root model presented in section 2.4 does not account for soil liquid and vapor flow. This was also not the primary intent of the present study.

~~.~~The observed RLD profile (Fig. 1a) showed a typical exponential shape, i.e., ~~maximal~~ maximum at the surface (5.42 ± 0.34 cm cm^{-3}) down to a ~~minimal~~ minimum at –1.10 m (0.540 ± 0.35 cm cm^{-3}), while it increased again from the latter depth up to a value of 1.660 cm cm^{-3} at –1.30 m. This significant trend was most probably a direct consequence of the high soil water content ~~level~~ value in this deeper layer.

3.1.2 Plant water and isotopic temporal dynamics

The temporal variation of δ_{tiller} (Fig. 3a) was found to be either (i) moderate during day and night, i.e., from DaS 167 - 06:00 to 11:00 (δ_{tiller} = –2.6 ± 1.4 ‰) and from DaS 167 - 21:30 to DaS 168 - 00:00 (δ_{tiller} = –2.7 ± 0.4 ‰), or (ii) strong during the day, i.e., from DaS 167 - 11:00 to 18:00 (maximum value of 20.9 ‰ at DaS 167 - 12:40), or else (iii) strong during the night, i.e., from DaS 167 - 04:00 to 06:00 (max = 36.4 ‰ at DaS 167 - 05:15) and from DaS 168 - 00:00 to 06:00 (max = 14.6 ‰ at 28:00, DaS 168). Note that transpiration (Fig. 3b) occurred also at night during the sampling period, due to relatively high temperature in the glasshouse leading to a value of atmospheric relative humidity smaller than 85%, Fig. 3b). From 12:00 to 14:00 and from 16:00 to 17:00 on DaS 167 (case (ii)) high values of leaf transpiration corresponded to high values of ~~δ_{tiller}~~δ_{tiller}.

3.1.3 Partial decorrelation between water and isotopic state variables

Figure 4 shows that variables describing plant water status, i.e., T and RH (Fig. 4a) and T and ψ_{leaf} (Fig. 4b) were well correlated: coefficient of determination R^2 was equal to 0.78 and 0.70 for the entire experimental duration, respectively. However, linear relationships between water status and isotopic variables were either ~~inexistent~~nonexistent, e.g., between T and δ_{tiller} (R^2=0.01, Fig. 4c) and between ψ_{leaf} and δ_{tiller} (R^2=0.00, Fig. 4h) or characterized by a low R^2 and high p-value (e.g., between T and δ_{leaf}, R^2=0.43, p>0.05, Fig. 4d). The partial temporal disconnection between δ_{leaf} and T could not be attributed to problems of the isotopic methodology, during e.g., the vacuum distillation of the water from the plant tillers and leaves: water recovery rate was always greater than 99 % and Rayleigh distillation corrections [Dansgaard, 1964; Galewsky et al., 2016] were applied to standardize the observed oxygen isotopic composition values to a 100 % water recovery (based on the comparison of sample weight loss during distillation and mass of collected ~~distillated~~distilled water). The evolution of δ_{leaf} was strongly correlated with that of δ_{tiller} during the day (R^2 = 0.90) whereas non-correlated during the night (R^2 = 0.00, Fig. 4j). These observed correlations are in agreement with the ~~Craig and Gordon (1965) model revisited by Dongmann (1974) and extensively used in the current literature (e.g., Dubbert et al., 2017): at isotopic steady-state, δ_{leaf} is a function of the input water~~ Craig and Gordon [1965] model revisited by Dongmann [1974] and extensively used in the current literature [e.g., Dubbert et al., 2017]: at isotopic steady-state, δ_{leaf} is a function of the input water oxygen isotopic composition (δ_{tiller}) among other variables, i.e., leaf temperature (not measured during the experiment), stomatal and boundary layer conductances, oxygen isotopic composition of atmospheric water vapor, and relative humidity.

It is generally difficult to observe a statistically significant δ_{leaf}-δ_{tiller} (Fig. 4j) relationship at this temporal scale under natural abundance conditions in the field since the soil water isotopic weak gradient translates into weaker δ_{tiller} temporal dynamics. The quality of linear fit between δ_{leaf} and δ_{tiller} data collected during the day (R^2=0.90) was made possible in this specific experiment by the artificial isotopic labeling pulse that enhanced the soil water isotopic gradient, which in turn increased the range of variation of δ_{tiller}, ultimately highlighting the δ_{leaf}-δ_{tiller} temporal correlation. Air relative humidity is a driving variable of δ_{leaf} in the model of ~~Dongmann (1974) via the competing terms (1–RH)·δ_{tiller} et RH·δ_{atm}, where δ_{atm} is the atmospheric water vapor isotopic composition inside the glasshouse.~~Dongmann [1974] via the competing terms (1–RH)·δ_{tiller} and RH·δ_{atm}, where δ_{atm} is the atmospheric water vapor isotopic composition inside the glasshouse. An overall significant linear correlation was observed between RH and δ_{leaf} during the experiment (R^2=0.57, Fig. 4g). During the two night periods (i.e., from 04:00-06:00 and from 20:30-07:00), as relative humidity increased in the glasshouse (51 % < RH < 85 %, Fig. 3b), the influence of the isotopic labeling of the tiller water (due to the labeling of deep soil water) through term (1–RH)·δ_{tiller} decreased to the benefit of term RH·δ_{atm} (with δ_{atm} values ranging from –15.9 to –10.7 ‰, mean = –13.1±1.6‰, data not shown). This was especially visible between 04:50 and 06:00 on DaS 167 and between 01:00 to 06:00 on DaS 168, when δ_{tiller} reached greater values than δ_{leaf}.

From a different perspective, as three plant water samples were pooled to reach a workable volume for the isotopic analysis at each observation time without replicates, the isotopic signal fluctuations may reflect both its temporal dynamics and its variability within the plant population.

3.2 Simulations

3.2.1 Rooting depth and transpiration rate control δ_{tiller} and ψ_{leaf} fluctuations, respectively

Despite the use of a global optimizer and 4 degrees of freedom (L_{pr}, k_{axial}, k_{sat}, λ, see optimal values in Table 1) specifically aiming at matching the simulated and observed temporal dynamics of δ_{tiller}, none of the 60 root system ~~classes~~ groups or average population could reproduce the measured fluctuations in time (R^2=0.00, Fig. 5a), regardless of the weight attributed to this criterion in the objective function. ~~However, the~~ The predicted versus observed ~~average~~ δ_{tiller} distributions including all plant groups and ~~its standard deviation for the overall dataset were~~observation times differed noticeably but not significantly ~~different~~ (6.6 ± 8.4 ‰ and 3.7 ± 8.4 ‰, respectively~~).~~) when pooling 3 simulated δ_{tiller} randomly at each observation time (P>0.01 in 92 cases out of 100 repeated drawings), as in measurements. Besides, the simulated ψ_{leaf} fitted well the observations (R^2=0.67, overall distributions: –0.175 ± 0.053 MPa and –0.177 ± 0.053 MPa, respectively, Fig. 5c). When analyzing the distributions of ψ_{leaf} and δ_{tiller} per maximum root system depth (Fig. 5b and d), it appears that the ψ_{leaf} signal is not sensitive to the rooting depth (Fig. 5d), while δ_{tiller} is more sensitive to rooting depth than to the temporal evolution of the plant environment (Fig. 5b).

This leaves us with two hypotheses. The “rollercoaster hypothesis”: δ_{tiller} rapidly goes up and down with all ~~the population~~ individuals on board of the same car (i.e. little variability within the population, unlike predictions in Fig. 5a, but like the simulated ψ_{leaf} in Fig. 5c). If that is correct, the physical model lacks a process that would capture the observed temporal fluctuations of δ_{tiller}. The “swarm pattern hypothesis”: δ_{tiller} is rather stable in time but its values within the plant population are dispersed like in a flying swarm, so that δ_{tiller} values sampled at different times fluctuate, not due to temporal dynamics but to the fact that different individuals are sampled (Fig. 5a).

The model suggests that the tall fescue population ψ_{leaf} follows a “rollercoaster” dynamics driven by transpiration rate, while the population δ_{tiller} follows a “swarm” pattern driven by the maximum rooting depth of the sampled plants. As no correlation could be expected between the drivers~~, transpiration rate and~~ (the maximum rooting depth of the sample plants~~, discrepancies~~ and canopy transpiration rate), our analysis explains the absence of correlation between ~~fluctuations of isotopic~~δ_{tiller} and ψ_{leaf} or transpiration rate.~~hydraulic variables are not surprising any more.~~

In future experiments and in the specific context of labeling pulses, sampling more plants at each observation time would help disentangle the spatial from temporal sources of variability of ψ_{leaf} and δ_{tiller}. It would however be at the cost of the temporal resolution of observations, or would necessitate a larger setup with more plants in the case of controlled conditions experiments.

3.2.2 Independent observations support the validity of the hydraulic model predictions

In the last 12 hours of the experiment (DaS 167 – 17:00 to DaS 168 – 05:00), the measured soil water content increased by ~~2.9%~~0.029 m^3 m^{-3} at –0.9 m depth, which could be a sign of nighttime hydraulic redistribution. During the same period, the physical model predicted a cumulative water exudation sufficient to increase soil water content by 0.003 m^3 m^{-3},~~38 %,~~ as soil water potential was sufficiently low to generate reverse flow, but high enough not to disrupt the hydraulic continuity between soil and roots ~~(Carminati and Vetterlein, 2013; Meunier et al., 2017a).~~[Carminati and Vetterlein, 2013; Meunier et al., 2017a]. While this increase is smaller than the observed water content change, it is only a component in the soil water mass balance. Given the soil water potential vertical gradient, upward soil capillary water flow may have accounted for another part of the observed moisture change. Experimental observations also show that δ_{soil} increased by 1.0 ‰ at 0.9 m depth during that time (–6.2 ‰, a value significantly higher than –7.1 ‰ ± 0.1 ‰ at earlier times based on ANOVA analysis, $P<0.01$), while our simulations of hydraulic redistribution generated an increase of δ_{soil} by 0.~~41~~34 ‰. As soil capillary flow may not generate local maxima of δ_{soil} (no enrichment observed at surrounding ~~heights~~depths, see Fig. 2b), and soil evaporation is assumed negligible at that depth, it is likely that the observed local enrichment was entirely due to hydraulic redistribution, which would then be underestimated by a factor of about ~~2.5~~3 in our simulations. Increasing water exudation by a factor ~~2.5~~3 would imply a simulated water content change due to exudation of 0.0090 m^3 m^{-3}~~95%~~ absolute water content, which remains compatible with the experimental observation.

Between –1.1 m and –0.9 depths, the nighttime water flow pattern transitioned from exudation to uptake in both measurements and predictions. At –1.1 m, the model predicted a cumulative water uptake sufficient to decrease soil water content by 0.0101 m^3 m^{-3},~~89 %,~~ as compared to the observed ~~1.41 %~~0.0141 m^3 m^{-3} total soil water content decrease. The remaining 0.~~5%~~004 m^3 m^{-3} water content decrease may have contributed to the recharge to the soil layers above through capillary flow, which was not simulated. Therefore, all relevant measurements (local increase of soil water content, local enrichment of water isotopic signature) and simulation results (S<0, i.e. local water release from roots) clearly converge to the conclusion that hydraulic lift occurred in the vicinity of -0.9 m depth in the early morning of DaS 168.

~~The fitted L_{pr} value (2.3 10^{-7} m MPa^{-1} s^{-1}) was in the range found by Martre et al. (2001) in tall fescue (2.2 10^{-7} ± 0.1 m MPa^{-1} s^{-1}) and falls in the range obtained by Meunier et al. (2017a)~~As far as fitted parameter values are concerned, L_{pr} (2.3 10^{-7} m MPa^{-1} s^{-1}) was in the range found by Martre et al. [2001] in tall fescue (2.2 10^{-7} ± 0.1 m MPa^{-1} s^{-1}) and falls in the range obtained by Meunier et al. [2017a] for another grass (*Lolium multiflorum* Lam., 6.8 10^{-8} to 6.8 10^{-7} m MPa^{-1} s^{-1}). Our k_{axial} value cannot be compared to values of axial root conductance from the literature as it transfers the water absorbed by roots ~~from 5 plants~~ in a single “big root” per ~~class~~group of ~~root system.~~5 identical plants. The optimal value of k_{sat} was quite high (Table 1) but reportedly very correlated to λ (i.e. soil unsaturated hydraulic conductivity is proportional to k_{sat}, but also to Se^{λ} [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.

3.2.3 Other sources of variability and observational error

Our treatment of the soil medium in this experiment (sieving, irrigation from the bottom) makes it laterally more homogeneous than natural soils. This method allowed us to study specifically the impact of the vertical gradients of δ_{soil} on δ_{tiller}. It also justified the use of a simplistic 1-D model adapted to the vertically resolved measurements. If lateral heterogeneity of soil water content remained and was accounted for, our predictions of root water uptake distribution, δ_{tiller} and ψ_{leaf} would be altered. Observational errors in the gravimetric soil water content measurement (turned into soil water potential using the soil water retention curve) would as well alter these predictions. In order to quantify the sensitivity of our simulated results to such heterogeneity or observational error, we varied the soil water content input by ± 0.02 m^3 m^{-3} at three critical depths (–0.9, –1.1 and –1.3 m, before interpolation), at the last observation time, during which measurements and simulations suggested that hydraulic lift occurred. Our results were mostly sensitive to soil water content alterations at –0.9 m, and barely differed in response to alterations at –1.1 and –1.3 m, though the conclusions were not affected qualitatively. No statistically significant difference between predicted and observed δ_{tiller} distributions for the overall dataset could be found when pooling 3 simulated δ_{tiller} randomly at each observation time (predicted and observed δ_{tiller} distributions were closest to differ when soil water content was reduced by 0.02 m^3 m^{-3} at 0.9 m depth; P>0.01 in 76 cases out of 100 repeated drawings). Measured and simulated ψ_{leaf} remained very correlated in all cases (from R^2=0.69 to 0.74 when adding or removing 0.02 m^3 m^{-3} at 0.9 m depth, respectively). Furthermore, when adding or removing 0.02 m^3 m^{-3} at 0.9 m depth, cumulative water exudation at –0.9 m varied between

0.0019 and 0.0035 m^3 m^{-3}, uptake at –1.1 m varied between 0.0080 and 0.0108 m^3 m^{-3}, and the simulated change of δ_{soil} ranged between 0.28 and 0.40 ‰, respectively.

Lateral heterogeneity of soil water isotopic composition may as well occur at the microscopic scale. As water in micropores is less mobile than water in meso- and macropores [Alletto et al., 2006], it is likely that, in the lower half of the profile, the capillary rise of labelled water affected the signature of water in meso- and macropores more than in micropores. If roots have more access to meso- and macropore water, then the water absorbed by roots would be isotopically enriched, as compared to the "bulk soil water" characterized experimentally. The importance of this possible bias depends on soil texture and heterogeneity (e.g. existence of more isolated "pockets" of soil or compact clusters), as well as on the speed of water mixing between mobile and immobile water fractions [Gazis and Feng, 2004]. Including this process in the modelling would necessitate sufficient observations to estimate the aforementioned properties, and ideally some quantification of the lateral heterogeneity of soil water isotopic composition at the micro-scale.

The lateral heterogeneity of soil hydraulic properties and root distribution may also have participated to the generation of lateral soil water potential heterogeneities, particularly in undisturbed soils. If one had access to data on lateral heterogeneity of soil properties and rooting density, it would be possible to simulate 3D soil-root water flow with a tool such as R-SWMS [Javaux et al., 2008], using a randomization technique for soil properties distribution as in Kuhlmann et al. [2012], in order to obtain estimations of the relative importance of this type of heterogeneity on δ_{tiller} and ψ_{leaf} variability.

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 d). This suggests that in this setup the hydraulic conductance of the soil-root system limited shoot water supply more than the distribution of roots, as in Sulis et al. [2019]. Simulated baseline (i.e. for uniform transpiration rates) leaf water potentials are shown as grey lines in Figure 5 panel c, and measured leaf water potentials as a green line in the same panel. The fact that they match well, despite the high sensitivity of leaf water potential to transpiration rate, reinforces the idea that transpiration rate was likely not spatially heterogeneous among the plant population. Therefore, the tiller water isotopic signature, whose sensitivity to transpiration rate is already very low, was likely not affected by transpiration rate heterogeneity.

3.2.4 Do root water uptake profiles predicted by hydraulic and Bayesian models differ?

The root water uptake dynamics predicted by the mechanistic model are shown in Fig. 6a. The overall pattern of peaking water uptake in the lower part of the profile during daytime matched that of the statistical model, and the correlation coefficient of both ~~models~~model predictions was relatively high (R^2=0.53) in average over the simulation period, see Figure ~~of Appendix E~~7). The main differences were the following: (i) in the upper soil layers where the soil water potential was lower –1.5 MPa, the statistical model predicted water uptake, which is theoretically impossible given the leaf water potential above –0.4 MPa ~~(van Den Honert, 1948); (ii) In the top~~ [van Den Honert, 1948]; (ii) In the upper half of the profile, the physical model predicted exudation at a rate limited by the low hydraulic conductivity between root surface and bulk soil, with a peak at night, at –0.9

m depth (quantitative analysis in previous section); (iii) Below –1.0 m depth, the water uptake rate predicted by the statistical model steadily increased with depth while that of the physical model was more uniform, likely due to axial hydraulic limitation [e.g., Bouda et al., 2018] counteracting the increasing soil water potential with depth. Note that the outcome of the statistical model may significantly depend on the definition of the a priori relative RWU (rRWU) profile. In the present study, we set it to follow a "flat" uniform distribution (i.e., rRWUj = 1/10, see Appendix E), in other words, each layer was initially defined to contribute equally to RWU. To the contrary of other studies [e.g., Mahindawansha et al., 2018], where the a priori rRWU profile was empirically constructed on basis of soil water content and root length density profiles, we decided not to further arbitrarily constrain the Bayesian model for the sake of comparison with the physically-based soil-root model.

~~(e.g., Bouda et al., 2018) counteracting the increasing soil water potential with depth.~~

3.3 Progresses and Challenges in soil water isotopic labeling for RWU determination

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., 2017a; Kühnhammer et al., 2019].

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 worlds: 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 mechanistic 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 [e.g., Rothfuss et al., 2013; Quade et al., 2019; Volkmann et al., 2016a] as alternatives of the widely used combination of destructive sampling and offline isotopic analysis following cryogenic vacuum extraction [Orlowski et al., 2016b] 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.

4 Conclusion

In the present study, light could be shed on RWU of *Festuca arundinacae* by specifically manipulating the lower boundary conditions for water content and oxygen isotopic composition. The new version of the one-dimensional model of Couvreur et al. (2014) implemented here accounted for both root and soil hydraulics in a population of “big” root systems of known root length density profile. This approach underlined the high sensitivity of δ_{tiller} to rooting depth and suggested that if δ_{tiller} is measured on a limited number of individuals, its variations in time may reflect the heterogeneity of rooting depth within the population, rather than temporal dynamics which was minor in our simulations. The model avoided the prediction of water uptake at locations where it was physically unavailable (e.g., in the ~~first~~ top half of the soil profile), by accounting for water potential differences observed between the leaves and the soil, and explained quantitatively the local isotopic enrichment of soil water as the occurrence of nighttime Hydraulic Lift at ~~–~~−0.9 m depth. On the other hand, the Bayesian statistical approach tested for comparison, which was driven by isotopic information solely, naturally translated the observed changes of δ_{tiller} into profound temporal dynamics of RWU, at the expense of eco-physiological consideration (e. g., temporal dynamics of leaf water potential and transpiration rate).

This case study highlights (i) the potential limitations of water isotopic labeling techniques for studying RWU~~, especially when water addition is localized and not broadcasted in~~ : the soil ~~therefore creates strong~~ water isotopic ~~depth~~ 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. As already pointed out in the review of Rothfuss and Javaux (2017), the study also (ii) underlines the interest of complementing in-situ isotopic observations in soil and plant water with information on soil water status and plant ~~eco-physiology~~ecophysiology; it finally (iii) calls for the use of simple soil-root models (though requiring additional water status measurements and making more explicit assumptions on the description of the soil-plant system, as compared to the traditional Bayesian approach) for inversing isotopic data and gain insights into the RWU process.

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Data sets

Upon acceptance, all research data needed for creating plots will be available in reliable FAIR-aligned data repositories with assigned DOIs.

Author contribution

TB, JLD, and PB designed the experiments and TB, JLD, PB, and YR carried them out. VC, FM, and MJ~~FM~~ developed the physically-based root water uptake model code and VC and FM performed the simulations. YR performed the statistical simulations. VC ~~and~~ , YR, FM, and MJ prepared the manuscript with contributions from all co-authors.

Competing interests

The authors declare that they have no conflict of interest.

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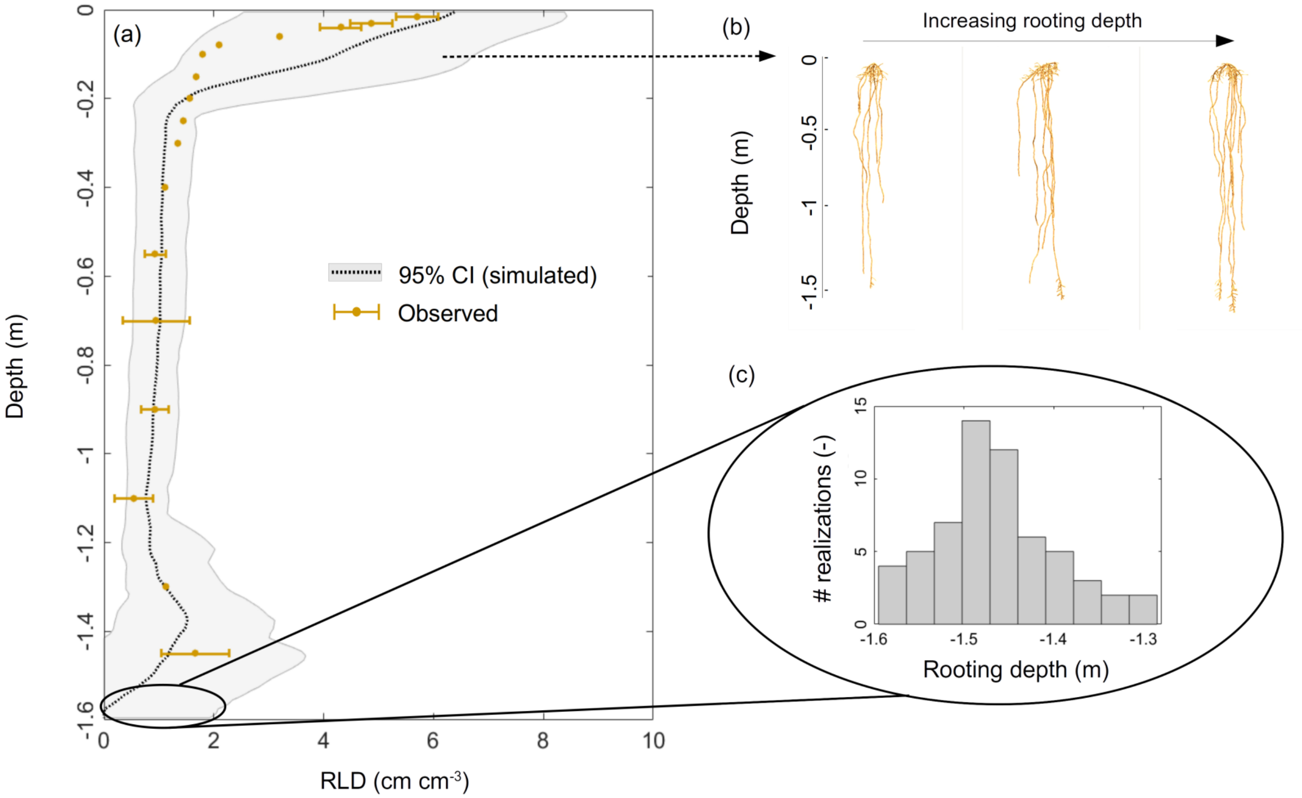
5 Tables

	L_{pr} (m MPa^{-1}s^{-1})	k_{axial} (m^4 MPa^{-1} s^{-1})	k_{sat} (m^2 MPa^{-1} s^{-1})	λ (-)
Lower limit	10^{-11}	10^{-13}	10^{-5}	–5
Upper limit	10^{-6}	10^{-8}	10^{-2}	2
Value at best fit	$2.3\ 10^{-7}$	$4.5\ 10^{-11}$	$9.5\ 10^{-3}$	–4.9

Table 1. Optimum and limits of the four-dimensional parametric space explored by the global optimization algorithm aiming at minimizing the difference between simulated and observed δ_{tiller} and ψ_{leaf}, as well as their standard deviation from average values during the full experiment.

6 Figures


(a)
Depth (m)
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
0
2
4
6
8
10
RLD (cm cm^{-3})
95% CI (simulated)
Observed
(b)
Increasing rooting depth
Depth (m)
0
-0.5
-1
-1.5
(c)
realizations (-)
0
5
10
15
-1.6
-1.5
-1.4
-1.3
Rooting depth (m)

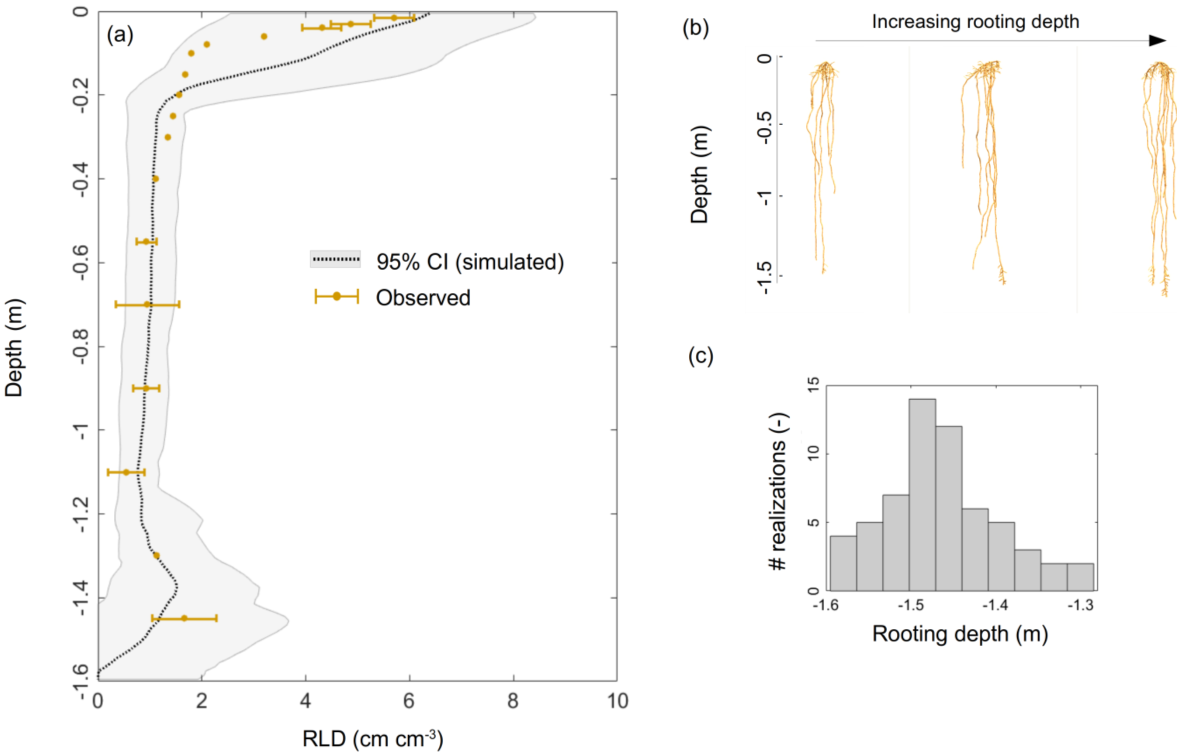
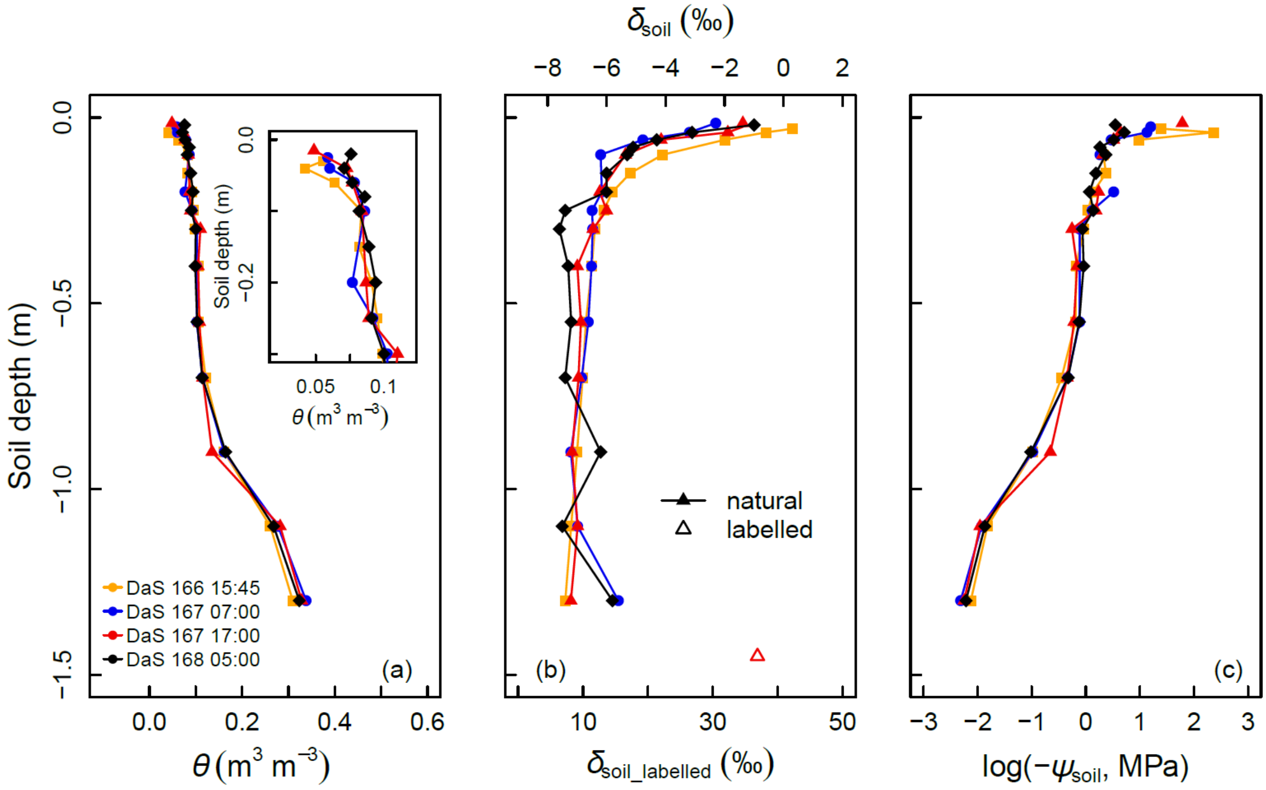


Figure 1. (a) Simulated (grey envelopes) and observed (brown dots) root length density profiles. Panels (b) and (c) illustrate the variability in modelled root system architectures and rooting depths, respectively.


δsoil (‰)
−8
−6
−4
−2
0
2
Soil depth (m)
0.0
−0.5
−1.0
−1.5
Soil depth (m)
0.0
−0.2
0.05
0.1
θ (m3 m−3)
DaS 166 15:45
DaS 167 07:00
DaS 167 17:00
DaS 168 05:00
(a)
0.0
0.2
0.4
0.6
θ (m3 m−3)
natural
labelled
(b)
10
30
50
δsoil_labelled (‰)
(c)
−3
−2
−1
0
1
2
3
log(−ψsoil, MPa)

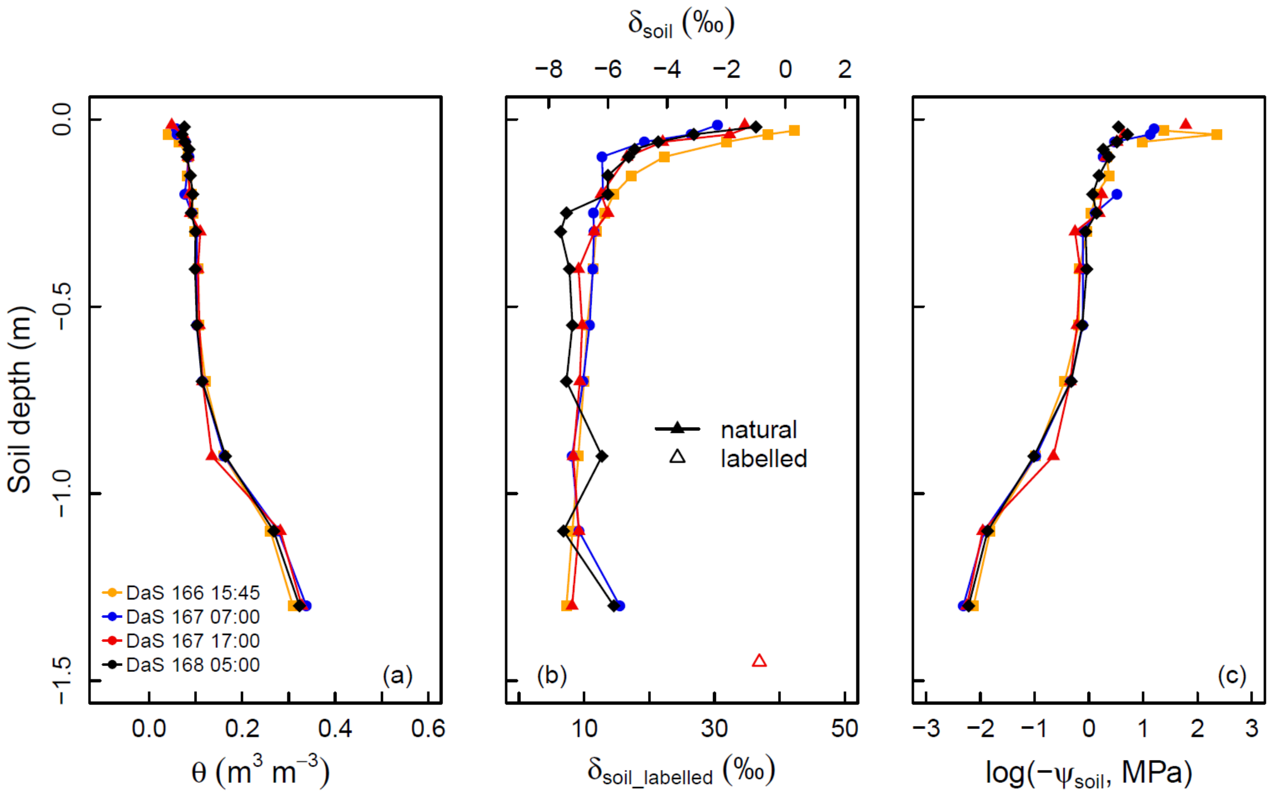
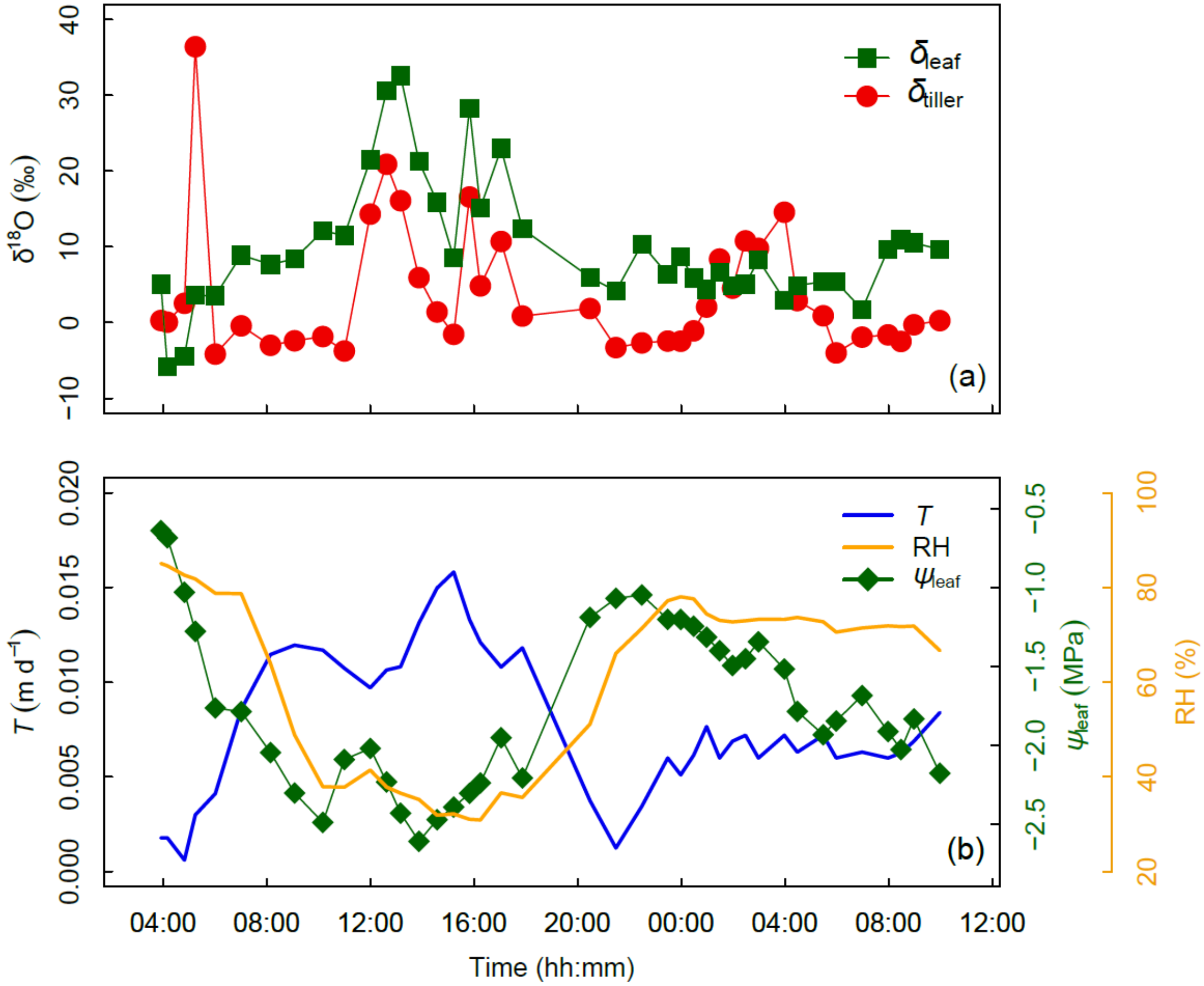


Figure 2. ~~Soil~~Measured soil volumetric water content (θ, panel a), oxygen isotopic composition (δ_{soil}, panel b), and calculated soil matric potential (ψ_{soil}, panel c) profiles during the sampling period.


δ¹⁸O (‰)
40
30
20
10
0
−10
δleaf
δtiller
(a)
T (m d⁻¹)
0.020
0.015
0.010
0.005
0.000
T
RH
ψleaf
(b)
−0.5
−1.0
−1.5
−2.0
−2.5
ψleaf (MPa)
100
80
60
40
20
RH (%)
04:00
08:00
12:00
16:00
20:00
00:00
04:00
08:00
12:00
Time (hh:mm)

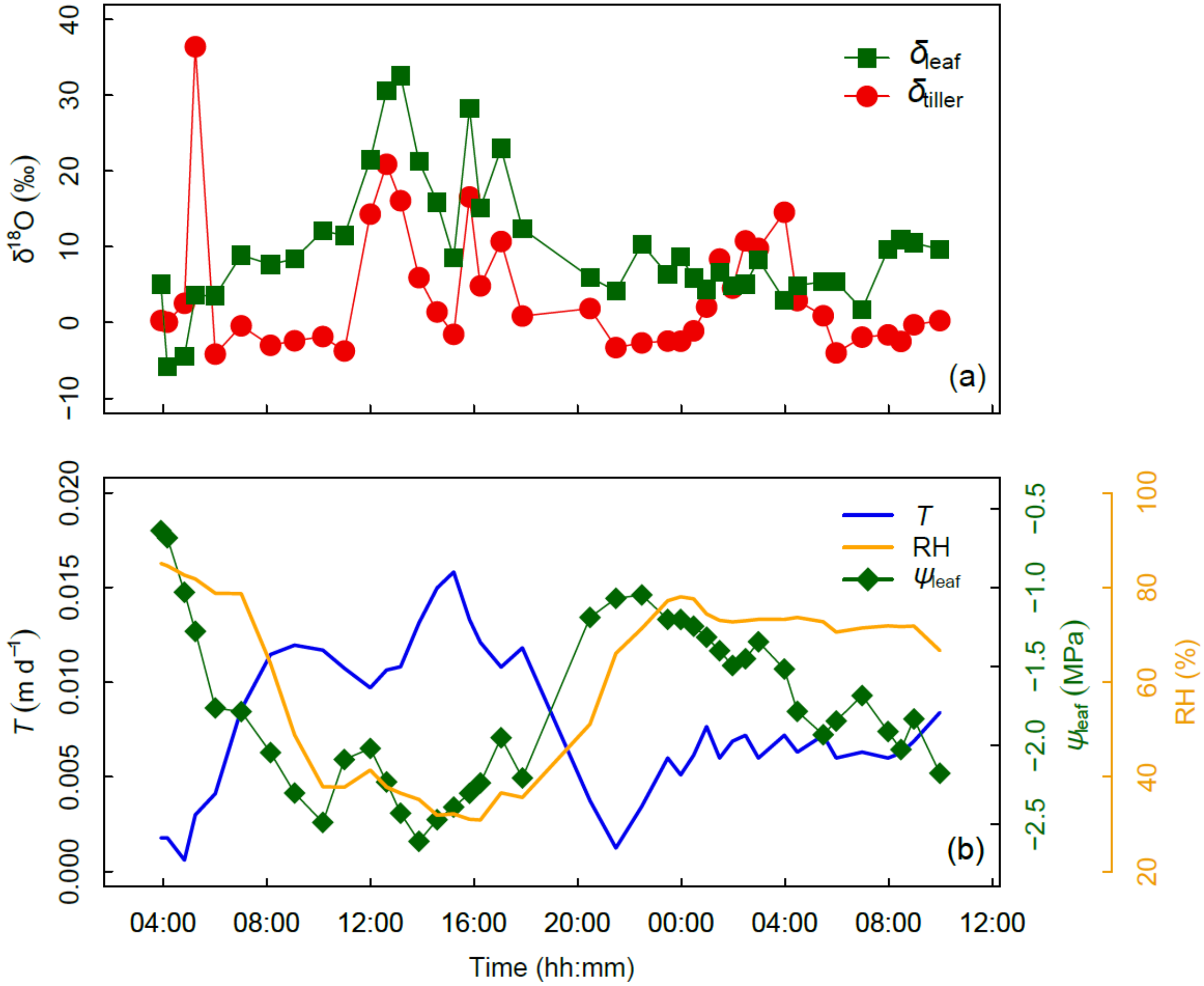
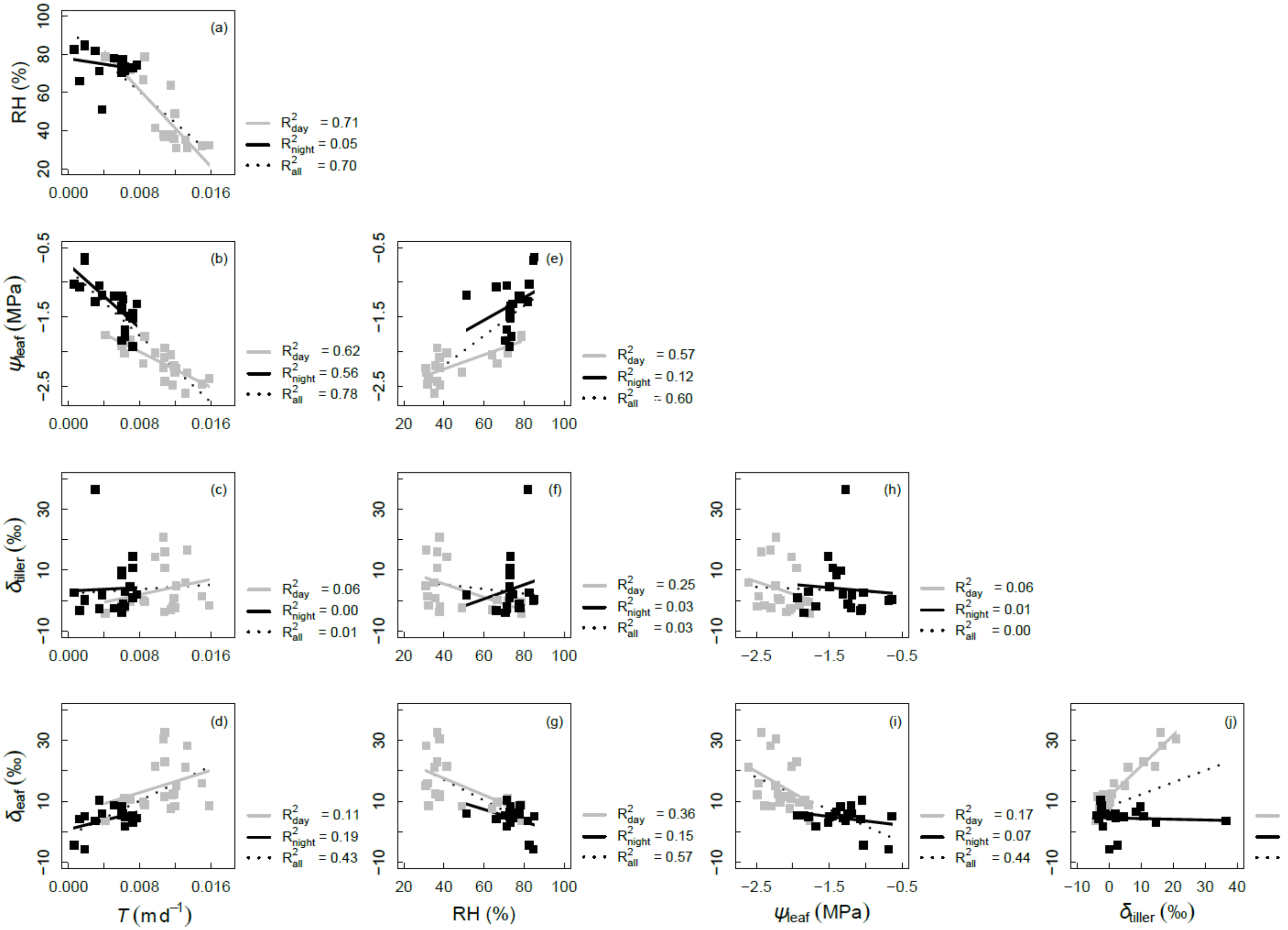


Figure 3. (a) Time series of tiller and leaf water oxygen isotopic compositions (δtiller and δsoil, ‰). (b) Transpiration flux (T, in m d–1), relative humidity (HR, %), and leaf water potential (ψleaf, in MPa, panel b) from days after seeding DaS 167 – 04:00 to DaS 168 – 11:00. Time of Labeling was DaS 166 – 17:00.


(a)
RH (%)
20
40
60
80
100
0.000
0.008
0.016
R^2_{day} = 0.71
R^2_{night} = 0.05
R^2_{all} = 0.70
(b)
ψ_{leaf} (MPa)
−2.5
−1.5
−0.5
R^2_{day} = 0.62
R^2_{night} = 0.56
R^2_{all} = 0.78
(e)
R^2_{day} = 0.57
R^2_{night} = 0.12
R^2_{all} = 0.60
(c)
δ_{tiller} (‰)
−10
10
30
R^2_{day} = 0.06
R^2_{night} = 0.00
R^2_{all} = 0.01
(f)
R^2_{day} = 0.25
R^2_{night} = 0.03
R^2_{all} = 0.03
(h)
R^2_{day} = 0.06
R^2_{night} = 0.01
R^2_{all} = 0.00
(d)
δ_{leaf} (‰)
R^2_{day} = 0.11
R^2_{night} = 0.19
R^2_{all} = 0.43
(g)
R^2_{day} = 0.36
R^2_{night} = 0.15
R^2_{all} = 0.57
(i)
R^2_{day} = 0.17
R^2_{night} = 0.07
R^2_{all} = 0.44
(j)
−10
0
10
20
30
40
20
40
60
80
100
−2.5
−1.5
−0.5
T (m d^{-1})
RH (%)
ψ_{leaf} (MPa)
δ_{tiller} (‰)

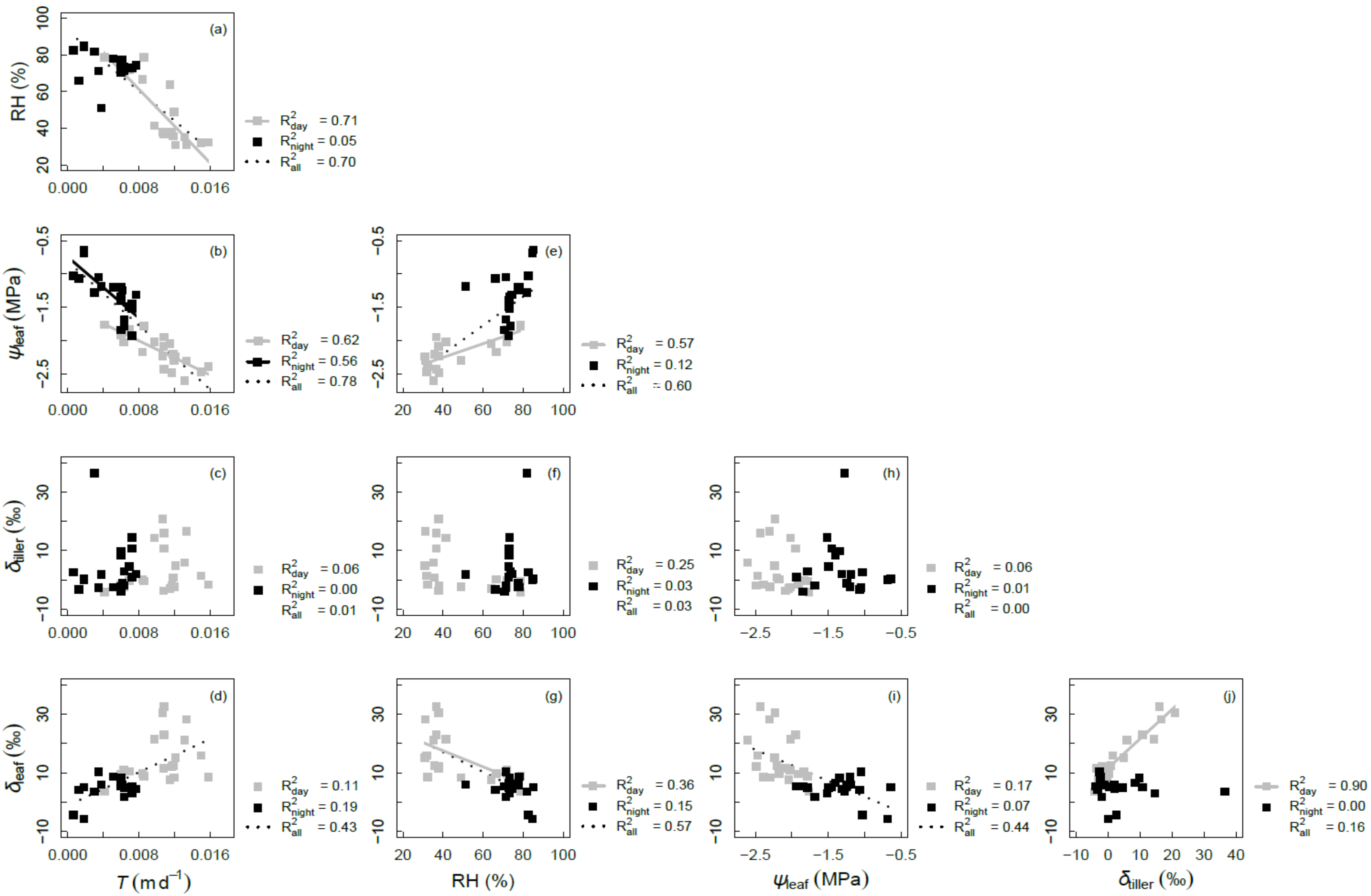
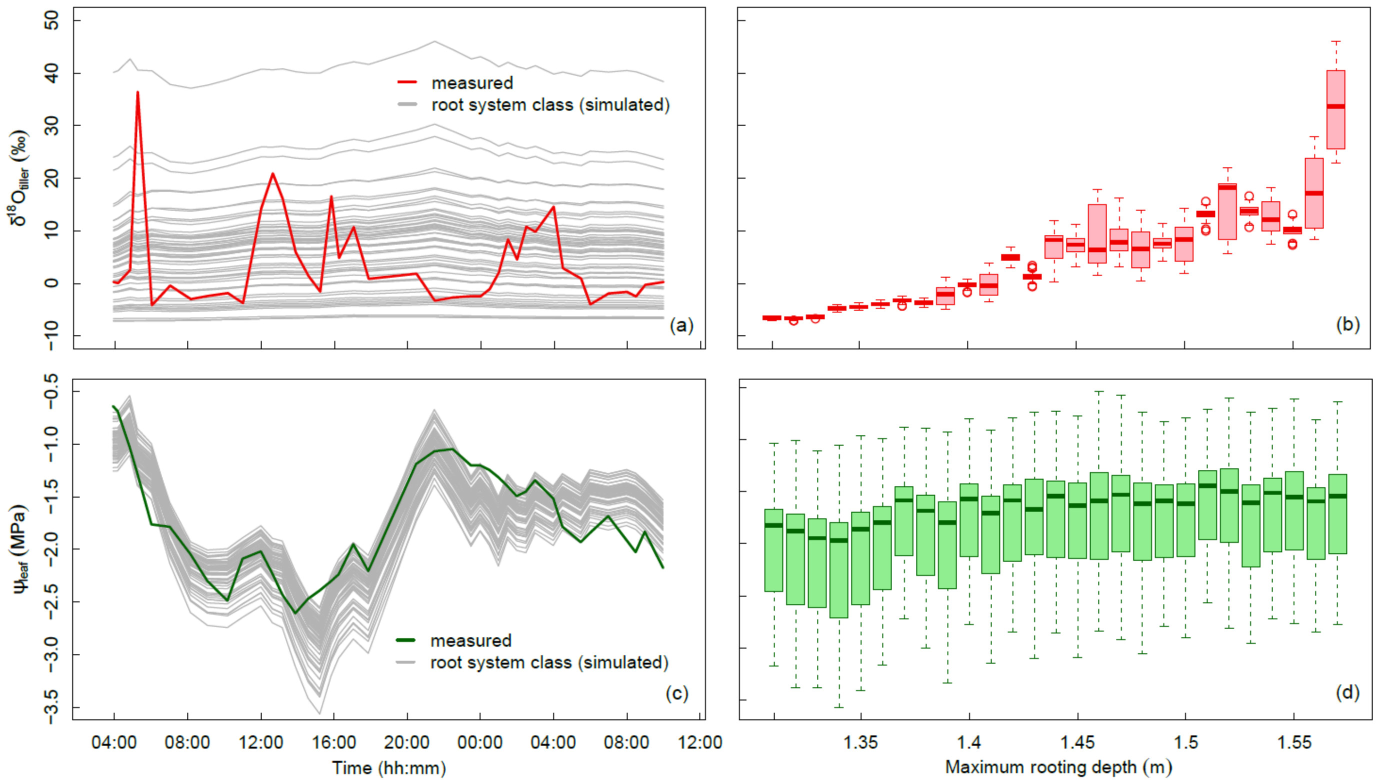


Figure 4. Correlations between measured variables: oxygen isotopic compositions of xylem and leaf waters (δ_{tiller} and δ_{leaf}, in ‰), transpiration rate (T, in m d^{-1}), relative humidity (RH, %), and leaf water potential (ψ_{leaf}, in MPa). Coefficient of determinations (R^2) are reported for all data, and separately for 'day' data (gray symbols) and 'night' data (black symbols) (see Appendix C for definition of 'day' and 'night' experimental periods).



Regression lines are drawn for linear models with p-value < 0.01

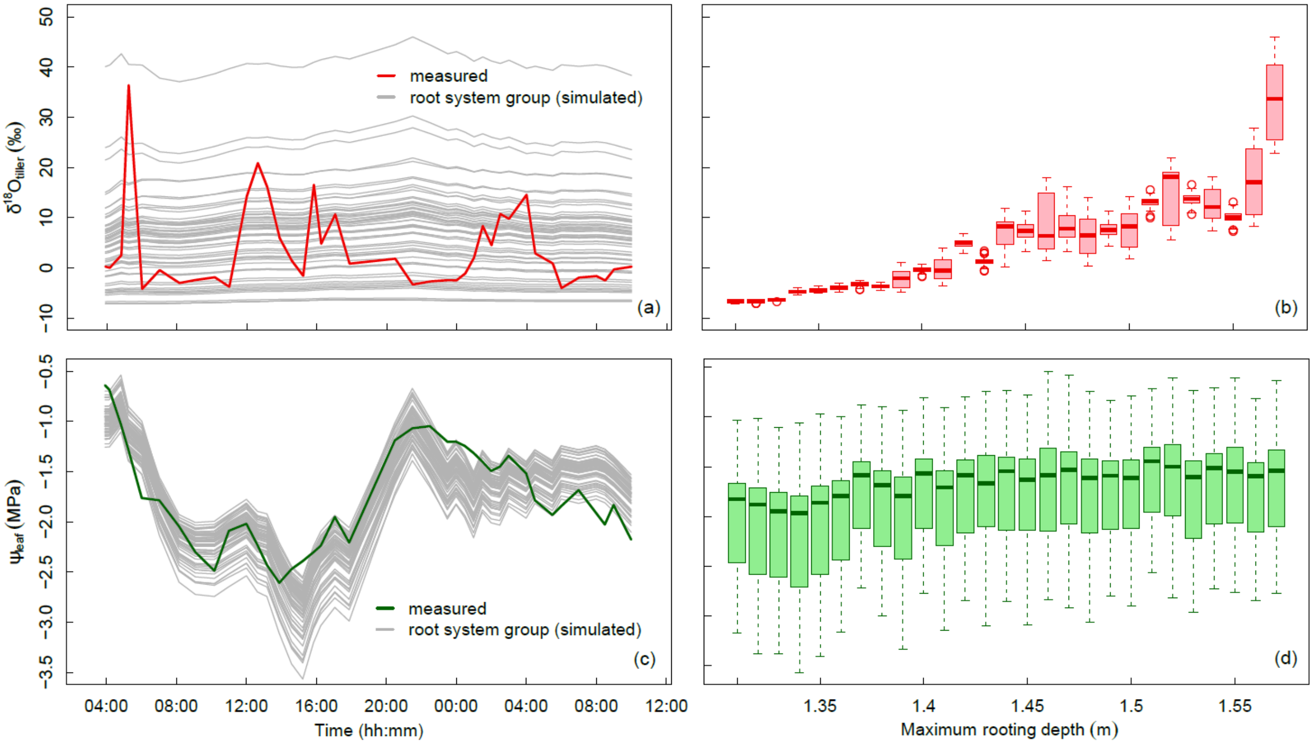
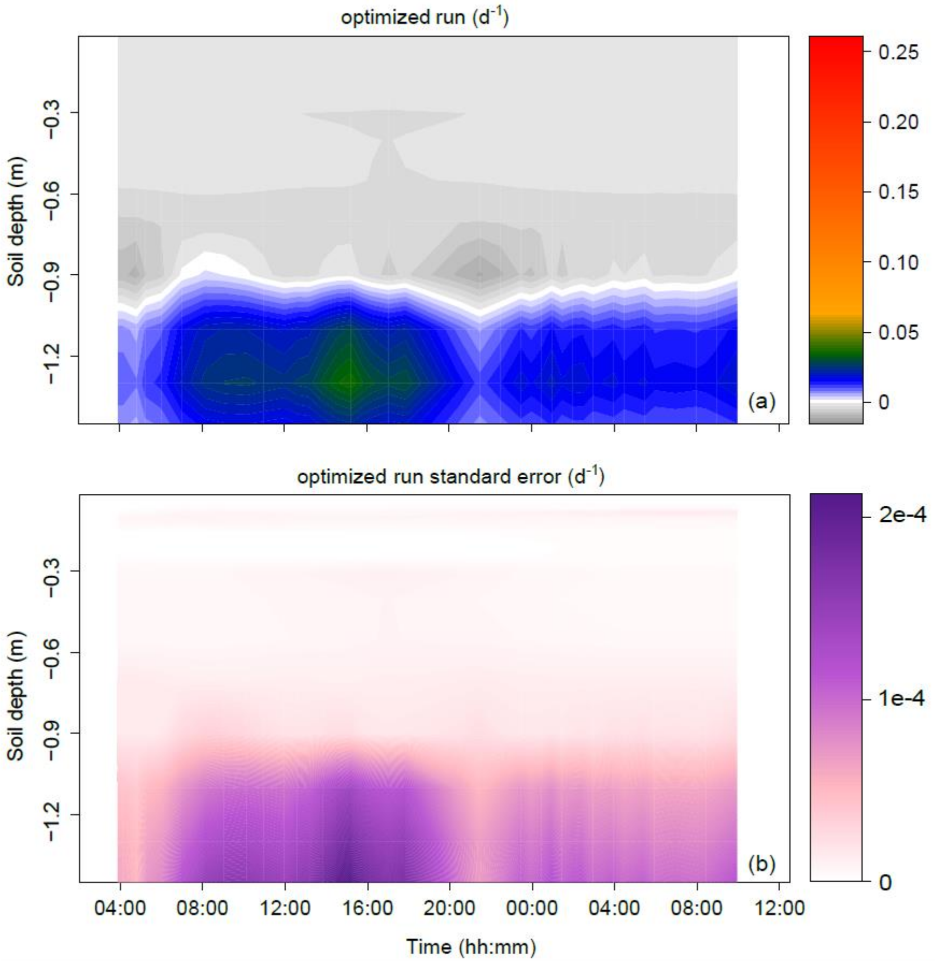


Figure 5. Variation of δ_{tiller} and ψ_{leaf} in time and across the 60 ~~classes~~ groups of simulated root systems. (a) Temporal dynamics of δ_{tiller} measured (thick red line) and simulated (thin grey lines, one line per root system ~~class).~~ group, following a "swarm" pattern). (b) Boxplot of simulated δ_{tiller} values for each root system maximum depth, by 1 cm increment. (c) Temporal dynamics of ψ_{leaf} measured (thick green line) and simulated (thin grey lines, one line per root system ~~class).~~ group, following a "rollercoaster" pattern). (d) Boxplot of simulated ψ_{leaf} values for each root system maximum depth, by 1 cm increment.


optimized run (d^{-1})
Soil depth (m)
−0.3
−0.6
−0.9
−1.2
(a)
0.25
0.20
0.15
0.10
0.05
0
optimized run standard error (d^{-1})
Soil depth (m)
−0.3
−0.6
−0.9
−1.2
(b)
2e-4
1e-4
0
04:00
08:00
12:00
16:00
20:00
00:00
04:00
08:00
12:00
Time (hh:mm)

1

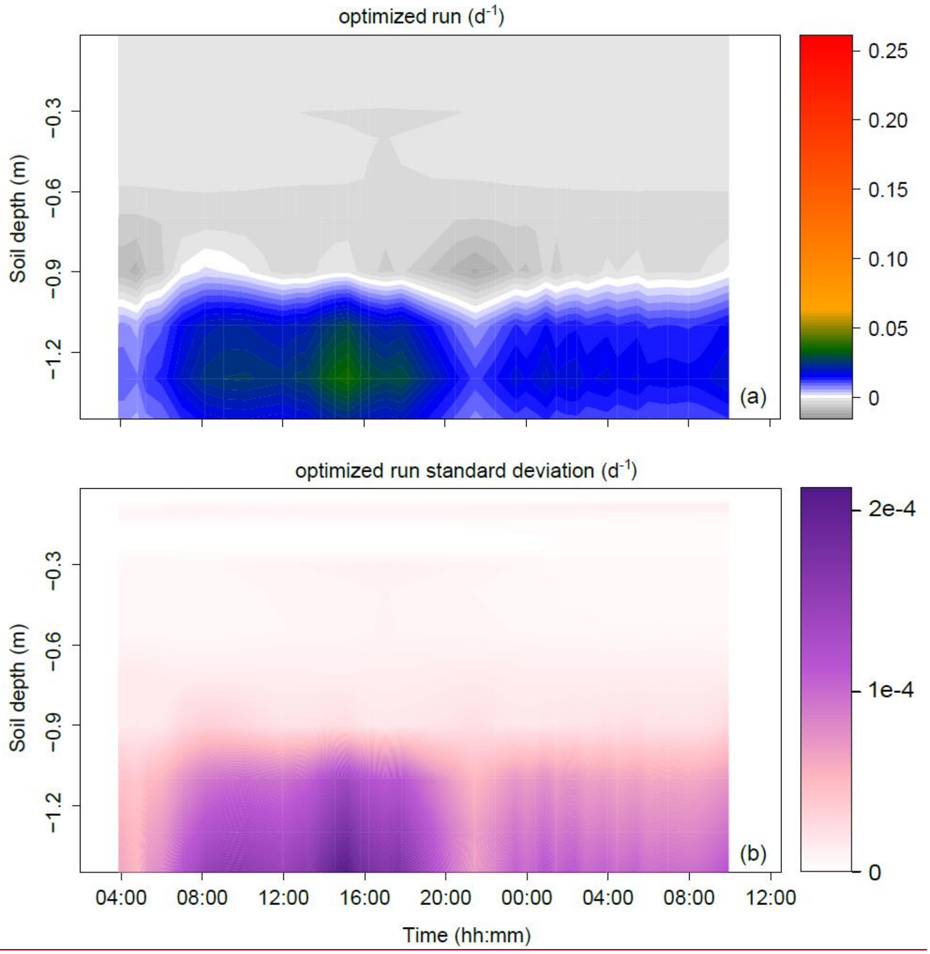


Figure 6. Time series of the profiles of root water uptake per unit soil volume (sink term, d^{-1}) computed with the physically-based model. (a) ~~Average~~ Sum of sink terms across the 60 ~~classes~~groups of the population. (b) Variability of sink terms within the 60 ~~classes~~groups of the population (1 standard deviation).

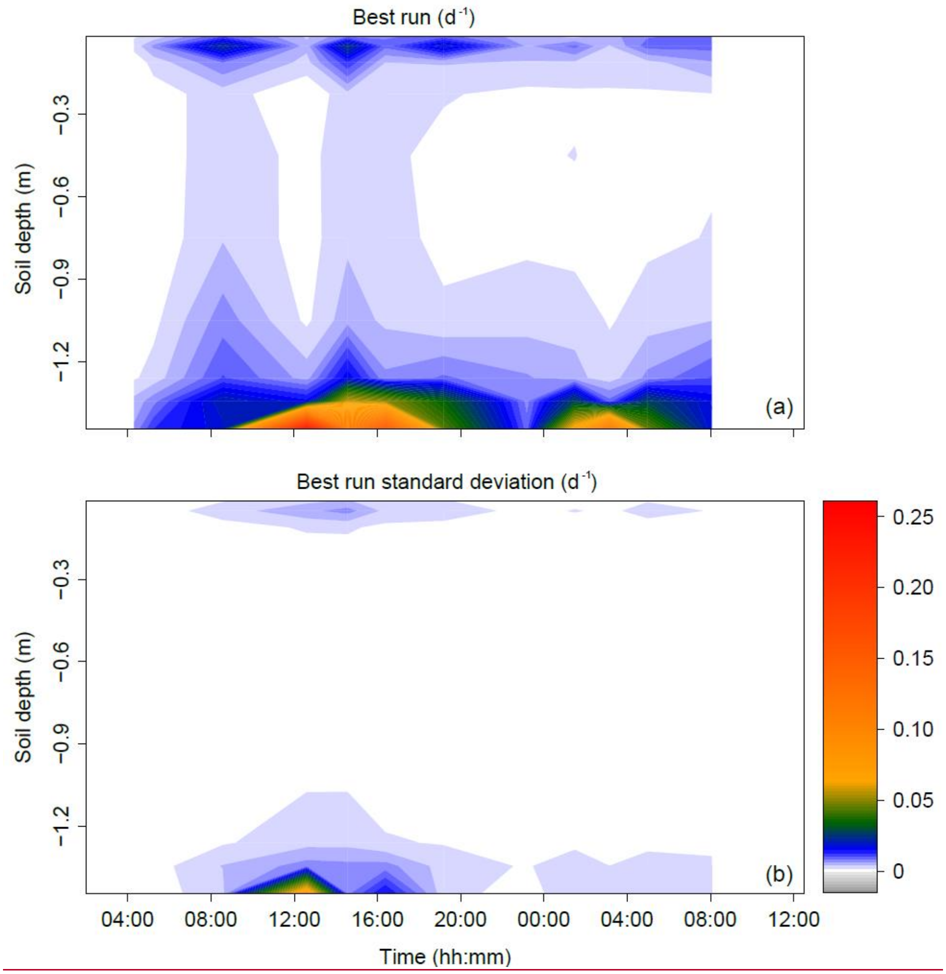
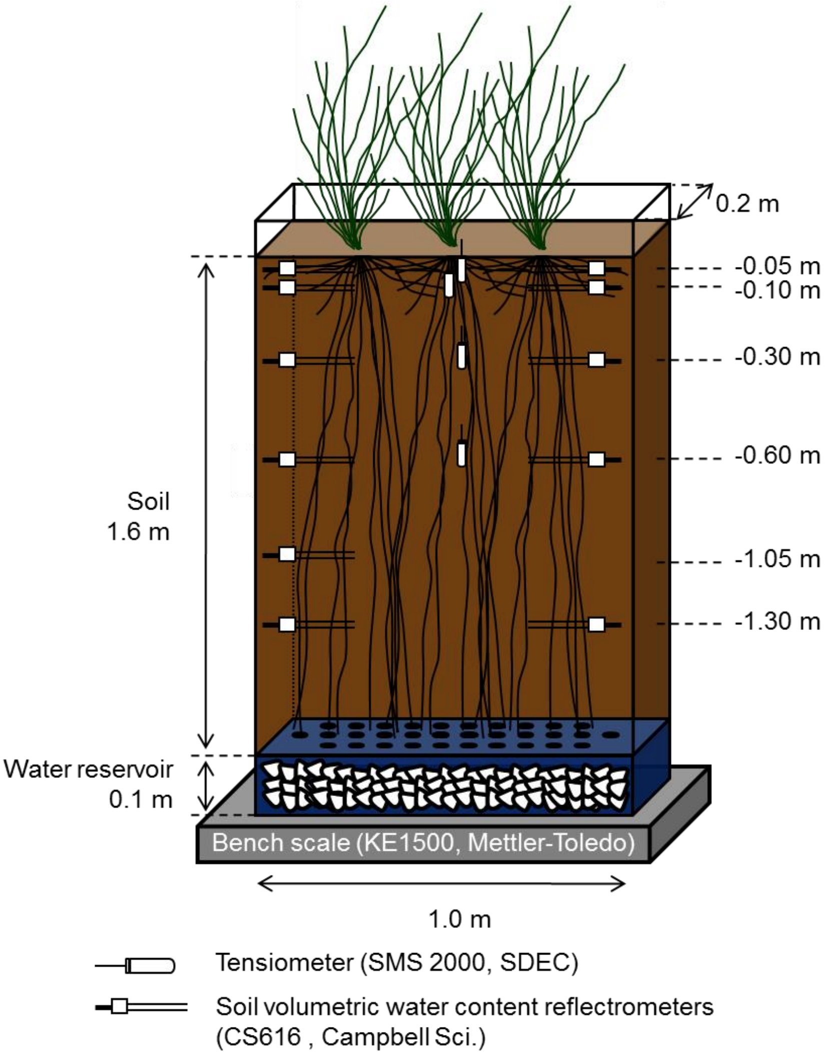


Figure 7. Time series of the profiles of root water uptake per unit soil volume (sink term, d^{-1}) computed with the statistical model SIAR (a). Panel (b) reports the variance of the estimated sink term (1 standard deviation).

7 Appendix



0.2 m
-0.05 m
-0.10 m
-0.30 m
-0.60 m
-1.05 m
-1.30 m
Soil
1.6 m
Water reservoir
0.1 m
Bench scale (KE1500, Mettler-Toledo)
1.0 m
Tensiometer (SMS 2000, SDEC)
Soil volumetric water content reflectrometers
(CS616 , Campbell Sci.)

Appendix A. Soil macro-rhizotron experimental setup with tall fescue cover

θ_{sat} (m^3 m^{-3})	θ_{res} (m^3 m^{-3})	α (m^{-1})	n (-)
0.4	0.044	0.0285	2.29

Appendix B. ~~**Soil retention curve and parameters optimized values (van Genuchten, 1980 - Burdine) (Meunier et al., 2017a)**~~

Soil retention curve and parameters optimized values [van Genuchten, 1980 - Burdine] [Meunier et al., 2017a]

Appendix C. Timeline of destructive sampling

	DAS 166	DAS 167																							
		'night' data					'day' data															'night data'			
Time	15:45	03:55	04:10	04:50	05:15	06:00	07:00	08:10	09:05	10:10	11:00	12:00	12:40	13:10	13:55	14:35	15:15	15:50	16:15	17:00	17:50	20:30	21:30	22:30	23:30
Soil	x						x													x					
Leaves		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
roots	x																								

	DAS 168																
	'night data'												'day' data				
Time	00:00	00:30	01:00	01:30	02:00	02:30	03:00	04:00	04:30	05:00	05:30	06:00	07:00	08:00	08:30	09:00	10:00
Soil										x							
Leaves	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x

Appendix D. Inverse modeling scheme

The parametrization method was inverse modeling, with four targets: (i) minimizing the differences between observed and predicted δ_{tiller} in each pool p, (ii) minimizing the difference between the standard deviations of observed and predicted δ_{tiller} (temporal and population deviations altogether), (iii) minimizing the differences between observed and predicted ψ_{leaf} in each root system ~~class~~group i, (iv) minimizing the difference between the standard deviations of observed and predicted δ_{tiller} (temporal and population deviations altogether). These targets translated as an objective function (*OF*) to be minimized, where differences were normalized by the standard deviation (*SD*) of observations in order to make the error function dimensionless:

$OF =$ ~~$\sqrt{\frac{1}{2}\left(\frac{1}{N_p N_t}\sum_i \sum_t \left(\frac{\delta_{\mathrm{tiller,obs}}(t) - \delta_{\mathrm{tiller},p,\mathrm{sim}}(t)}{SD\left(\delta_{\mathrm{tiller,obs}}(t)\right)}\right)^2 + \frac{1}{N_i N_t}\sum_i \sum_t \left(\frac{\psi_{\mathrm{leaf,obs}}(t) - \psi_{\mathrm{leaf},i,\mathrm{sim}}(t)}{SD\left(\psi_{\mathrm{leaf,obs}}(t)\right)}\right)^2\right)}$~~

~~$+\left|\frac{SD\left(\delta_{\mathrm{tiller,obs}}(t)\right) - SD\left(\delta_{\mathrm{tiller},p,\mathrm{sim}}(t)\right)}{SD\left(\delta_{\mathrm{tiller,obs}}(t)\right)}\right| + \left|\frac{SD\left(\psi_{\mathrm{leaf,obs}}(t)\right) - SD\left(\psi_{\mathrm{leaf},i,\mathrm{sim}}(t)\right)}{SD\left(\psi_{\mathrm{leaf,obs}}(t)\right)}\right|$~~

$$\sqrt{\frac{1}{2}\left(\frac{1}{N_p N_t}\sum_i \sum_t \left(\frac{\delta_{tiller,obs}(t) - \delta_{tiller,p,sim}(t)}{SD\left(\delta_{tiller,obs}(t)\right)}\right)^2 + \frac{1}{N_i N_t}\sum_i \sum_t \left(\frac{\psi_{leaf,obs}(t) - \psi_{leaf,i,sim}(t)}{SD\left(\psi_{leaf,obs}(t)\right)}\right)^2\right)}$$

~~(D1)~~

$$+\left|\frac{SD\left(\delta_{tiller,obs}(t)\right) - SD\left(\delta_{tiller,p,sim}(t)\right)}{SD\left(\delta_{tiller,obs}(t)\right)}\right| + \left|\frac{SD\left(\psi_{leaf,obs}(t)\right) - SD\left(\psi_{leaf,i,sim}(t)\right)}{SD\left(\psi_{leaf,obs}(t)\right)}\right| \qquad \text{(D1)}$$

where N_p is the number of ~~δ_{tiller}~~δ_{tiller} pools simulated (100) at each observation time, N_i is the number of plant ~~classes~~groups simulated (60), and N_t the total number of observation times (40).

The global optimizer Multistart heuristic algorithm OQNLP (Optimal Methods Inc.) of the MATLAB (The MathWorks, Inc., USA) optimization toolbox was used to minimize the error function within the lower and upper limits of the parametric space reported in Table 1.

Appendix E. Statistical determination of relative RWU profiles with SIAR

~~The Bayesian inference statistical model SIAR (Parnell et al., 2013) was used to determine the profiles of relative contributions to RWU (rRWU, dimensionless) of ten identified potential water sources. These water sources were defined to originate from the 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. Their corresponding isotopic compositions were obtained from the measured soil water isotopic compositions (δ_{soil}) and volumetric content (θ) values following Eq. (E1) (Rothfuss and Javaux, 2017):~~

~~$\delta_{soil,J} = \frac{\sum_{j\in J} \delta_{soil,j} \cdot \theta_j \cdot \Delta Z_j}{\sum_{j\in J} \theta_j \cdot \Delta Z_j}$~~The Bayesian inference statistical model SIAR [Parnell et al., 2013] was used to determine the profiles of relative contributions to RWU (rRWU, dimensionless) of ten identified potential water sources. These water sources were defined to originate from the 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. Their corresponding isotopic compositions were obtained from the measured soil water isotopic compositions (δ_{soil}) and volumetric content (θ) values following Eq. (E1) [Rothfuss and Javaux, 2017]:

$$\delta_{soil,J} = \frac{\sum_{j\in J} \quad \delta_{soil,j} \cdot \theta_j \cdot \Delta Z_j}{\sum_{j\in J} \quad \theta_j \cdot \Delta Z_j} \tag{E1}$$

where J is the soil layer index, j is the soil sub-layer index, and ΔZ_j is the thickness of the soil sub-layer j. Therefore, equation (E1) translates the soil water isotopic composition measured across sub-layers j into representative isotopic compositions of the different sources (i.e., across layers J). The computed $\delta_{soil,J}$ were compared to δ_{tiller} values. For this, δ_{tiller} measurements were pooled in twelve groups corresponding to different time periods. These groups were defined to best reflect the apparent temporal dynamics of δ_{tiller}.

For each of the twelve time periods:

(i) the function *siarmcmcdirichletv4* of the SIAR R package (https://cran.r-project.org/web/packages/siar/index.html) was run 500,000 times with prescribed burnin and thinby equal to 50000 and 15, respectively. The output of the model (i.e., the *a posteriori* rRWU distribution across the ten soil water sources J) was obtained from a flat Dirichlet *a priori* rRWU distribution (i.e., $rRWU_J=1/10$);

(ii) the 'best run' (*br*, dimensionless) was selected from SIAR's output. It was defined as the closest solution of relative contributions across sources from the set of most frequent values (*mfv*, dimensionless), i.e., the relative contribution with the greatest probability of occurrence. The best run was identified as minimizing the objective function below, i.e., the RMSE (root mean square error) with respect to the set of mfv_J:

$$OF = \sqrt{\frac{\cancel{\sum_{J=1}^{10}(\mathrm{mfv}_J-\mathrm{br}_J)^2}}{\cancel{10}}}\sqrt{\frac{\sum_{J=1}^{10}(mfv_J-br_J)^2}{10}}$$

(E2)

(iii) *br* was then multiplied by transpiration rate (in m d^{-1}) and divided by soil layer thicknesses (ΔZ_J, in m) to obtain sink terms (S_J, i.e. root water uptake rate per unit soil volume, expressed in d^{-1}). The interest of sink terms in a comparison is that they do not vary with soil vertical discretization.

Steps (i)-(iii) were repeated a 1,000 times to estimate the variance of the best run for each time period and soil water source J.

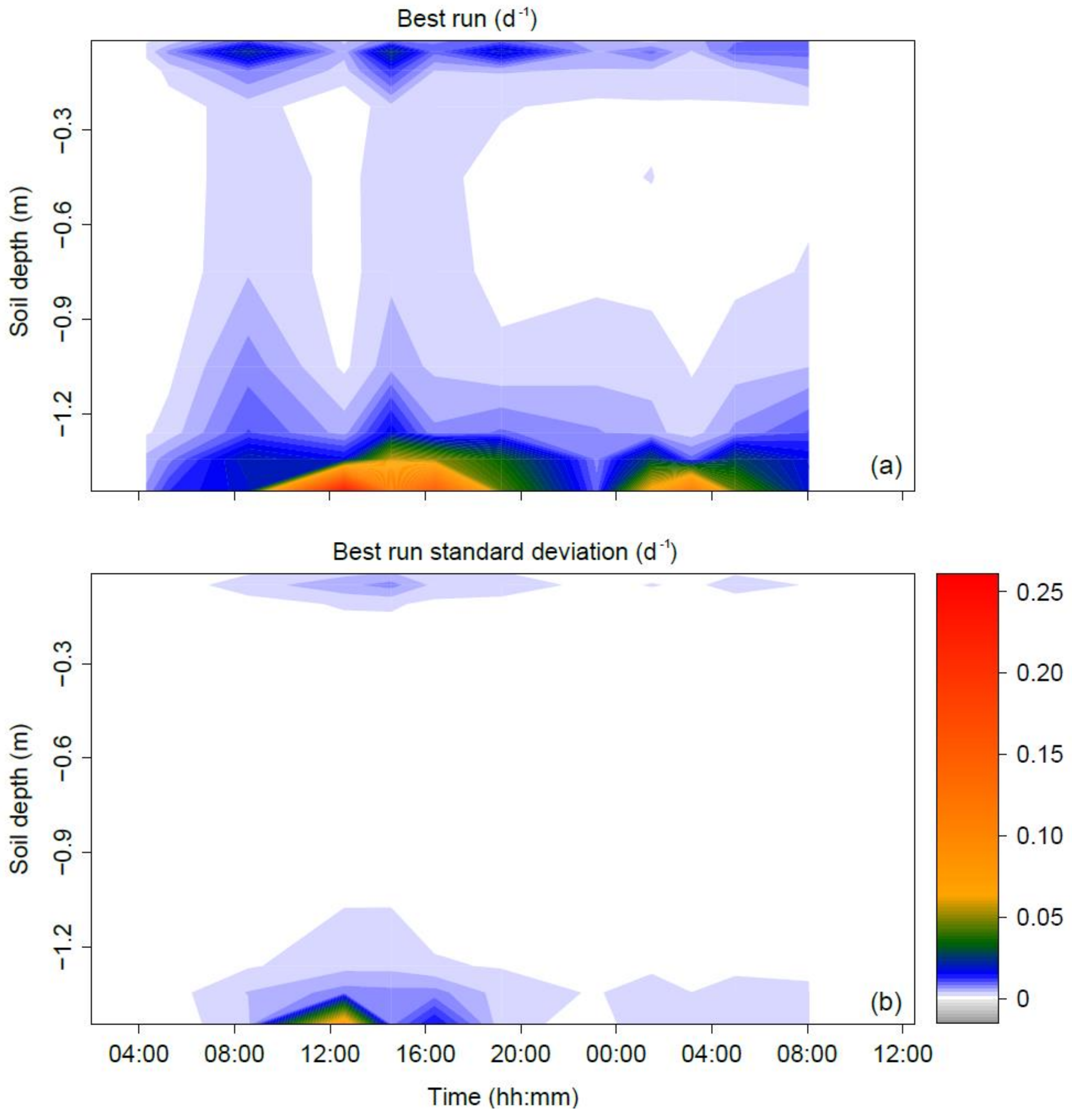


Figure E. ~~Time series of the profiles of root water uptake per unit soil volume (sink term, d^{-1}) computed with the statistical model SIAR (a). Panel (b) reports the variance of the estimated sink term (1 standard deviation).~~