EGUsphere:

Isotopic insights into the dynamics of soil water pools along an elevation gradient

Jiří Kocum et al.

AUTHORS' RESPONSE

REVIEWER 1

Reviewer's Comments:

In this work, the authors compared isotopic dynamics and seasonal origins of different soil water pools at four study areas at various elevations. The novelty of this study lies in the use of a new extraction method to determine tightly bound soil water. This new technique appears promising, but no spike-experiment results and no comparisons with other soil-water-extraction methods are presented. Furthermore, the uncertainty associated with the mass-balance mixing model was not estimated. A detailed description of the technique and its critical evaluation is necessary to assess the quality of the results. In addition to this major comment, other descriptions of the methods should be improved (e.g., the determination of soil water content) and/or moved to other sections (e.g., the use of RMA-based regression).

Authors' Response:

AR:

We thank the reviewer for these detailed comments. In response, we will add the results of spike experiments and explicitly include uncertainty propagation for the mass-balance mixing model. A complete description and critical evaluation of the new extraction technique, together with all associated test results, will be added to the Supplement section to maintain the primary focus of the manuscript on soil water dynamics. In addition, the main text will be revised according to the reviewer's suggestions, including improvements to the description of soil water content and the relocation of method details (e.g., RMA-based regression) to the Methods section. All of these points are addressed in detail in the responses below.

Specific Comments:

RC:

Line 65: Maybe I missed that information, but I do not see a definitive protocol proposed in Ceperley et al. (2024).

AR:

We thank the reviewer for pointing this out. After re-evaluating the cited study, we agree that Ceperley et al. (2024) does not propose a definitive protocol in this context. Therefore, we will revise the text accordingly and remove this reference to avoid potential misinterpretation.

RC:

Table 2: Please group soil cores and mobile soil water by sampling depth, and please indicate the number of locations used for each water source in each study area.

AR:

We took this comment into account and we will edit the table based on the recommendation.

RC:

Section 3.2: The main findings of this research depend on the application of this method to extract tightly bound soil water (TBW). Conventional spike experiments were performed (lines 156-157), but the manuscript does not present evidence of these results or a critical evaluation of the method (including comparisons with other approaches). The use of glass balls for air removal (are they effective?), and the choice of the labelled water seem fundamental for the method and to determine the isotopic composition of TBW based on a mass balance mixing model (and to minimize the uncertainty in the estimations of TBW). Uncertainties arising from the application of the mixing model (e.g., calculations can be based on the error propagation method) should also be presented and discussed. Finally, it would have been helpful to compare the obtained results with those derived from soil water extracted by other methods (e.g., by cryogenic vacuum distillation).

AR:

Extraction method

We thank the reviewer for this important comment. We agree that the robustness of the TBW extraction method needs to be clearly demonstrated, as it underpins the main findings of the study. We will therefore add the results of the conventional spike experiments to the Supplementary section. These experiments will demonstrate that the applied extraction and mixing approach is able to reliably recover the isotopic composition of tightly bound soil water. While the isotope mixing equation itself is a well-established concept in hydrology, we will explicitly discuss the assumptions and potential sources of uncertainty associated with its application to tightly bound soil water.

Furthermore, we acknowledge that cryogenic vacuum distilation (CVD) is currently the most widely used method for soil water extraction and is often considered a reference approach. However, we consider a direct comparison between our method and CVD to be methodologically problematic, as individual CVD setups differ substantially among laboratories in terms of design, operational parameters, and achievable accuracy, as repeatedly documented in the literature (e.g., Orlowski et al., 2018; Kocum et al., 2025).

Such a comparison would therefore not allow for an unambiguous separation of differences arising from the methodological principles themselves from those caused by specific laboratory implementations, and could lead to misleading conclusions. For this reason, we will adopt an alternative approach based on quantifying the systematic offset of the method and its variability using controlled spike experiments. These parameters provide a transparent and transferable measure of method performance, allowing subsequent comparison with specific CVD setups (or other extraction methods) validated in individual laboratories, without confounding the interpretation by uncertainties associated with the heterogeneity of CVD approaches. To clarify this rationale, we will expand the discussion section of the manuscript to explicitly address the limitations of direct CVD comparisons and to justify the chosen validation strategy.

We believe that these additions strengthen the transparency and reproducibility of the method while preserving the primary focus of the manuscript on soil water dynamics rather than on the development or benchmarking of a new extraction method.

Glass balls

Glass balls, typically 10 to 100 per sample, serve two complementary purposes within the applied methodology. First, they help to minimize or completely eliminate air bubbles within the sample. The presence of dissolved oxygen in the added isotopically labelled water is further reduced by using freshly prepared distilled water.

Second, due to the design of the experimental setup (see Fig. A1 in this response) and the applied mixing principle, the glass balls are repeatedly displaced within the sample under the influence of gravity. This mechanical movement facilitates the disruption of larger soil aggregates and promotes effective mixing of the resulting solution. This approach has proven effective in achieving a homogeneous mixture without any observable influence on the isotopic composition of the water.

These methodological details will be added to the revised manuscript to improve clarity and reproducibility.

Figure A1: Custom-built laboratory apparatus designed to ensure homogeneous mixing of soil and added water through controlled mechanical motion of sealed sample vials prior to analysis.

Labelled water

We agree with the reviewer that the isotopic composition of the labelled water is a critical parameter of the mixing approach. While a strong isotopic contrast between the labelled water and soil water can, in principle, facilitate source discrimination, it also amplifies the propagation of mass-balance uncertainties when the contribution of tightly bound water represents a relatively small fraction of the total water pool.

In our approach, distilled tap water was therefore deliberately selected as the labelled water because its isotopic composition lies approximately in the middle of the seasonal range of soil water isotopic variability at the study site. This choice represents a compromise that minimizes error propagation in the mixing equation: even very small uncertainties in the weighed masses of the individual components (in the order of hundredths of grams) can lead to disproportionately large uncertainties in the estimated TBW isotopic composition when the isotopic contrast between the labelled and target water is large (Fig. A2a,b in this response).

By using labelled water with an intermediate isotopic signature, we reduce the sensitivity of the mass-balance model to small weighing errors, thereby improving the overall robustness of the TBW estimation. This rationale has now been clarified in the revised manuscript.

Error propagation

We agree with the reviewer that uncertainties associated with the application of the mixing model should be explicitly quantified and discussed. In the revised manuscript, we will therefore account for both (i) the analytical uncertainty of the isotopic measurements and (ii) the uncertainty arising from the mass-balance mixing procedure. The total uncertainty of the estimated isotopic composition of tightly bound water will be quantified using standard Gaussian error propagation. Analytical isotope measurement uncertainty will be assumed to be constant across samples, whereas mass-related uncertainty was propagated individually for each sample according to its specific mixing ratio. All uncertainty terms will be assumed to be independent. This approach results in higher sensitivity of the estimated isotopic composition at low soil water contents and large dilution ratios.

$$\delta^{18}O_{TBW} = \frac{m_M}{m_{TBW}} \cdot \delta^{18}O_M - \frac{m_L}{m_{TBW}} \cdot \delta^{18}O_L \tag{1}$$

$$\delta^{2}H_{TBW} = \frac{m_M}{m_{TBW}} \cdot \delta^{2}H_M - \frac{m_L}{m_{TBW}} \cdot \delta^{2}H_L \tag{2}$$

$$m_{TBW} = m_M - m_L \tag{3}$$

$$\sigma^2_{\delta^{18}O_{TBW}} = \left(\frac{m_M}{m_{TBW}}\right)^2 \sigma^2_{\delta^{18}O_M} + \left(\frac{m_L}{m_{TBW}}\right)^2 \sigma^2_{\delta^{18}O_L} + \left(\frac{\delta^{18}O_M - \delta^{18}O_{TBW}}{m_{TBW}}\right)^2 \sigma^2_{m_M} + \left(\frac{\delta^{18}O_{TBW} - \delta^{18}O_L}{m_{TBW}}\right)^2 \sigma^2_{m_L} \tag{4}$$

where TBW stands for tightly bound soil water, M for mixture and L for isotopically labelled water.

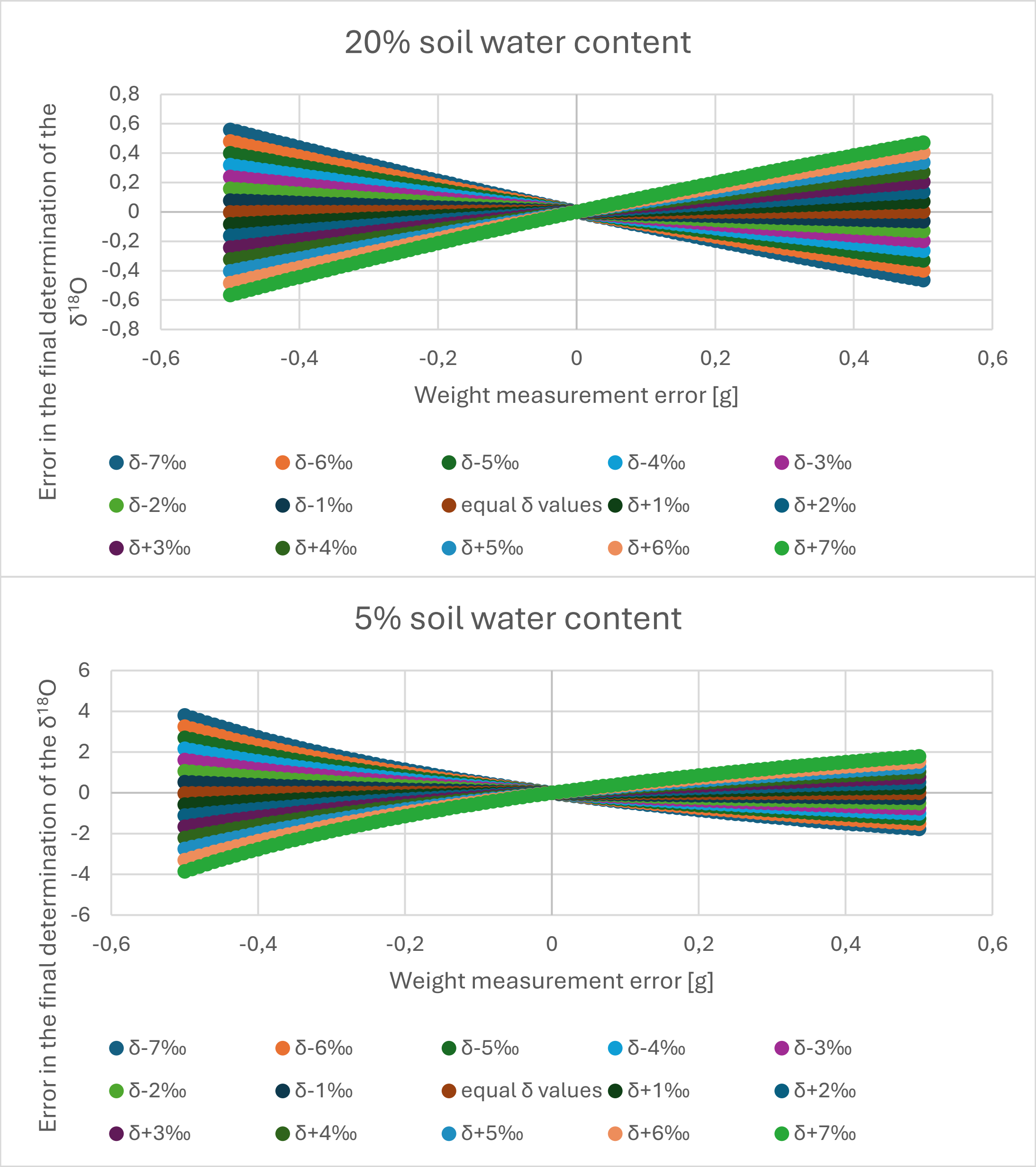

Figure A2: Sensitivity of the calculated isotopic composition of tightly bound water to weighing uncertainties at different soil moisture contents. The figures show the resulting error in $\delta^{18}O$ (Y axis) as a function of cumulative weighing error (X axis) for different isotopic contrasts between soil water and labelled water ($\Delta\delta$, coloured lines), illustrated for soil water contents of 20% and 5%, respectively.

RC:

Line 147: Please provide the isotopic composition of the traced water. I also suggest using 'isotopically labelled water' instead of 'traced water' to improve the clarity. Furthermore, I expect that the isotopic signature of the traced water is significantly different from that of the samples. Otherwise, it would be challenging to discriminate between TBW and the traced water.

AR:

Thank you for the suggestion. We will revise the terminology to "isotopically labelled water", and will use this term consistently throughout the revised manuscript. The isotopic composition of the labelled water will be added to the manuscript. The stable isotopic composition of labelled water used in this study was ranging from -7.84 to -9.64 ‰ and -60.47 to -68.20 ‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. The choice of isotopically labelled water is explained in the previous answer.

RC:

Line 158: Do the values between parentheses represent isotopic shifts or isotopic compositions?

AR:

These are isotope shifts. The sentence will be reworded for clarity.

RC:

Lines 195-196: Besides a better visual representation, is there a more quantitative purpose for the application of a 3rd-degree polynomial fit? Given that you cannot use it to obtain an amplitude for the specific water sources, I would suggest removing such analysis based on polynomial fits.

AR:

As Fig. 5 will be largely modified in the revised version to improve readability (as described in one of the following comments), the 3rd-degree polynomial analysis will be removed. Nevertheless, we prefer to keep it in Figure 8, where it provides a suitable approximation of the data and effectively highlights differences in the annual cycle dynamics between sites.

RC:

Lines 197-206: The description of RMA-based regression lines should be moved to section 3.3, before Equation 3. Please clarify whether RMA was applied to determine the $\delta^{2}H$-$\delta^{18}O$ regressions of all water sources.

AR:

The description of RMA-based regression lines will be moved to section 3.3. A statement will be added to indicate that RMA will be applied to determine the $\delta^{2}H$-$\delta^{18}O$ regressions for all water sources.

RC:

Section 3.5: Did you apply any isotopic correction for samples with an evaporative signature? If so, please describe it. If not, you should consider it for samples with a negative lc-excess.

AR:

We thank the reviewer for pointing out this oversight. In the revised version, the correction will be applied according to Benettin et al. (2018).

RC:

Section 3.6: I do not understand very well the aim of this statistical analysis, and particularly of the 'differences in the annual courses of isotopic composition of individual waters'. Are you comparing δ^2H-δ^{18}O regression lines or time series of different water sources? The current text is unclear.

AR:

The intention was not to compare δ^2H–δ^{18}O regression lines, but to statistically evaluate differences between the seasonal time series of isotopic composition among individual water sources.

Specifically, sinusoidal functions were fitted to the isotope time series, and a robust bootstrap approach was used to assess whether the fitted seasonal patterns (i.e., their amplitudes and phases) differed significantly between water sources.

As Figure 5 will be completely redesigned and the fitting of individual isotope signals will be omitted, this statistical analysis will no longer be relevant and will therefore be removed from the revised manuscript.

RC:

Section 4.1 and Figure 3: Are the regression slopes significantly different or not? Many TBW samples fall on the left side of the LMWL; do they lie within the 95% prediction interval for precipitation? If not, why do they plot there? Were there any issues (e.g., organic contamination) observed during the isotopic analysis?

AR:

In the revised manuscript, we will complement the regression analysis with a bootstrap-based assessment of RMA slope differences, including the calculation of 95% confidence intervals. The results of this analysis and their implications for the interpretation of the isotopic relationships among the investigated water types will be explicitly discussed in the revised manuscript.

RC:

Figure 4: I recommend showing the sample size associated with each boxplot, as well as which water sources are significantly different.

AR:

Thank you for this suggestion. The requested information will be added to the revised manuscript.

RC:

Line 274: Please provide the name of the statistical test when you state that there are (or there are not) significant differences among samples.

AR:

The manuscript will be revised to explicitly specify the statistical tests used when reporting significant differences.

RC:

Section 4.3: Soil water content is mentioned many times here, but details about the measurements (sensors, locations, spatial and temporal resolution) were not reported in earlier sections of the manuscript.

AR:

In Section 2, information will be added regarding soil moisture measurements, including a control tensiometer within the VS-Pro system and with other tensiometers (T8, UMS, Germany) located at each site in various depths.

RC:

Figure 5: In this figure, there are too many dots, lines, and vertical bars that make the interpretation very difficult. For instance, vertical bars for precipitation amount are not very visible, and symbols or lines for soil water content are not clearly represented. If soil water content is indicated by the vertical bars for TBW and MW, I do not think these values are representative of soil water content at the monthly scale (or of the temporal variability of wetness conditions) in the study areas. Moreover, it seems that the fitted lines do not capture well the temporal dynamics of the various water sources, but their efficiency needs to be quantified and deserves an explanation.

AR:

Thank you for pointing this out. This figure will be completely redesigned to enhance clarity.

RC:

Lines 317-320 and Table 3: The comparison between the fitted lines does not make much sense if the sine function can model a small fraction of the total variability. I also do not think this analysis is particularly informative in supporting the key messages of this work; therefore, this method and these results can be removed.

AR:

Along with the revision of Figure 5, this section of the text will be completely rewritten, and the method mentioned will be removed.

RC:

Figure 6: How many SOI values are there for each month? Please report the sample size inside each cell.

AR:

Values indicating sample size within each cell will be added to the figure depicting SOI values, which will also be redesigned based on a suggestion from the second reviewer.

RC:

Lines 351-355: This text belongs to the discussion. Please move it.

AR:

The text will be moved to the Discussion section.

RC:

Line 397: The previous text and the figures do not present the estimation of the transit times. Please revise the text and refer only to the phase shift.

AR:

We will revise this part of the text so that it will no longer include the reference to transit times.

RC:

Line 18: Please replace ‘intimate’ with another term (e.g., ‘comprehensive’, ‘detailed’).

AR:

We will replace the word "intimate" with the word "comprehensive."

RC:

Line 115: ‘lower sampled soil layer’ instead of ‘lower soil layer samples’.

AR:

This part of the text will be rewritten and, based on the suggestion of the second reviewer, the terms "lower" and "deeper" will be replaced with numerical values indicating depth in centimeters.

RC:

Line 142: ‘and’ instead of ‘with’.

AR:

Thank you for pointing this out. The suggested stylistic change will be implemented.

RC:

Equations 1 and 2: Please use ‘TBW’ instead of ‘S’.

AR:

Thank you for this suggestion. We will revise the indexing in Eqs. (1) and (2) and replace the symbol S with TBW as recommended. In addition, to maintain consistent terminology throughout the manuscript and in response to a previous comment, we will replace the symbol T (traced water) with L (labelled water).

RC:

Line 189: Zuecco et al. (2024) is not present in the list of references.

AR:

Thank you for pointing this out. The citation will be added.

RC:

Line 274: The term 'absolute quantities' is unclear (not very precise); please replace it with another term.

AR:

The term 'absolute quantities' will be replaced with 'relative contributions to soil water' to improve clarity and precision.

Benettin, P., Volkmann, T. H. M., von Freyberg, J., Frentress, J., Penna, D., Dawson, T. E., and Kirchner, J. W.: Effects of climatic seasonality on the isotopic composition of evaporating soil waters, Hydrol. Earth Syst. Sci., 22, 2881–2890, https://doi.org/10.5194/hess-22-2881-2018, 2018.

Kocum, J., Haidl, J., Gebousky, O., Falatkova, K., Sipek, V., Sanda, M., Orlowski, N., and Vlcek, L.: Technical note: A new laboratory approach to extract soil water for stable isotope analysis from large soil samples, Hydrology and Earth System Sciences, 29, 2863–2880, https://doi.org/10.5194/hess-29-2863-2025, 2025.

Orlowski, N., Breuer, L., Angeli, N., Boeckx, P., Brumbt, C., Cook, C. S., Dubbert, M., Dyckmans, J., Gallagher, B., Gralher, B., Herbstritt, B., Hervé-Fernández, P., Hissler, C., Koeniger, P., Legout, A., Macdonald, C. J., Oyarzún, C., Redelstein, R., Seidler, C., Siegwolf, R., Stumpp, C., Thomsen, S., Weiler, M., Werner, C., and McDonnell, J. J.: Inter-laboratory comparison of cryogenic water extraction systems for stable isotope analysis of soil water, Hydrology and Earth System Sciences., 22, 3619–3637, https://doi.org/10.5194/hess-22-3619-2018, 2018.