

General comments to the Editor and Reviewer

We thank the Editor Matijn Westhoff and both Reviewer for their time to provide critical feedback to our manuscript. We believe that their specific suggestions, which we widely implemented into the revised manuscript, improved the clarity of our study.

To make the assessment of our revision as easy as possible for the Editor, we list below first the main six changes done for the revised manuscript. We also added the responses to the two reviewers below and the minor changes to the responses already published in the HESS discussion forum are highlighted via track changes. Examples from text that was changed in the revised manuscript are in quotation marks and the font is in *italic*. The reviewer's comments are given in Times New Roman font.

The revised manuscript with changes highlighted via track changes is at the end of this document.

We hope that this will allow the editor to assess the revision without another round of peer-review, since the requested changes were minor and all remarks of the reviewers were accounted for.

The main changes done were:

1.) Rephrasing parts of the abstract and introduction to highlight the novelty (see examples below)

We rephrased some parts of the abstract:

“Some studies found based on stable isotope (^2H and ^{18}O) data that water infiltrating into soils can bypass older pore water. Though, the mechanisms leading to a separation between water routed to the streams and water held tightly in smaller pores are yet unclear. Here, we address the current limitations of the understanding in subsurface mixing and its consequences for the application of stable isotopes in ecohydrological studies. We present an extensive data set,...”

We changed in the introduction:

“It has been hypothesized by Brooks et al. (2010) that rainwater refilling the dry soil at the end of a dry season would lead to a distinct signal between mobile and tightly bound water. Though, the mechanisms leading to a separation between water routed to the streams and water held tightly in smaller pores are unclear and currently under debate (Sprenger et al., 2016b; Berry et al., 2017; Dubbert et al., 2019)”

And we added the limited sampling numbers in older studies:

“So far, the experimental set up of studies addressing the disconnection between water flow across different pore spaces had limited sampling frequencies and sample numbers (maximum twice per year in Brooks et al., 2010; Goldsmith et al., 2012, and five per year in Hervé-Fernández et al., 2016), which impeded progress in understanding of the mechanisms explaining the disjunct subsurface water pools.”

2.) Adding a figure showing the locations of the hydrometric measurements and stable isotope sampling. (Reviewer #1)

3.) Adding info on the suction lysimeter. (Reviewer #1)

4.) Adding discussion on water volumes stored in the tightly bound water pool (Reviewer #2)

5.) Rephrasing sections where we used expressions like “no mixing” or “disconnected” and changed it to “little mixing” and “disjunct”, as we cannot exclude that there is no exchange at all taking place.

6.) To better explanation of Figure 7 (former Figure 6):

“Figure 7 summarizes our field observations and their potential implications. Due to the nature of two disjunct pore spaces, a seasonally varying isotopic composition in the rainfall will not be preserved across the soil profile (as in Sprenger et al. (2016a) and Figure S1), but the fast and slow flow domains contain waters of different isotopic compositions with higher seasonal variation in the mobile water (Figure 7b) than in the tightly bound water (Figure 7c). The different water retention characteristics (as derived in section 2.4) for the fast and slow flow domain (e.g., Gerke and van Genuchten, 1993) are directly linked to the soil pore diameters (Schjonning, 1992). The different flow pattern and the lack of exchange between the mobile and tightly bound water result in the distinct isotopic compositions across the pore space at the same soil depth (red and blue shaded in Figure 7e).”

Responses to the reviewer comments. Differences to the already published responses in the HESS discussion forum are highlighted via track changes.

Reviewer #1

The manuscript titled “Mechanisms of consistently disconnected soil water pools over (pore) space and time” describes a study that uses the stable isotopes of hydrogen and oxygen in soil water, precipitation, local streams, and groundwater to identify apparently separate soil water pools and assess specific processes that result in bypass flow in soils. This study adds to the increasing amount of evidence that confirms the occurrence of the partitioning of soil water between tightly bound immobile water and mobile water that moves downward to discharge in streams and recharge groundwater, as suggested by the two water worlds hypothesis. In addition, this study puts effort into assessing the mechanisms that result in this partitioning.

Overall, this paper is very well written. It addresses a very relevant scientific question that has implications for determining the proper way to interpret stable isotope data in ecohydrology and for better understanding the local water balance in different areas. The scientific methods and assumptions are valid and sound, and the conclusions, which are supported by the results, add significantly to our understanding of ecohydrology.

Response: We are glad to hear that the reviewer believes that our work contributes to a better understanding of ecohydrological processes.

The only significant modification to the paper that I would suggest is the addition of a figure that shows:

- 1) The global, regional, and local location of the study area
- 2) Soil and water sample locations

Response: In the revised manuscript, we ~~will~~added a figure that shows the location of the Can Vila research catchment in Spain, the study site, where the soil water sampling, piezometer sampling and rainfall sampling were done within the catchment, and the outlet, where stream water isotopes and discharge were measured.

~~The figure will look similar to the preliminary Figure 1.~~

Other minor suggested edits include:

1) Abstract – The second sentence is awkward and should be revised. The third sentence appears to describe an observation from the study before the study objectives and other details are defined. This sentence (and one or two additional sentences) should describe observations of other researchers to define the problem that this study addresses.

Response: We ~~will~~ rephrased the second sentence and we actually intended to refer to earlier studies in the third sentence. We ~~will~~ revised this part of the abstract to be clear about that. The revised ~~part will~~ reads as follows:

“However, how much subsurface mixing of water occurs, how much of the water is available for plants or otherwise percolating to streams and the groundwater is not yet understood. Some studies found based on stable isotope (^2H and ^{18}O) data that water infiltrating into soils can bypass older pore water. Though, the mechanisms leading to a separation between water routed to the streams and water held tightly in smaller pores are yet unclear. Here, we address the current limitations of the understanding in subsurface mixing and its consequences for the application of stable isotopes in ecohydrological studies.”

2) Page 1, line 28 – Change the word “since” to “for.”

Response: ~~Will be e~~Changed as suggested.

3) Page 1, line 30 – Change the word “unraveled” to “of interest” or something similar.

Response: ~~Will be e~~Changed as suggested.

4) Page 3, line 2 – Add a citation for a reference that describes suction lysimeters or include more details about them.

Response: We ~~will~~ added and adjusted the following sentences to better describe provide a reference for the suction lysimeters: *“The lysimeters consisted of 15 cm long porous cups (RSK ADAS Ltd., UK) with 2 inserted tubes that allow to create the vacuum in the lysimeter and to sample soil water by injecting air into the lysimeter. We applied a suction of -700 hPa and sampled the water extracted within a few hours.”*

5) Page 3, line 3 – Include a citation of the cryogenic extraction procedure.

Response: We ~~will~~ added a reference to (Martín-Gómez et al., 2015), where more details on the extraction can be found from the laboratory that conducted the extraction and isotope analysis.

6) Page 12, line 17 – change wording to “...mechanisms by which...”

Response: ~~Will be e~~changed as suggested.

Other comments: The figures are quite complex, and it takes time to fully understand them, but they do show a lot of valid information. The authors state that tightly bound water is composed of relatively old water. I am wondering if they could suggest an actual age or range of ages for this tightly bound water (months, years, decades??).

Response: On Page 9, line 5 – 10, we briefly discuss the difficulty to assess how much the mobile and tightly bound water are in exchange with regard to their isotopic composition and we argue that numerical models would be a way to test different hypotheses of inter-pore mixing. We are currently not aware of an isotope enabled soil hydraulic model that could account for both a dual-permeability representation and a variable lower boundary condition, that accounts for temporarily pressure heads that reach the soil surface (as observed in the piezometer). However, we know that we are limited in our interpretation of stable isotope data to a few years due to the annual cycle of the rainfall isotope signal. Thus, if the tightly bound water would be older than two years, there would be no way in detecting that with the current data set. We ~~are planning to add~~ed on page 9 the following to the discussion to pick up the idea of Reviewer #1 and a potential solution to this question:

“Thus, based on our field data, we can currently not assess the actual age (months or years?) of the tightly bound water, but experimental approaches with deuterated (enriched in 2H) water could help assessing how much of exchange between the mobile and tightly bound water can occur (Evaristo et al., 2019).”

References:

Evaristo, J., Kim, M., Haren, J., Pangle, L. A., Harman, C. J., Troch, P. A., and McDonnell, J. J.: Characterizing the fluxes and age distribution of soil water, plant water, and deep percolation in a model tropical ecosystem, *Water Resour. Res.*, doi:10.1029/2018WR023265, 2019.

Martín-Gómez, P., Barbeta, A., Voltas, J., Peñuelas, J., Dennis, K., Palacio, S., Dawson, T. E., and Ferrio, J. P.: Isotope-ratio infrared spectroscopy: a reliable tool for the investigation of plant-water sources?, *New Phytologist*, 207, 914–927, doi:10.1111/nph.13376, 2015.

Reviewer #2

Content: The paper examines isotopic differences in water pools across depth and time to quantify the extent of mixing. They find that tightly bound water, defined as water not sampled via suction lysimeters, differed consistently from more tightly bound water.

Contextually, the paper is a comparison of two soil water extraction methodologies, it begs the question of whether the extraction technique equals the label applied here (and by many others!), i.e. if ‘mobile’ water and ‘immobile’ water (lysimeter and cryogenically sampled water, respectively) are truly so.

Response: Following Brooks et al. (2010) we refer to the differently sampled waters as “mobile” and “tightly bound” waters (Page 1 Line 15) and did not use the term “immobile” water in the paper. Indeed,

we assume that the water extraction methods (suction lysimeters and cryogenic vacuum extraction) correspond to two actual different degrees of soil water mobility.

The authors transform the isotopic signatures using a mass-balance approach but this is ultimately based on differences between extraction techniques. Others have reported similar observations of the differences between soil water, and attributed it to the same process where small pores retain water. This derives similar conclusions from a larger dataset and expands on dynamics in time. However, it does seem to re-hash, in greater detail, the observations and conclusions drawn by Brooks 2010 and leaves the reader questioning the novelty of the results (indeed, many papers have noted consistent differences between cryogenically-extracted water and soil lysimeters).

Response: We are surprised to read that the novelty is questioned. To our knowledge there are no studies that provide in a similar way an explanation on the differences in the stable isotopic composition of waters in the unsaturated zone. We refer to several studies that show these differences in mobile and bulk waters, but we did not find in any of these studies an explanation laying out the processes that lead to the observations. Brooks et al. (2010) suggested the filling of smaller pores during low soil moisture conditions but they could not underline the hypothesis with sufficient data (3 soil sampling campaigns and NO rainfall data prior to soil sampling). As pointed out by Reviewer #2, our study presents results “in greater detail”. We truly believe that this “greater detail” allows us to go further in our observations and conclusions and therefore we think that our contribution do not deserve to be qualified as a “re-hash”. We have seen numerous studies referring to “ecohydrological separation” and often limiting the analysis to comparisons of soil water and xylem water in dual isotope plots and then concluding that there is either an ecohydrological separation if they do not plot on top of each other, or there is (partially) no ecohydrological separation if they plot on top of each other. Therefore, we think that the fact that our study “expands on dynamics in time” (as acknowledged by Reviewer #2) is a truly new view on the issue.

Overall, the paper is a nice contribution to our understanding of partitioning of water in the subsurface. They refer to this as ecohydrologic separation but it seems more a function of meteorology and geology, with potential impacts on cycling of water and uptake by plants.

Response: We really only use the term “ecohydrological separation” once in our manuscript (Page 9 Line 30) when we discuss our findings in the context of the study by Brooks et al. (2010). We are glad that Reviewer #2 could follow our discussion that the observed separation stems from the interplay of the hydro-meteorological seasonality and the soil characteristics. This was one of our main messages and deriving this conclusion was only possible due to the long-term rainfall isotope and soil moisture observations.

Moreover, e.g. p9, line 5 (and elsewhere referred to) How many mm of water does this ‘tightly bound’, ‘immobile’ portion represent? What portion of the annual water budget at this field site is ‘locked’ away as suggested? Ultimately, how important is this water that doesn’t mix? The various conclusions and potential impacts highlighted in the discussion section very much depend on an implicit assumption that this ‘portion’ is somehow significant.

Response: As indicated above, following Brooks et al. (2010) we are not using the term “immobile” water, but we highlight that this tightly bound water seems to play a minor role in the groundwater recharge and stream water contributions, as it does not mix well with the mobile water and has a lower hydraulic conductivity compared to the mobile water (compare thin blue and red lines in Figure S2).

According to the water retention curve (as shown with thick lines in Figure S2) the volumetric soil moisture of the more tightly bound water ($h < -700$ hPa) is $0.29 \text{ cm}^3/\text{cm}^3$. If we consider the studied upper 1 meter we would have about 290 mm stored in the soil matrix, which cannot be sampled with suction lysimeters. However, this water is not “locked away”, as it would be partly available for evaporation and transpiration (permanent wilting point is often assumed to be about -15.000 hPa) and percolates according to subsurface pressure differences. 290 mm are about 1/3 of the annual rainfall. Why this water is relevant is discussed on Page 11 Line 31 and following. However, the actual volume does not seem to be most important here, but the strong “non-uniform” character of subsurface flow that can be recognized with our data set is the most relevant point.

We ~~will add~~ed to the revised manuscript the 290 mm of water in tightly bound water. ~~However, we will also~~We further rephrased the manuscript in a way to clarify that we cannot claim that the observed water pools do not mix at all, but mixing is very limited given the very distinct stable isotopic compositions between mobile and bulk soil waters.

Example of where we changed: “The maximum water volume stored in the tightly bound water pool is about 290 mm if we consider the studied upper 100 cm (max $\theta_{TW} = 29$ %, Figure S2). This represents about 1/3 of the annual average rainfall, but it cannot be considered as being in an inactive storage, since it would be partly available for evaporation and transpiration (permanent wilting point is often assumed to be about -15.000 hPa) and percolates according to subsurface pressure differences (likely at lower conductivities as shown in Figure S2).”

Grammar: There were a fair number of mistakes in grammar and punctuation. Please revise professionally for verb tense agreement and use of imperfect tense, i.e. ‘we got it or we took it’ are informal and temporally less explicit than the perfect tenses.

Response: We ~~will~~carefully revised the grammar in our manuscript. So far, we used a lot “We sampled...” or “We took...” as active voice is a more natural style. We will discuss with the Editor if that should be changed.

Mechanisms of consistently ~~disjunct~~^{connected} soil water pools over (pore)space and time

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Abstract. Storage and release of water in the soils is critical for sustaining plant transpiration and groundwater recharge. However, ~~how much~~^{the} subsurface mixing of water ~~occurs, how much of the water is~~ available for plants or ~~quickly~~^{otherwise} percolating–flowing to streams and ~~the~~ groundwater is not yet understood. ~~Moreover, while~~ Some studies found based on stable isotope (²H and ¹⁸O) data that water infiltrating into soils ~~was shown to can~~ bypass older pore water. ~~Though~~, the mechanisms leading to a separation between water routed to the streams and water held tightly in smaller pores are ~~yet~~ unclear. ~~Here, we address the current limitations of the understanding in~~ ~~subsurface mixing and its consequences for the application of stable isotopes in ecohydrological studies. Here w~~We present an extensive data set, for which we sampled fortnightly the isotopic composition (²H and ¹⁸O) of mobile and bulk soil water in parallel with groundwater, stream water and rainfall in ~~the a~~ Mediterranean long-term research catchment, ~~in~~ Vallcebre, ~~in~~ Spain. The data revealed that mobile and tightly bound water of a silty loam soil in a Scots pine forest do not mix ~~well~~, but they constitute two ~~separate-disjunct~~ subsurface water pools ~~with little~~ ~~exchange~~; despite intense rainfall events leading to high soil wetness. We show that the isotopic compartmentation results from rewetting of small soil pores with isotopically depleted winter/spring rain. Thus, stable isotopes, and therefore water residence times too, do not only vary across soil depth, but also across soil pores. Our findings have important implications for stable isotope applications in ecohydrological studies assessing water uptake by plants or process realism of hydrological models, as the observed processes are currently rarely implemented in the simulation of water partitioning into evapotranspiration and recharge in the critical zone.

1 Introduction

Rainfall infiltrating into soils often does not replace already stored water in the soil pores as described by translatory flow (Hewlett and Hibbert, 1967) (Figure S1), but it can bypass large amounts of water that were held in pores prior to infiltration (Beven and Germann, 1982). It is important to understand which of these two processes is dominating the subsurface water flow due to its impact on plant water availability, nutrient or contamination transport, and groundwater recharge. Bypass flow has been studied ~~since-for~~ decades using artificial tracers and hydrometric methods (Beven and Germann, 2013), but its relevance for the partitioning between transpiration and groundwater recharge from plot to global scale has been ~~unreveled-of interest~~ over the last ten years based on stable isotope (²H and ¹⁸O) data (Brooks et al., 2010; Good et al., 2015). Several studies found different isotopic compositions between mobile soil water, which predominantly contributes to groundwater recharge and stream runoff, and water that is

more tightly bound to the soil matrix (Brooks et al., 2010; Goldsmith et al., 2012; Hervé-Fernández et al., 2016; Gierke et al., 2016; Sprenger et al., 2018b). Here we define in line with Sprenger et al. (2018b) mobile water as soil water sampled with suction lysimeters, bulk water as soil water sampled with cryogenic extraction, and tightly bound water as the difference between mobile water bulk soil water, since it is the water in the pore space fraction that is not accessible by suction lysimeter. It has been hypothesized by Brooks et al. (2010) that rainwater refilling the dry soil at the end of a dry season would lead to a distinct signal between mobile and tightly bound water. Though, the mechanisms leading to a separation between water routed to the streams and water held tightly in smaller pores are unclear and currently under debate (Sprenger et al., 2016b; Berry et al., 2017; Dubbert et al., 2019). However, understanding the stable isotopic composition and its variation in space and time is essential in the application of stable isotopes of water for palaeoclimatological tree ring analyses (Gessler et al., 2014), plant water uptake assessments (Dubbert and Werner, 2019), partitioning between soil evaporation and transpiration (Wang et al., 2010), testing atmosphere – land surface models (Haese et al., 2013), runoff processes understanding (Klaus and McDonnell, 2013), and water residence time estimates (Sprenger et al., 2019).

Field Code Changed

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So far, the experimental set up of studies addressing the disconnection between water flow across different pore spaces had limited sampling frequencies and sample numbers (maximum twice per year in Brooks et al., 2010; Goldsmith et al., 2012, and five per year in Hervé-Fernández et al., 2016), which impeded progress in understanding of the mechanisms explaining the disjunct subsurface water pools. Here we therefore address the following research questions: 1.) How do the isotopic compositions of mobile and tightly bound water vary over time and different hydro-meteorological conditions? 2.) Can we identify the source of the water more tightly bound to the soil pores? 3.) What do the isotopic differences in subsurface water pools tell us about the hydrological processes such as pore scale variability in water transport and age?

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Field Code Changed

Our objective was to identify the dynamics of subsurface transport and flow by combining fortnightly isotope sampling of groundwater, mobile and bulk (encompassing both mobile and tightly bound water) soil water during various hydrometeorological conditions (May to December 2015) with long-term rainfall and runoff isotope sampling and soil moisture and groundwater measurements (2011-2015).

2 Methods

2.1 Sampling site

Our study site is in the Can Vila catchment (0.56 Km²) in the Vallcebre research area in the South-eastern part of the Pyrenees (Spain, 42°11'43"N, 1°49'13"E) on 1200 m a.s.l. (Figure 1). The climate is characterized as humid Mediterranean with an average precipitation of 880 mm/year distributed over about 90 days per year (Latron et al., 2009). Least precipitation occurs during winter and most rain falls in May, October, and November, while the most intense rain events occur during summer. Snow plays a minor role with <5% of precipitation. The average air temperature is 9.1°C and varies seasonally. The potential evaporation is about 823 mm/year with a strong seasonal dynamic ranging from about 20 mm/month in winter to up to 150 mm/month in summer (Llorens et al., 2018). The dry winters and negative water balance during summer result in a succession of wet and dry periods with soils

usually wetting up in early spring and early fall (Gallart et al., 2002). The studied soils are of silty loam texture (4% sand, 72% silt, 24% clay) with about 3% gravel content and 6% organic matter content (Molina et al., 2019). The soil's bulk density is 0.99 g cm⁻³ and its porosity is 62% (Molina et al., 2019). The study area was terraced before and during the 19th and abandoned in the second half of the 20th century. The slope at the study site is about 15°. The vegetation is Scots pine (*Pinus sylvestris*). The Scots pine trees are about 17 m high, basal area is 45 m²ha⁻¹ and their canopy cover is 70% (Molina et al., 2019).

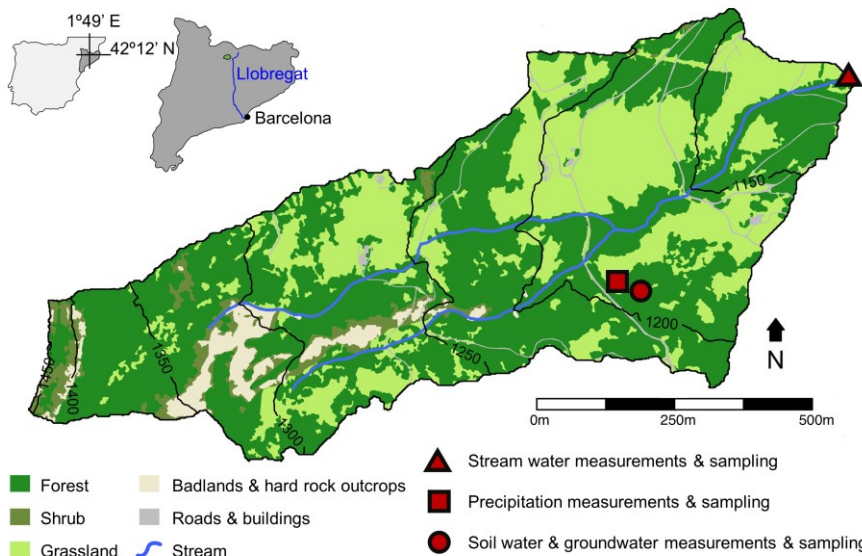


Figure 1 Locations of hydrometric measurements and isotope sampling of soil water, groundwater (red circle), precipitation (red square), and stream water (red triangle) within the Can Vila catchment. The elevation is shown with contour lines and the colours represent land use classes. The inset on the top left indicate the location within Spain (light grey) and Catalonia (dark grey).

2.2 Stable isotope data

We sampled ~~between May and December 2015~~ in approximately fortnightly frequency the mobile soil water at 20, 50, and 100 cm depths with suction lysimeters ~~between May and December 2015. The lysimeters consisted of 15 cm~~ long porous cups (RSK ADAS Ltd., UK) with 2 inserted tubes that allow to create the vacuum in the lysimeter and to sample soil water by injecting air into the lysimeter. (We applied a suction of -700 hPa ~~applied~~ and sampled the water extracted within a few hours). In parallel, we took soil samples at 10, 20, 30, 50, 100 for bulk water cryogenic extraction (temperature: 110-120 °C, duration: 120 minutes, vacuum: 10⁻² mbar, Martín-Gómez et al., 2015). We further sampled water from a piezometer that reached down to 263 cm and was screened along the bottom 60 cm. We took rain-water samples on event basis from January 2011 to May 2013 and again from May 2015 on. We also

sampled the stream at the outlet of the Can Vila catchment at least every 12 hr and at higher frequency during high flows with a discharge triggered autosampler throughout 2015 (Cayuela et al., 2018b). We retrieved monthly long-term rainfall stable isotope data from the Global Network of Isotopes in Precipitation (GNIP) (IAEA/WMO, 2019) database for the station Girona (Long: 41.91, Lat: 2.76; 129 m a.s.l., ca. 100 km SE of Vallcebre). For the Vallcebre rainfall isotopes, we calculated the weighted monthly averages and weighted averages over 30 days prior to each day of soil sampling. We used the GNIP data to reveal long-term relationships between soil moisture and rainfall stable isotope compositions and to fill the gap in the Vallcebre data set (August 2013 to May 2015) via linear regression on monthly data: $\delta^2\text{H}$ in Vallcebre = 1.1512 $\delta^2\text{H}$ in Girona - 5.1584 ‰ ($r=0.79$; $p<0.01$). We sampled throughfall and stemflow on an event basis between May 2015 and May 2016, but we do not present this information here. The isotopic composition of throughfall and stemflow was in general more enriched than that of rainfall, and all samples fell along the LMWL (Cayuela et al., 2018a). All water samples were analyzed for their stable isotopic composition (^2H and ^{18}O) using Cavity Ring-Down Spectroscopy (Picarro L2120-I, Picarro Inc., USA). The precision of the measurements is <0.1 for $\delta^{18}\text{O}$ and <0.4 ‰ for $\delta^2\text{H}$. All isotope data are expressed in the δ -notation as parts per mil (‰) relative to Vienna Standard Mean Ocean Water (Coplen, 2011).

Since cryogenic extraction was found to be reliable for clayey loams of soil moisture >30 % (Orlowski et al., 2016), we can exclude methodological issues with the isotopic analysis for our silty loam soils that had moisture contents >30 % on the sampling days (Figure 2Figure 4d). Further, we neither see a fixed offset between the isotopic compositions of mobile and bulk water, nor does the difference between the mobile and bulk soil water correlate with soil moisture. Thus, there are no signs of a bias introduced by the applied methods.

2.3 Hydrometric data

We measured ~~from January 2013 on~~ the volumetric soil moisture at 0-30 cm, 30-60, and 60-90 cm depth with 30 cm-long TDR probes (CS605, Campbell Scientific) in 20 minute intervals ~~from January 2013 on~~ (Molina et al., 2019) and computed daily and monthly averages. We continuously gauged the depth to the groundwater level with a Mini-Diver (Van Essen Instruments B.V.) compensated for barometric pressure in a PVC tube reaching down to 263 cm and screened along the bottom 60 cm. Catchment runoff was monitored at a 90° V-notch weir using a water pressure sensor and a rating curve to get discharge values. Rainfall was measured in an open field adjacent to the study site using a 0.2 mm tipping bucket rain gauge (AW-P, Institut Analitic, Spain).

2.4 Data analysis

We used non-parametric Spearman rank correlation to describe relationships between two variables, because the isotope data was not normally distributed according to the Shapiro-Wilk test. We assessed if the isotopic compositions of water samples were significantly different from long-term weighted mean values for precipitation using the Wilcoxon signed-rank test. Comparisons among piezometer, bulk and mobile soil water isotopic compositions were done with the Kruskal Wallis test and a subsequent posthoc test after Dunn. The significance level for all statistical tests was set to the 95% confidence interval.

We calculated for each water sample the lc-excess as defined by Landwehr and Coplen (2006) as: $lc-excess = \delta^2H - a \cdot \delta^{18}O - b$, with a being the slope of the local meteoric water line (LMWL) ($a = 7.96$) and b being the intercept of the LMWL ($b = 12.89$ ‰). A negative lc-excess value indicates kinetic fractionation of the isotopic composition of a water sample (Craig, 1961), which is depicted with plotting to the right of the LMWL in a dual isotope plot.

5 We estimated the isotopic composition (both 2H and ^{18}O) of tightly bound water (δ_{TW}) based on an isotope mass balance approach. First, we derived the water retention curve ($\theta(h)$) using a pedotransfer function based on the soil texture and bulk density information (Schaap et al., 2001) (see black line in Figure S2). Afterwards, based on $\theta(h)$ and the pressure limit of the suction lysimeter, h_{MW} ($= -700$ hPa), we determined the maximum soil moisture of tightly bound water ($\theta_{TW}=29$ ‰). The soil moisture of the mobile water fraction (θ_{MW}) is the difference between the measured (bulk) soil moisture θ_{BW} and θ_{TW} . (note that θ_{TW} was always bigger than θ_{MW}).

We derived δ_{TW} as a function of δ_{BW} and δ_{MW} , which were the bulk and mobile water stable isotopic compositions, respectively:

$$\delta_{TW} = \frac{\theta_{BW} \cdot \delta_{BW} - \theta_{MW} \cdot \delta_{MW}}{\theta_{TW}} \quad \text{Equation (1)}$$

We calculated δ_{TW} for the depths at which bulk and mobile water were sampled in parallel (20, 50, and 100 cm).

15 We used the above mentioned pedotransfer function and applied the concept of dual-permeability as suggested by Gerke and van Genuchten (1993) to derive $\theta(h)$ and the hydraulic conductivity curve ($K(h)$) for the fast flow domain (soil fracture), the slow flow domain (soil matrix), and the bulk soil. We calculated based on $\theta(h)$ for each soil sampling day the pressure head according to the measured soil moisture. We further calculated the hydraulic conductivity for the long-term rainfall isotope data set based on observed monthly mean soil moisture (as both plotted in Figure S2).

20 We calculated for each sampling time (t) the fraction of event water (f_e [-]), which we defined as the share of water that was newly infiltrated from rainfall since the last sampling time ($t-1$). These calculations were done for sampling time steps in which the total soil moisture volume (V_a [L]= θ [L³·L⁻³] · z , with z being the representative depth of soil moisture measurements; $z = 30$ cm) increased ($V_t > V_{t-1}$) and the soil water isotopic composition changed from δ_{t-1} to δ_t into the direction of the rainfall event (δ_e). We estimated the share of event water (V_e) from all soil water (V_a) as follows:

$$f_e = \frac{V_e}{V_a} = \frac{\delta_t \cdot \theta_t - \delta_{t-1} \cdot \theta_{t-1}}{\delta_e \cdot \theta_t} \quad \text{Equation (2)}$$

3 Results and discussion

30 3.1 Continuous isotopic separation between mobile and bulk soil water

Mobile and bulk soil waters were significantly distinct in their stable isotopic composition (^2H and ^{18}O) at all depths throughout the study period (Figure 2Figure-1c) (Kruskal-Wallis post-hoc Dunn test, $p < 0.02$). We found for each sampling campaign that the mobile water was more enriched in heavy isotopes than the bulk soil water. At every sampled depth, bulk soil water $\delta^2\text{H}$ was significantly more depleted than mobile water, and bulk soil water below 10 cm was also significantly more depleted than groundwater (Kruskal-Wallis post-hoc Dunn test, $p < 0.01$). Mobile water $\delta^2\text{H}$ was at all depths significantly more enriched than groundwater (Kruskal-Wallis post-hoc Dunn test, $p < 0.05$). With soil depth, we observed a depletion of heavy isotopes in soil water, a decrease in temporal variability (Table S1), and less difference between mobile and bulk soil water (Figure 3Figure-2). Our groundwater samples had intermediate $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values and generally little variation (Figure 2Figure-1b). Groundwater was not significantly different to the weighted mean $\delta^2\text{H}$ values for spring (one-sample Wilcoxon signed rank test, p -value = 0.09377) and fall precipitation (p -value = 0.7982) and to the weighted mean $\delta^{18}\text{O}$ values of the annual precipitation (p -value = 0.4144). Stream water base flow isotopic compositions were similar to groundwater in terms of values and variability during base flow conditions. However, during rain events, the runoff isotopic composition responded immediately according to the rainfall $\delta^2\text{H}$ and $\delta^{18}\text{O}$ input (Figure 2Figure-1b).

The general pattern of disjunct isotopic compositions in mobile and bulk soil water was persistent, despite variable hydrometeorological conditions during our sampling period: There was a dry period in between Mid-June and Mid-July leading to a cease in catchment runoff (cyan line in Figure 2Figure-1a) and a strong depletion of the soil moisture (Figure 2Figure-1d). We also covered with our sampling period four intense rain events between End-July and End-October that resulted in short-term increases of the water table in the piezometer up to the ground surface (grey line in Figure 2Figure-1a). We further observed continuously high soil moisture volumes between August and October with little variation (Figure 2Figure-1d).

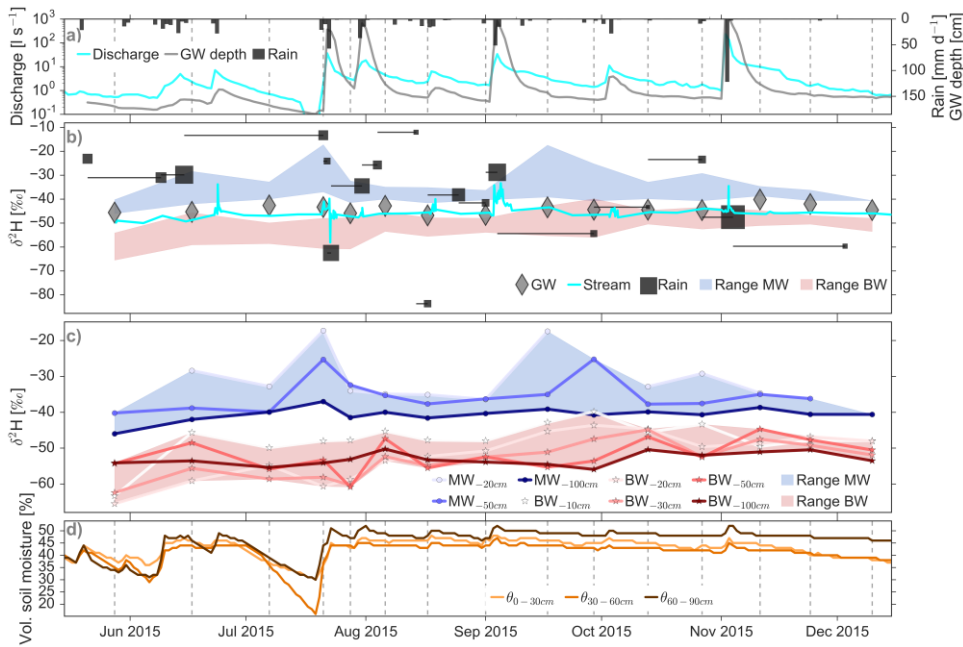


Figure 24 Temporal dynamics of hydrological conditions and stable isotopes during soil water sampling period in 2015. a) Catchment runoff at outlet (cyan), groundwater depth (grey) and daily rainfall volumes (black) at the study site. Note the logarithmic scale for discharge. b) Stable isotope composition ($\delta^2\text{H}$) of stream water, groundwater, and rainfall. Blue and red shaded areas show the range of mobile and bulk soil water. Note that the size of the black squares indicates the rainfall amount and the black lines represent the time span rain-water was cumulatively sampled. c) Stable isotope composition of mobile water (MW) and bulk soil water (BW) at different depths. d) Volumetric soil moisture at three depth intervals.

$\delta^2\text{H}$ values of mobile soil water at 20 cm depth were significantly correlated to 30-day weighted averages in $\delta^2\text{H}$ of the antecedent rainfall of each sampling day ($\rho=0.67$, $p=0.02$). Such a relationship did neither exist for the 50 and 100 cm mobile water sampling depths nor for any of the sampling depths of bulk soil water. Averages over shorter time spans (e.g., 7 days or 14 days) for the weighted average input did not correlate with soil water stable isotope compositions. Notably, we observed a significant trend in isotopic enrichment of bulk soil water during the growing season (May to September) at 10, 20, and 30 cm depth ($\rho=0.68$, $\rho=0.94$, $\rho=0.85$, respectively; $p<0.03$). This relationship indicates a slow refill of small pore spaces with isotopically enriched summer precipitation at 20 and 30 cm depth, but at 10 cm depth, the enrichment is caused by evaporation fractionation, since $1c$ -excess decreased over the growing season ($\rho=0.72$, $p=0.02$). We also saw an increasing trend in $\delta^2\text{H}$ values for mobile water at 50 cm ($\rho=0.65$, $p=0.04$), but no trends for $1c$ -excess or samples taken at 20 cm and 100 cm. Mobile water $\delta^2\text{H}$ values at 20 and 50 cm depth were similar to the long-term weighted average for summer rainfalls (Figure 4Figure-3). Towards 100 cm depth, the mobile water approached the isotopic compositions of the piezometer samples; though, significantly different in their $\delta^2\text{H}$ values (Wilcoxon rank sum test, $p < 0.001$). Contrary, bulk soil water isotope

values ranged mainly between average winter and annual rainfall isotope values. We observed in the bulk water samples at 10 cm soil depth kinetic fractionation (samples plot to the right of the local meteoric water line (LMWL) in Figure 4Figure-3), which is indicative for soil water evaporation. This deviation from the LMWL, described as the $\delta^2\text{H}$ -excess, was significantly more intense for the bulk water at 10 cm compared to other bulk water samples at deeper depths (Kruskal-Wallis post-hoc Dunn test, $p < 0.05$), and all mobile water, piezometer, and stream water samples, which all plotted along the LMWL.

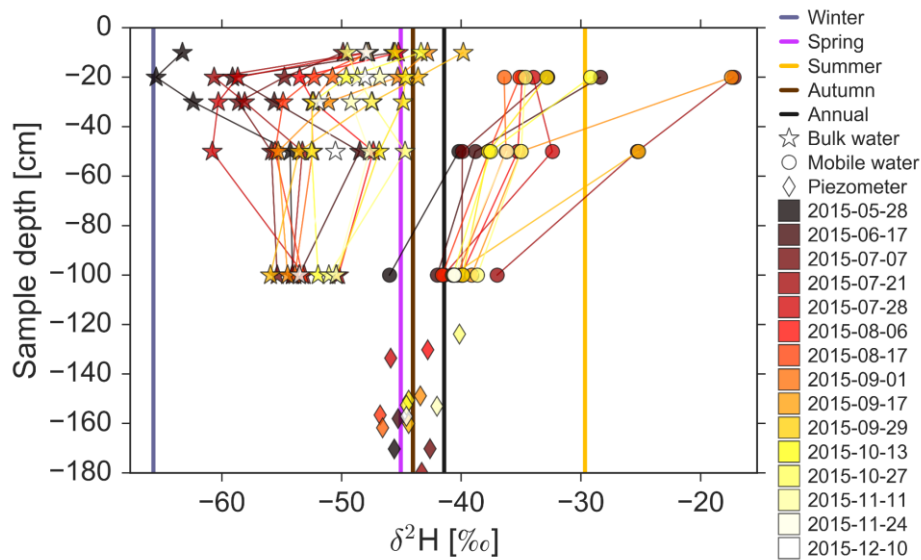


Figure 32 Depth profiles showing the variability of the stable isotopic composition across the soil profile over the sampling period (color coded) for bulk soil water (stars), mobile soil water (circles), and groundwater sampled from the piezometer (diamonds). Vertical lines represent seasonal and annual weighted averages of precipitation. Location of the piezometer samples according to the depth of the water table in the piezometer.

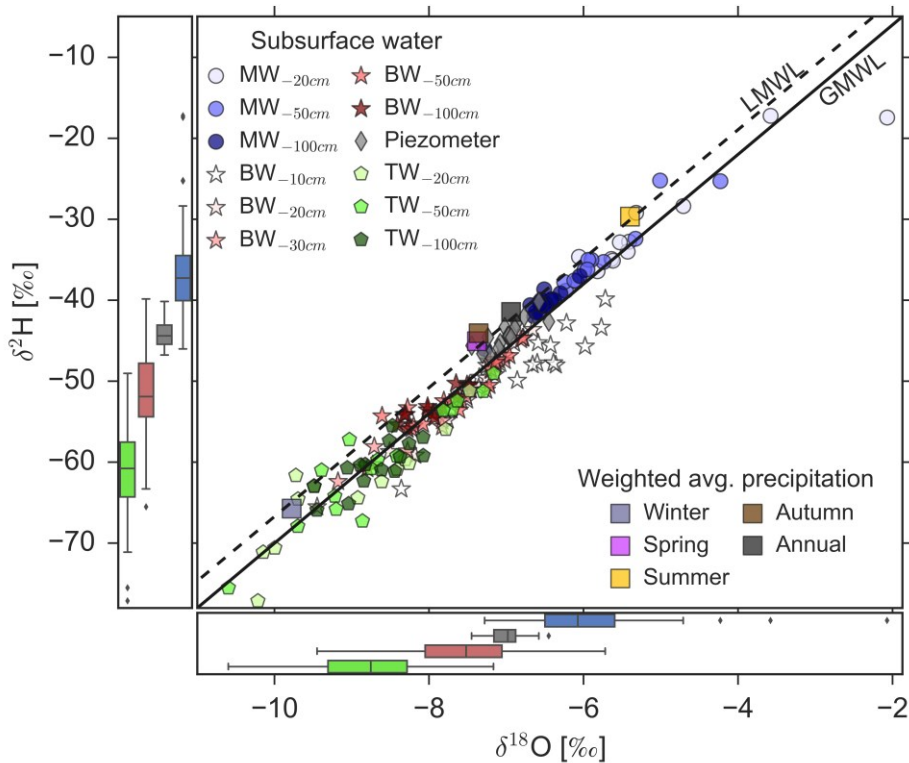


Figure 43 Water isotopes ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) of mobile (MW) and bulk soil water (BW) and piezometer water. We further show weighted seasonal and annual averages of rainfall stable isotopes (squares), the local meteoric water line (LMWL; $\delta^2\text{H} = 7.96 \delta^{18}\text{O} + 12.89 \text{‰}$), the global meteoric water line (GMWL; $\delta^2\text{H} = 8 \delta^{18}\text{O} + 10 \text{‰}$), and mass balance derived tightly bound water (TW). The box and whisker plots represent the mean (black line), the interquartile (box), the range (whiskers), and outliers (black dots) of all MW (blue), piezometer (grey), BW (red), and TW (green) water samples.

We conclude that there is little ~~to no~~ mixing of tightly bound water (here defined as water that cannot be accessed via suction lysimeter) ~~neither~~ with recently infiltrated rainfall ~~nor~~ with mobile soil water. While it was recently shown for a silty clay soil that mobile and tightly bound water would be in exchange during wet conditions (Hervé-Fernández et al., 2016), our observation for a silty loam soil does not support this, as the isotopic distinction persisted throughout extreme wet conditions with soil pores close to saturation. Our data does not indicate for any periodic well-mixed conditions; neither across soil depths (as frequently assumed in hydrological catchment models (McDonnell, 2014)) nor across the pore space at individual depths (as frequently assumed in soil hydraulic models (Sprenger et al., 2018b)). We recognized only for the shallow mobile water a replacement of older water with newly infiltrating water as suggested in the translatory flow concept (Hewlett and Hibbert, 1967). Further, the little variation in bulk water stable isotopes and its similarity to isotopically depleted winter rainfall, indicates that tightly bound water is composed of relatively old water (see also discussion in section 3.3). To underline this, we calculated

based on a mass balance approach (Equation 1) the isotopic composition of the tightly bound water. This water, which cannot be sampled with suction lysimeter had isotopic compositions in the range between weighted averages of spring/fall and winter rainfall (green points in [Figure 4](#)[Figure-3](#)). Therefore, our data set reveals that a large part of the subsurface water storage does not - or at least to a very limited amount and thus on long time scales - take part in the water flux towards groundwater and streams.

3.2 Filling of small pores during rewetting

To understand how the distinct isotopic composition in mobile and bulk soil water samples evolve, we combined our 8-month field data with multi-year monitoring data. The long-term water balance (2011-2017) is positive (rainfall > potential evapotranspiration) for the months between October and May. However, this is not the period of highest soil wetness, because there is little precipitation from December to March (<40 mm/month on average) resulting in a dry down of soils ([Figure 5](#)[Figure-4b](#)). Evapotranspirative losses clearly surpass rainfall inputs (> 10 mm/month) only between June and August, but rainfall in these months often occurs as intense precipitation events ([Figure 2](#)[Figure-1a](#)).

We connected these hydrometeorological and soil moisture data with rainfall stable isotopic compositions measured on event basis at the study site and monthly GNIP data. We found that on the long run, the rainfall happens to be more depleted in heavy isotopes when soil moisture is low ([Figure 6](#)[Figure-5a](#)). This significant relationship between rainfall isotopic composition and soil moisture ($\rho > 0.41$, $p < 0.05$) results from the combination of dry soil during winter, that get rewetted during early spring, and the temperature dependency of the rainfall isotopic composition that leads to its seasonality, with isotopically depleted rainwater during winter and spring ([Figure 5](#)[Figure-4b](#)). Rainfall was generally more depleted in heavy isotopes when monthly rainfall exceeded evapotranspiration ($\rho = -0.5$, $p < 0.05$, [Figure 6](#)[Figure-5b](#)). Thus, isotopically depleted rainfall from winter and spring will preferably infiltrate into deeper soil layers and not get recycled into the atmosphere, as it is the case for isotopically enriched summer rainfall. As a result of isotopically depleted rainfall infiltrating into dry soils when atmospheric demands are low, smaller pores will be preferentially filled with the isotopically depleted waters.

The fraction of newly infiltrated event water in soil pores based on an isotope mass balance (Equation 2) showed a strong negative relationship with the soil moisture before an event ~~both~~ for mobile ($\rho = -0.86$, $p < 0.05$) and a weaker relationship with bulk soil water ($\rho = -0.62$, $p < 0.1$). Mobile water was almost fully replaced by event water at low soil moisture and was less affected when rain infiltrated into wetter soil ([Figure 6](#)[Figure-5c](#)). The fraction of newly infiltrated event water in soil pores was generally lower for the bulk water than for the mobile water underlining the high impact of tightly bound water that ~~did not~~ mixed not well with infiltrating rain-water.

Thus, our findings highlight that the ecohydrological separation of mobile and tightly bound water, as defined by the isotope study of Brooks et al. (2010), will occur in all settings where a soil of relatively fine texture (high potential for tightly bound water) dries out occasionally and gets rewetted by an isotopic composition that is distinct from the rainfall isotopic composition during wet periods. However, if there is no or little isotopic variability in the

precipitation, tightly bound and mobile water may ~~be~~ not be hydraulically connected, but we would not be able to detect that separation via stable isotopes.

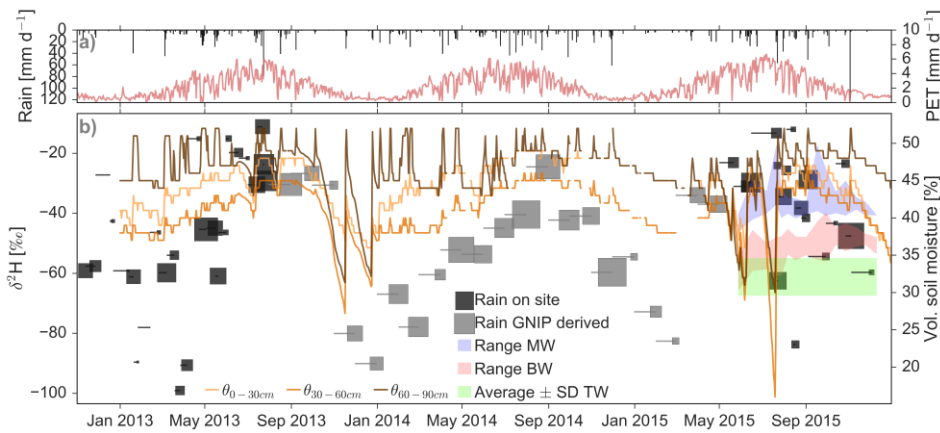


Figure 54 Long-term hydrological and stable isotopes dynamics. a) Daily rainfall (bars) and potential evapotranspiration (red line). b) Rain-water $\delta^2\text{H}$ values and soil moisture dynamics. Note that the size of the black and grey squares indicates the rainfall amount and the line represents the time span of the water sample. Black squares show rain-water sampled at the study site and grey squares show monthly values derived from GNIP data. The blue and red shaded area shows the range of mobile (MW) and bulk (BW) soil water, respectively. The green shade indicates the average \pm standard deviation value estimated for the tightly bound water (TW).

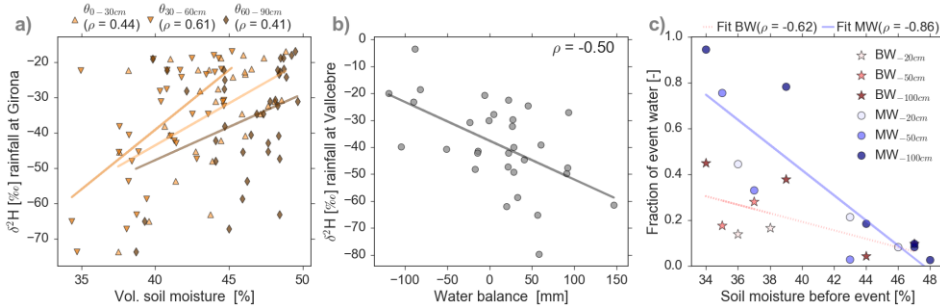


Figure 65 Relationships between wetness and rainfall stable isotopes and refilling of soil pores. a) Monthly rainfall stable isotopes (GNIP data) are more depleted when soils are drier. b) Monthly rainfall at the study site is more depleted when monthly rainfall volumes are higher than potential evapotranspiration (positive water balance). c) Fraction of newly infiltrated water (derived from mass balance) is higher when soil moisture has been low before the infiltration event. All relationships (ρ shows Spearman rank correlation coefficients) are significant ($p < 0.05$) with the exception of Fit BW with $p = 0.1$.

3.3 Tightly bound water is old

The combination of the above outlined mechanisms of I.) mobile and bulk water being ~~for-most-of-the~~ timecontinuously disjunct and II.) tightly bound water being refilled by isotopically depleted winter rainfall leads to

a pronounced variability in water ages across the soil's pore space. Water held in smaller soil pores of the clayey fraction appear to be considerably older than water in pores of the sandy soil texture within the same soil profile. The presented isotope data thus suggest that the subsurface flow at the investigated site cannot be conceptualized as a uniform flow with water being in exchange across the entire continuum from coarse to fine pores (e.g., as assumed in the Buckingham-Richards equation (van Genuchten, 1980), Figure S1d). Instead, we conclude that concepts based on e.g., the dual-porosity model as described by Gerke and van Genuchten (1993) (Figure 7Figure-6d) for soil physical modelling or a conceptual two parallel systems representing matrix and preferential flow paths (e.g., Stumpp et al., 2007) will provide a better representation of the pronounced variability in flow velocities across the pore space.

Figure 7 summarizes our field observations and their potential implications. Due to the nature of two disjunct pore spaces, a seasonally varying isotopic composition in the rainfall is not preserved across the soil profile (as in Sprenger et al. (2016a) and Figure S1), but the fast and slow flow domains contain waters of different isotopic compositions with higher seasonal variation in the mobile water (Figure 7b) than in the tightly bound water (Figure 7c). The different water retention characteristics (as derived in section 2.4) for the fast and slow flow domain (e.g., Gerke and van Genuchten, 1993) are directly linked to the soil pore diameters (Schjonning, 1992). The different flow pattern and the lack of exchange between the mobile and tightly bound water result in the distinct isotopic compositions across the pore space at the same soil depth (red and blue shaded in Figure 7e).

To what extent the isotopologues (i.e., $^2\text{H}^1\text{H}^{16}\text{O}$, $^1\text{H}_2^{18}\text{O}$) of mobile and tightly bound water exchange via gas phase or dispersion is unclear (Oerter and Bowen, 2017), but for the studied soil in the Valleebre-Can Vila catchment, this exchange appears to be very limited. One approach to this question could be testing the different hypotheses of mixing using a recently presented soil hydrologic isotope model that allows conceptualizing various intensities of isotopic exchange between a fast and a slow subsurface flow domain (i.e., mobile and tightly bound soil water) (Sprenger et al., 2018b). Thus, based on our field data, we can currently not assess the actual age (months or years?) of the tightly bound water, but experimental approaches with deuterated (enriched in ^2H) water could help assessing how much of exchange between the mobile and tightly bound water can occur (Evaristo et al., 2019).

The maximum water volume stored in the tightly bound water pool is about 290 mm if we consider the studied upper 100 cm (max $\theta_{TW} = 29\%$, Figure S2). This represents about 1/3 of the annual average rainfall, but it cannot be considered as being in an inactive storage and completely immobile, since it would be partly available for evaporation and transpiration (permanent wilting point is often assumed to be about -15.000 hPa) and percolates according to subsurface pressure differences (likely at lower conductivities as shown in Figure S2).

While there is increasing acknowledgement of subsurface water to be not well mixed and its consequences for hydrological modeling being tested (Fenicia et al., 2010; McMillan et al., 2012; Hrachowitz et al., 2013; Knighton et al., 2017; Cain et al., 2019), we propose to extend the notion from variability of isotopic tracers (and thus residence times) over depth (Figure 7Figure-6b,c) to the variability of tracers (and residence times) over the pore space continuum (Figure 7Figure-6e). Due to the highly non-linear relationship of soil moisture, pressure heads and hydraulic conductivity, the tightly bound soil water will be percolating several magnitudes slower than the mobile

water (Figure S2). The outlined mechanisms result in isotopically enriched rainwater infiltrating at higher soil hydraulic conductivities, which leads to a relatively rapid transport of young water to the groundwater and streams (Figure 7f). When isotopically depleted rain infiltrates during dry conditions, the hydraulic conductivity is generally lower and also the differences in hydraulic conductivity between mobile and tightly bound water will be lower than during wet conditions, resulting in lowered percolation rates of isotopically depleted rainfall (squares in Figure S2). Lysimeter experiments with weighable lysimeters (e.g., Stumpp and Maloszewski, 2010; Benettin et al., 2019), where the stable isotopes of the outflow were sampled, found results similarly to our field observations for of the mobile water, as well rapid percolation via preferential flow paths. However, the isotopic composition of the stored bulk soil water is unknown in such experiments, since destructive soil sampling is not possible in weighing lysimeter studies. Thus, for interaction (or the lack of it) between tightly bound and mobile water lysimeter experiments would need to be extended by in-situ soil water vapor isotope measurements.

Our observations of the pore scale flow velocity variability of disjunct subsurface water pools has consequences on the contact time with mineral surfaces and thus will affect nutrient concentrations (Asano et al., 2006), which will then be higher for the water in the slow flow domain. As hypothesized by Brantley et al. (2017), such nutrient concentration differences might be one reason why plants were shown to take up tightly bound water even though highly mobile water is available (Brooks et al., 2010). The pore scale water age differences outlined above could further explain, why plant stem water stable isotopic compositions indicate that trees preferentially use winter precipitation (relatively old water) during summer, as recently shown by Allen et al. (2019). Moreover, the different origin (timing of infiltration and associated isotopic composition) of mobile and tightly bound water (as shown in Figure 7c) can explain differences observed among different methods for soil water isotope analysis (Sprenger et al., 2015; Geris et al., 2015; Orłowski et al., 2016; Tsuruta et al., 2019). The distinct subsurface flow paths of a younger more mobile water domain and a domain of low flow velocities (consequently older soil water) has further implications for the interpretation of recharge estimates based on depth profiles of pore water stable isotopes (Koeniger et al., 2016) or tritium (Li et al., 2019). Moreover, residence time estimates based on isotope data sampled with suction lysimeter will overestimate the soil water turnover rates (Sprenger et al., 2018a).

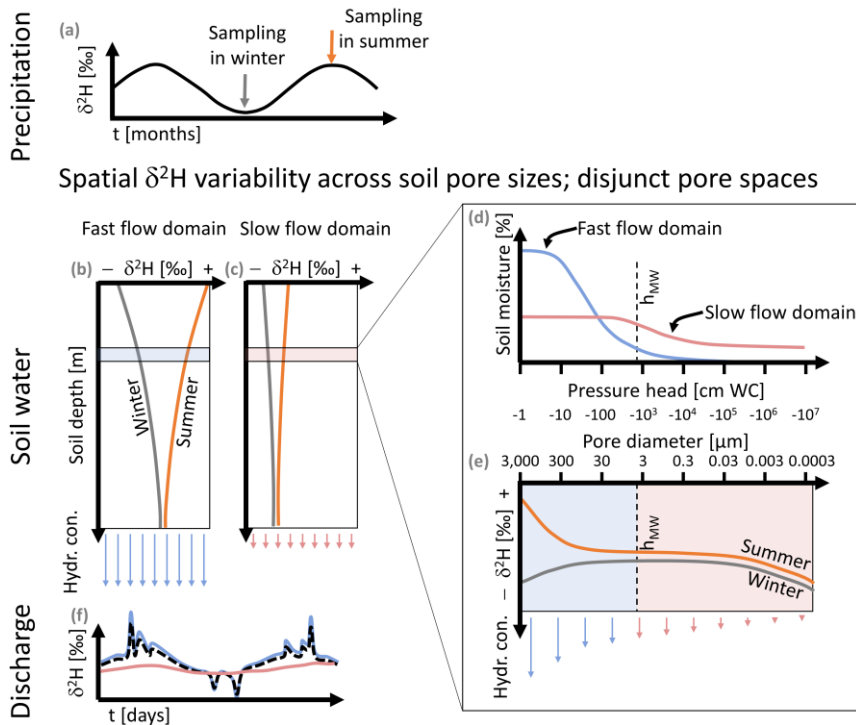


Figure 76 Conceptualization of pore space water stable isotope variability. a) Seasonal variation of the isotopic composition in the rainfall. Arrows indicate soil sampling days. b) Isotope depth profile of the fast flow domain (mobile water) during winter (grey) and summer (orange). The arrows below the profile represent the hydraulic conductivity. c) Same as (b), but for the slow flow domain (tightly bound water). d) Water retention curve based on the dual-porosity model as proposed by Gerke and van Genuchten (1993) for the fast (blue) and slow (red) flow domain with h_{MW} indicating the threshold for suction of lysimeter sampling. e) Isotopic compositions across the pore space at a specific depth as shown in (b) and (c) with blue and red shaded areas representing mobile and tightly bound water pore space, respectively. Note that (d) and (e) share the same x-axis representing pressure head and pore diameter based on Schjonning (1992). Vertical arrows in (b), (c), and (e) represent hydraulic conductivity in fast (blue) and slow (red) flow domain. f) Isotopic composition of the discharge from the soil subdivided into fast (blue) and slow (red) flow contributions and the resulting total isotopic composition (black dashed line).

4 Conclusions

The mechanism how-by which different isotopic compositions for the mobile and tightly bound soil water develop at the plot scale have not been presented before. Our extensive field data shows that mobile and tightly bound water of a silty loam soil in a Scots pine forest were continuously separated during various environmental conditions over the eight months of our study period. These differences resulted in preferential refilling of small soil pores by isotopically depleted rainfall during low soil moisture conditions. This water, replenishing smaller pores, is held more tightly and contributes to lesser extent to the soil water flux and is therefore of older ages. Thus, the variability of the stable isotopic composition and soil water ages across the soil's pore space continuum is contrary to the

common assumptions made in stable isotope applications in both hydrological modelling of water fluxes of-and ecohydrological studies that assess the root water uptake depth of trees.

In both applications of stable isotopes, it is usually presumed that the soil water is well mixed across the pore space, which means for example that the water of-within the sandy soil fraction has the same isotopic composition as the clayey soil fraction at the same soil depth in a soil profile, since the water exchanges over the entire pore space. Here we showed for our Mediterranean study site that this is not the case and we explained the pore scale isotopic variability by the synchronized water balance and rainfall stable isotope dynamics.

While the differences in stable isotopes between mobile and tightly bound soil water were observed in other studies, the explanation for this occurrence were limited so far. We suggest that the long-known soil physical processes in heterogeneously structured soils explain the variability of stable isotopes across the pore space continuum and that this will need to be acknowledged in ecohydrological field and modeling studies based on stable isotope data.

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5 Author contributions:

MS did the data analysis and wrote the initial draft of the manuscript, PL, JL, FG designed the experiment, PL obtained funding, PL, JL, FG, CC did the field and laboratory work and preliminary data analysis. All authors discussed the results and edited the manuscript.

Competing interests:

10 The authors declare no competing interests.

Data availability.

Request for materials should be addressed to P.L.

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