Mechanisms of consistently disjunct 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 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 (^{2}H 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, for which we sampled fortnightly the isotopic composition of mobile and bulk soil water in parallel with groundwater, stream water and rainfall in a Mediterranean long-term research catchment, in Vallcebre, 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 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 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 of interest over the last ten years based on stable isotope (^{2}H and ^{18}O) 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

5   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

10   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).

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;

15   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

20   scale variability in water transport and age?

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).

25   ## 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.,

30   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

35   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 m^{2}ha^{-1} and their canopy cover is 70% (Molina et al., 2019).
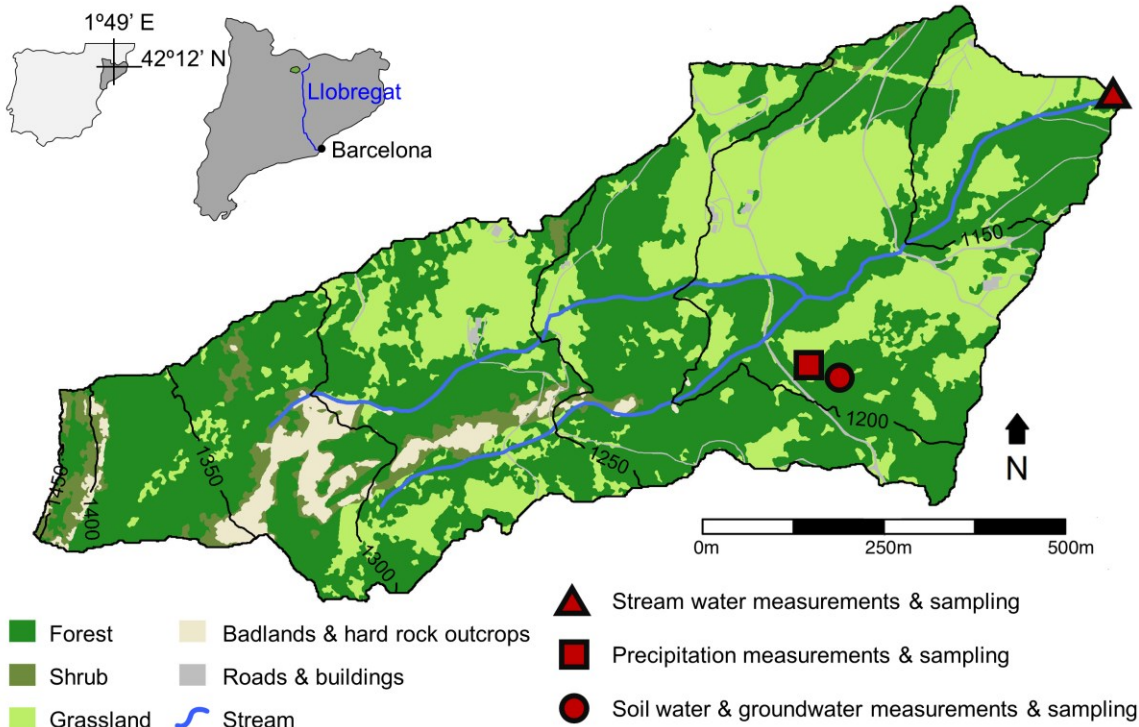
Figure 1 Locations of hydrometric measurements and isotope sampling of soil water, groundwater (red circle), precipitation (red square), and stream water (red triangle) within the Can Vila catchment. The elevation is shown with contour lines and the colours represent land use classes. The inset on the top left indicate the location within Spain (light grey) and Catalonia (dark grey).

2.2   Stable isotope data

We sampled 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 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 rainwater 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: δ^2H in Vallcebre = 1.1512 δ^2H 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 (^{2}H and ^{18}O) using Cavity Ring-Down Spectroscopy (Picarro L2120-I, Picarro Inc., USA). The precision of the measurements is <0.1 for δ^{18}O and <0.4‰ for δ^2H. 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 2d). 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 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 = δ^2H – a • δ^{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 LWML (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.

We estimated the isotopic composition (both ^{2}H 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 (θ_{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:

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

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

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).

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 [L^3·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:

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

3 Results and discussion

3.1 Continuous isotopic separation between mobile and bulk soil water

Mobile and bulk soil waters were significantly distinct in their stable isotopic composition (^{2}H and ^{18}O) at all depths throughout the study period (Figure 2c) (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 δ^2H 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 δ^2H 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 3). Our groundwater samples had intermediate δ^2H and $\delta^{18}O$ values and generally little variation (Figure 2b). Groundwater was not significantly different to the weighted mean δ^2H 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 $\delta^{18}O$ 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 δ^2H and $\delta^{18}O$ input (Figure 2b).

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 2a) and a strong depletion of the soil moisture (Figure 2d). 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 2a). We further observed continuously high soil moisture volumes between August and October with little variation (Figure 2d).
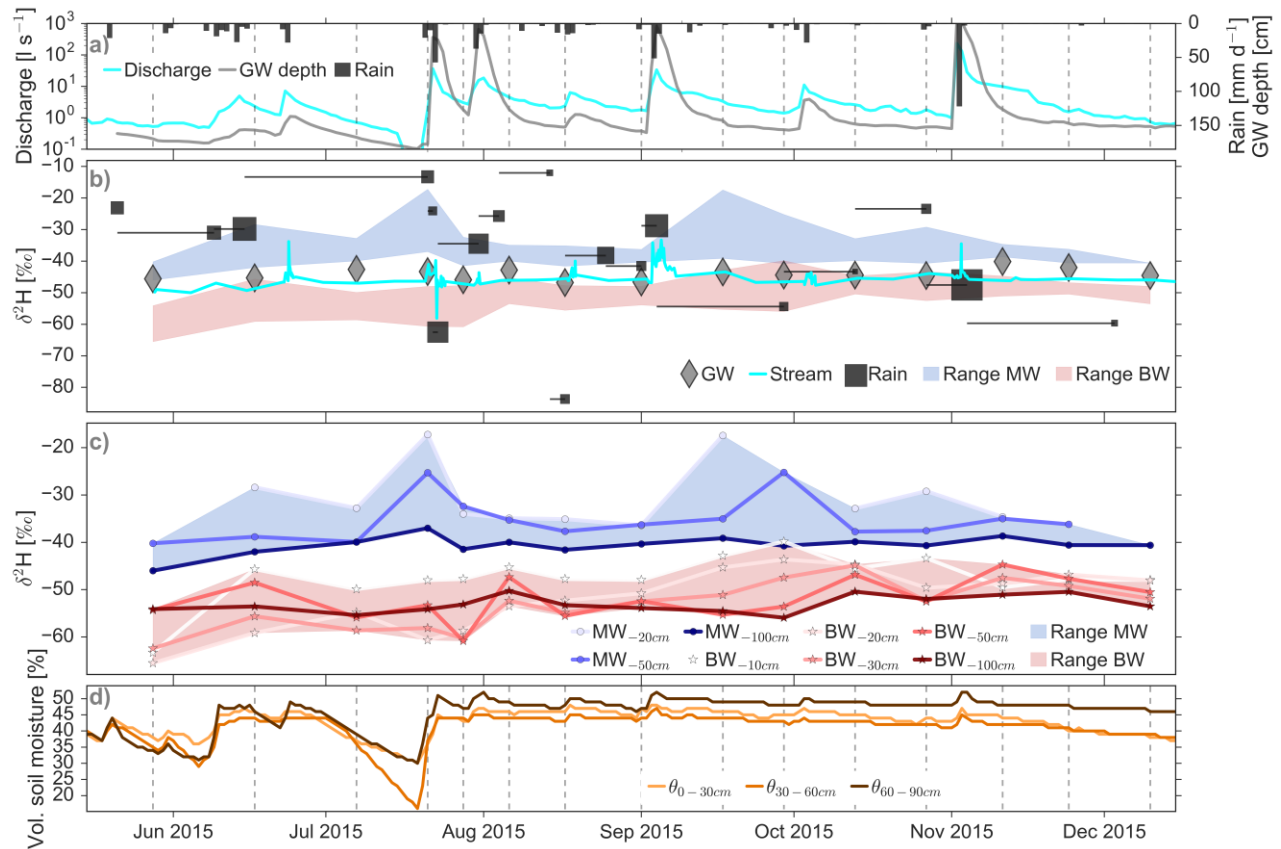
Figure 2 Temporal dynamics of hydrological conditions and stable isotopes during soil water sampling period in 2015. a) Catchment runoff at outlet (cyan), groundwater depth (grey) and daily rainfall volumes (black) at the study site. Note the logarithmic scale for discharge. b) Stable isotope composition (δ²H) 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 rainwater 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.

δ^2H values of mobile soil water at 20 cm depth were significantly correlated to 30-day weighted averages in δ^2H of the antecedent rainfall of each sampling day (ρ=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 (ρ=0.68, ρ=0.94, ρ=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 lc-excess decreased over the growing season (ρ=0.72, p = 0.02). We also saw an increasing trend in δ^2H values for mobile water at 50 cm (ρ= 0.65, p = 0.04), but no trends for lc-excess or samples taken at 20 cm and 100 cm. Mobile water δ^2H values at 20 and 50 cm depth were similar to the long-term weighted average for summer rainfalls (Figure 4). Towards 100 cm depth, the mobile water approached the isotopic compositions of the piezometer samples; though, significantly different in their δ^2H 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 4), which is indicative for soil water evaporation. This deviation from the LMWL, described as the lc-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.
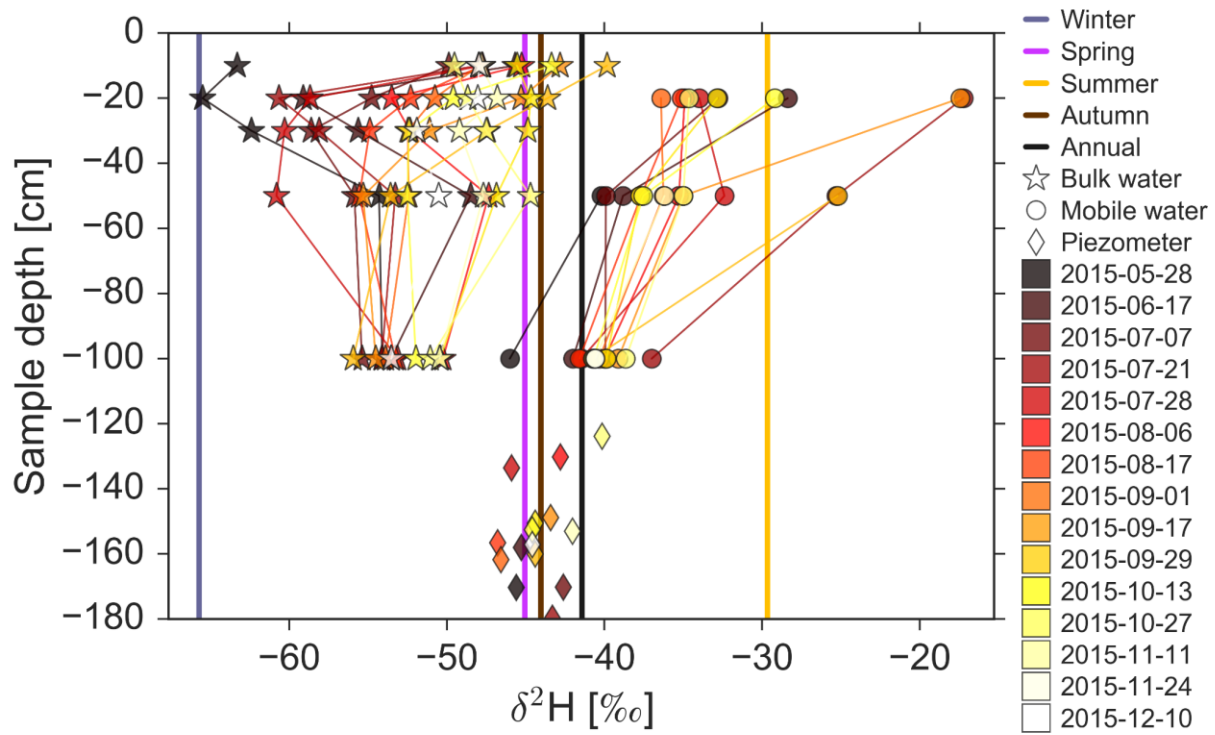
Figure 3 Depth profiles showing the variability of the stable isotopic composition across the soil profile over the sampling period (color coded) for bulk soil water (stars), mobile soil water (circles), and groundwater sampled from the piezometer (diamonds). Vertical lines represent seasonal and annual weighted averages of precipitation. Location of the piezometer samples according to the depth of the water table in the piezometer.
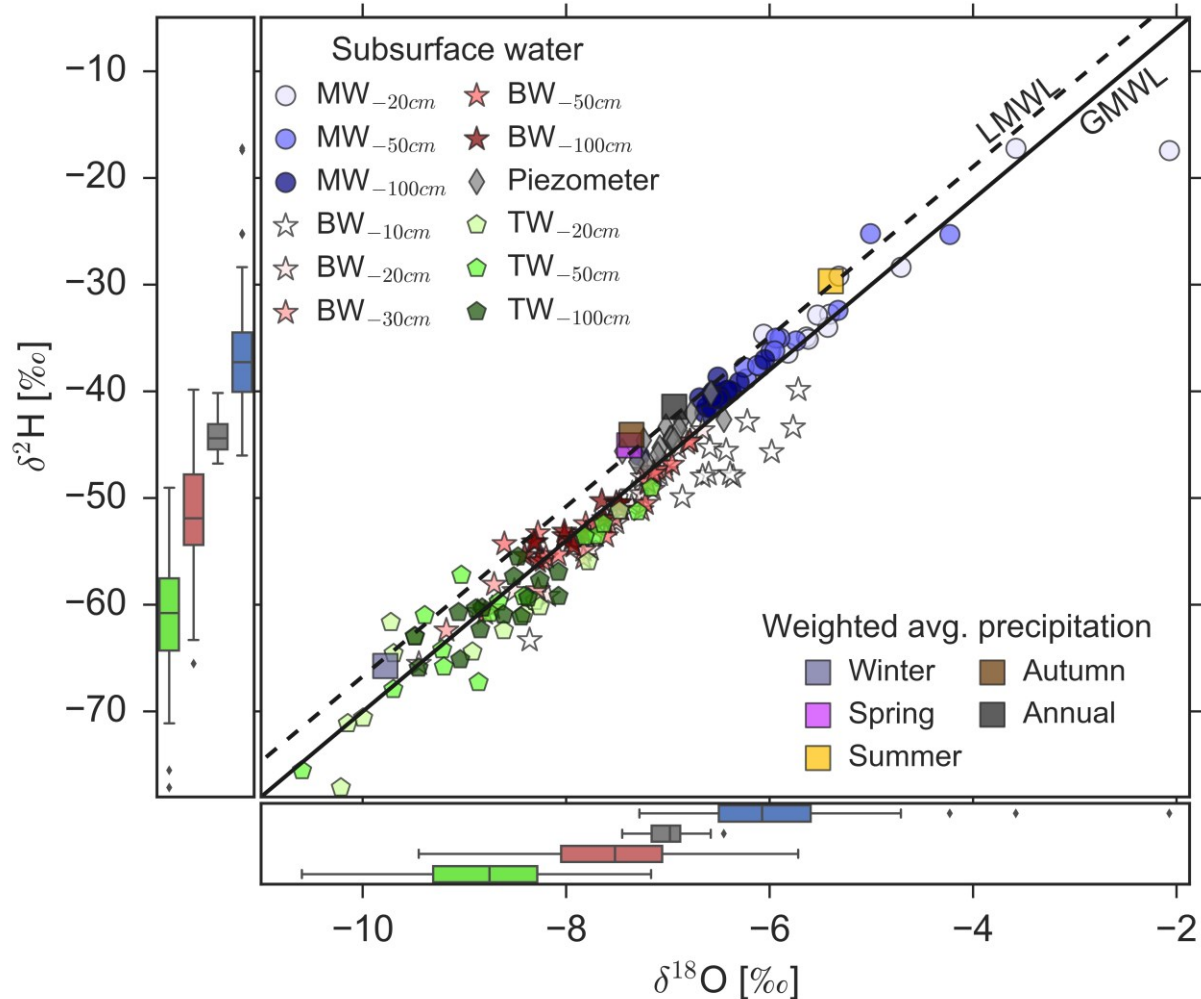
Figure 4 Water isotopes (δ ²H and δ ¹⁸O) of mobile (MW) and bulk soil water (BW) and piezometer water. We further show weighted seasonal and annual averages of rainfall stable isotopes (squares), the local meteoric water line (LMWL; δ²H = 7.96 δ¹⁸O + 12.89 ‰), the global meteoric water line (GMWL; δ²H = 8 δ¹⁸O + 10 ‰), 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 mixing of tightly bound water (here defined as water that cannot be accessed via suction lysimeter) with recently infiltrated rainfall or 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.,

10   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

15   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). 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 5b). 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 2a).

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 6a). 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 5b). Rainfall was generally more depleted in heavy isotopes when monthly rainfall exceeded evapotranspiration ($\rho=-0.5$, $p<0.05$, Figure 6b). 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 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 6c). 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 mixed not well with infiltrating rainwater.

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 not be hydraulically connected, but we would not be able to detect that separation via stable isotopes.
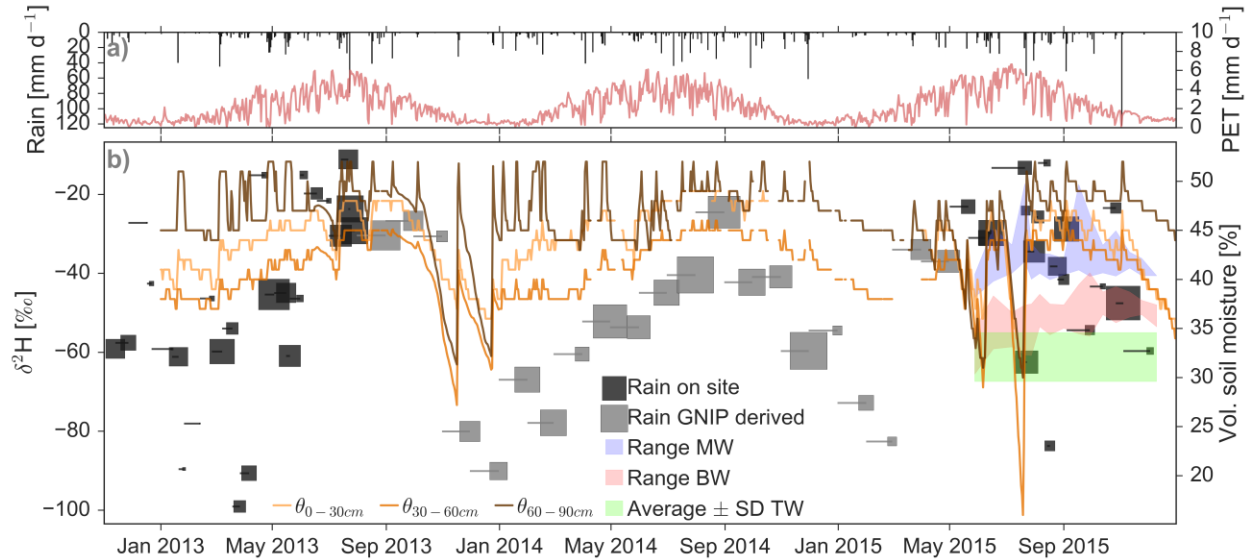
Figure 5 Long-term hydrological and stable isotopes dynamics. a) Daily rainfall (bars) and potential evapotranspiration (red line). b) Rainwater δ^2H values and soil moisture dynamics. Note that the size of the black and grey squares indicates the rainfall amount and the line represents the time span of the water sample. Black squares show rainwater 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 ± standard deviation value estimated for the tightly bound water (TW).
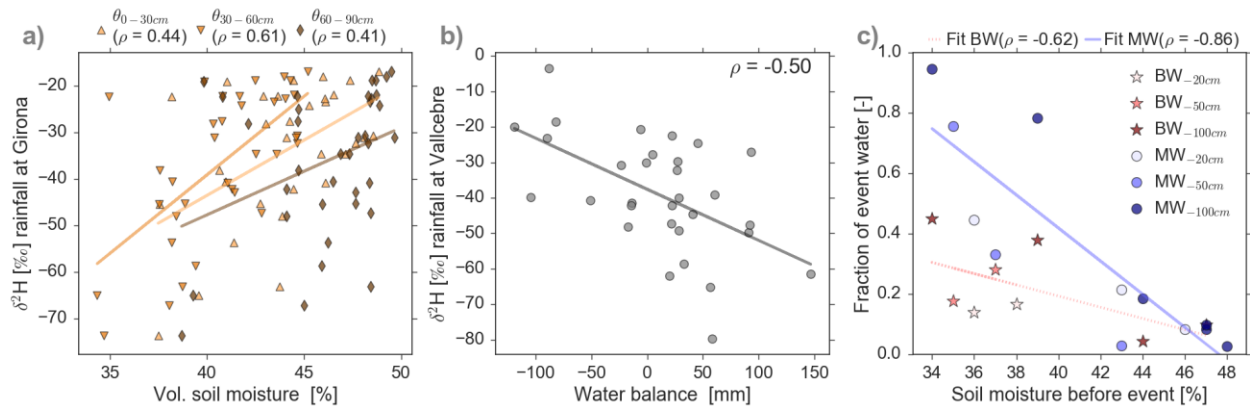
Figure 6 Relationships between wetness and rainfall stable isotopes and refilling of soil pores. a) Monthly rainfall stable isotopes (GNIP data) are more depleted when soils are drier. b) Monthly rainfall at the study site is more depleted when monthly rainfall volumes are higher than potential evapotranspiration (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 (dashed red line) 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 continuously 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 7d) 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

5   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

10   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 extend the isotopologues (i.e., $^2H^1H^{16}O$, $^1H_2^{18}O$) of mobile and tightly bound water exchange via gas phase

15   or dispersion is unclear (Oerter and Bowen, 2017), but for the studied soil in the 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

20   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 θ_{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

25   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

30   times) over depth (Figure 7b,c) to the variability of tracers (and residence times) over the pore space continuum (Figure 7e). 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

35   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). Experiments with weighable lysimeters (e.g., Stumpp and Maloszewski, 2010; Benettin et al., 2019), where the stable isotopes of the outflow were sampled, found similarly to our field observations for the mobile water, rapid percolation via preferential flow paths. However, the isotopic composition of the stored bulk soil water is unknown

5   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

10   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

15   origin (timing of infiltration and associated isotopic composition) of mobile and tightly bound water (as shown in Figure 7e) can explain differences observed among different methods for soil water isotope analysis (Sprenger et al., 2015; Geris et al., 2015; Orlowski 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

20   (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).
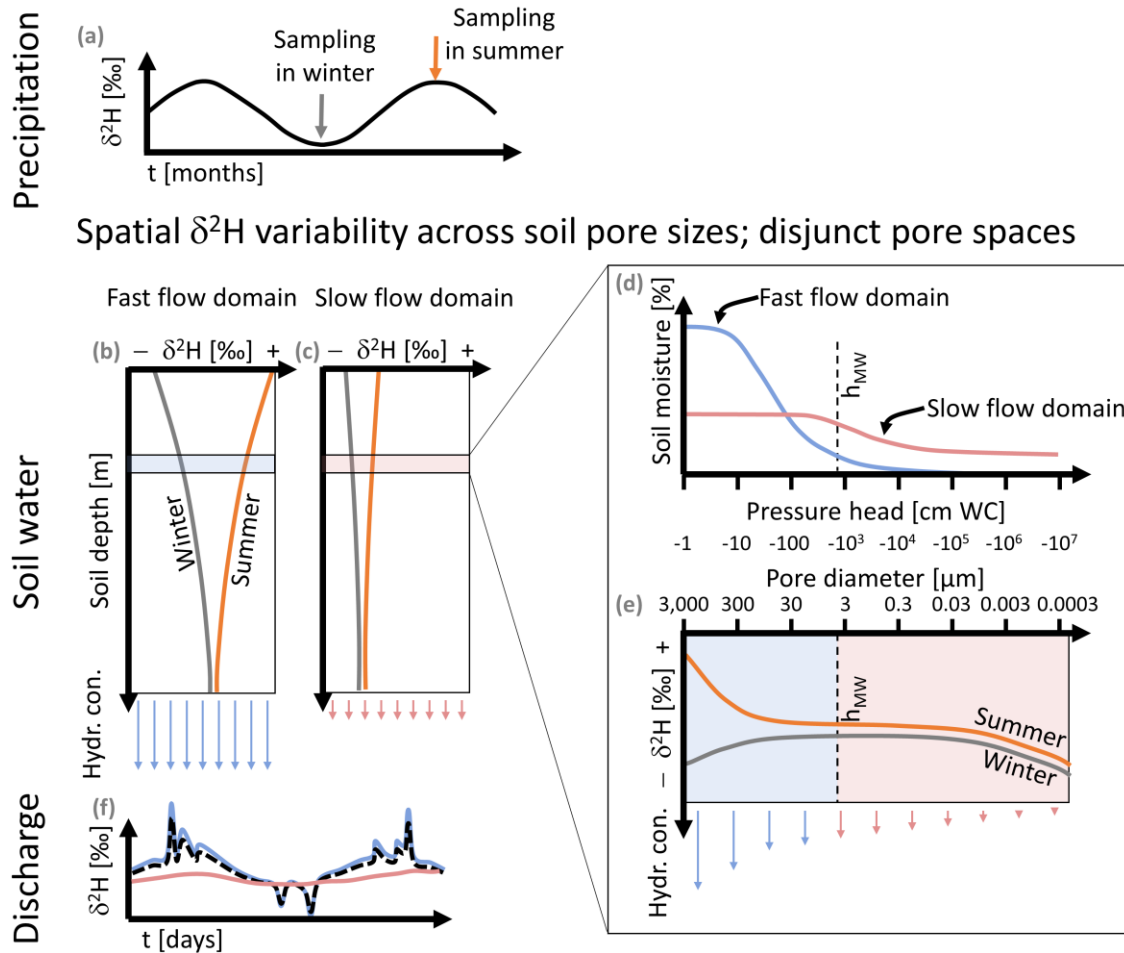
Figure 7 Conceptualization of pore space water stable isotope variability. a) Seasonal variation of the isotopic composition in the rainfall. Arrows indicate soil sampling days. b) Isotope depth profile of the fast flow domain (mobile water) during winter (grey) and summer (orange). The arrows below 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 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 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 within the sandy soil fraction has the same isotopic composition as the clayey soil fraction at the same 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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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:

The authors declare no competing interests.

Data availability.

Request for materials should be addressed to P.L.

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