Dynamic $^2$H irrigation pulse labelling reveals rapid infiltration and mixing of precipitation in the soil and species-specific water uptake depths of trees in a temperate forest

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Abstract
Understanding the movement of water in terrestrial ecosystems and determining the soil depths from which mature trees take up water has become an important research priority. Here, we test the suitability of a dynamic $^2$H pulse-labelling experiment for assessing (1) the fate of a simulated precipitation event as it moves through the eco-hydrological system and (2) the water uptake depths of different tree species in a mature temperate forest. We applied $^2$H-labelled water as a single pulse to the top soil using a sprinkler system and then allowed it to infiltrate into deeper soil layers by washing it through the soil column with a sequence of non-$^2$H-labelled irrigation pulses. We then followed this $^2$H-enriched irrigation pulse over a period of 81 days in different depths of the soil and in the xylem of four tree species (Fagus sylvatica, Quercus petraea, Picea abies and Pinus sylvestris). Our experiment shows infiltration and mixing of the irrigation pulse in the soil occurs within a few days. Furthermore, we found that tree species differed significantly in their use of shallow (~10- to ~30-cm soil depth) and deep (~80-cm soil depth) soil water. We also found immediate uptake of infiltrating mobile soil water by trees, which questions the recently established two-water-worlds hypothesis. Our study demonstrated that a dynamic $^2$H-labelled irrigation pulse is a useful approach to (1) assess how water from a precipitation event infiltrates into a forest ecosystem and (2) assess the water uptake depths of different temperate tree species.

KEYWORDS
deuterium isotopes, labelling, plant–water relations, preferential flow, rooting depth, soil, trees, two water worlds

1 | INTRODUCTION

Trees lose water from their leaves to the atmosphere when stomates are open for CO$_2$ uptake and when the vapour pressure in the atmosphere is lower than the vapour pressure in the leaves. The amount of water a tree can transpire to the atmosphere can be substantial and can amount up to several hundred litres per day (Larcher, 2003; Wullschleger et al., 1998). At the global scale, the water that is transpired by trees constitutes a critical component of the global water cycle (Jasechko et al., 2013; Lian et al., 2018; Schlesinger & Jasechko, 2014; Wei et al., 2017).

To maintain a hydrated water balance, trees need to replace the water that they have transpired to the atmosphere by taking up water via their roots from the soil. Trees have long been known to differ in
their rooting patterns and their water uptake depth from the soil (Canadell et al., 1996; Jackson et al., 1996). Understanding variation in soil water uptake depths among different trees species is important to assess sources of transpired water in the quantification of vegetation-atmosphere vapour fluxes at the ecosystem and global scale (Dawson et al., 2020; Evaristo et al., 2015; Javava et al., 2013; Werner & Dubbert, 2016). In addition, the rooting depth of trees has also been suggested to be a key determinant of a tree’s ability to withstand periods of reduced water supply in the soil and elevated water demand of the atmosphere (Brinkmann et al., 2016; David et al., 2007; Leuzinger et al., 2005; Nardini et al., 2015; Zapater et al., 2013). Assessing soil water uptake depths of trees will therefore also be critical to better characterize the drought sensitivity of different tree species and to predict the capability of different species to withstand future changes in the hydrological cycle.

The natural variability of the stable oxygen and hydrogen isotope composition ($\delta^{18}$O and $\delta^2$H values, respectively) in soil water and in water recovered from the conducting tissue of trees (i.e., the xylem), have long been used to assess the soil water uptake depths of trees (e.g., Amin et al., 2020; Dawson & Ehleringer, 1993; White et al., 1985). The approach relies on gradients in $\delta^{18}$O and/or $\delta^2$H values of soil water along a vertical soil profile. These gradients are generated by the evaporative $^{18}$O and/or $^2$H enrichment of water in shallow soil layers and by the increase of the residence times of water in the soil that typically increase with depth (Barnes & Allison, 1983; Sprenger et al., 2019). In temperate systems, this results in $^{18}$O- and/or $^2$H-enriched summer precipitation to be overrepresented in the upper soil layers at the peak of the growing season and reversed patterns in winter (Brinkmann et al., 2018). Since plants take up water from the soil with presumably no fractionation (Allison et al., 1983; Dawson & Ehleringer, 1991), the comparison of unfraccionated xylem water $\delta^{18}$O or $\delta^2$H values to $\delta^{18}$O or $\delta^2$H values along the soil profile allows identifying the soil water uptake depth of a tree (Rothfuss & Javava, 2017). Studies utilizing this method have revealed astonishing patterns in the water sources of trees. Meinzer et al. (1999), for example, were among the first to show marked differences in soil water uptake depths of different tropical tree species. Dawson and Ehleringer (1991) have shown that stream side trees do not use stream water. Meißner et al. (2012) and Brinkmann et al. (2019) have found that the soil water uptake depth of temperate trees can vary in response to the water availability in the soil. In a desert ecosystem, Ehleringer and Dawson (1992) have revealed that plants with different life history strategies utilize water sources that originate from different frontal systems in a desert ecosystem. Along the same lines, Brinkmann et al. (2018) have documented that winter precipitation contributes significantly to the total water used by trees throughout a growing season in temperate forests.

Despite the significant progress in our understanding of the water relation of trees that was made possible by using the natural abundance of stable isotopes in soil and xylem water, this approach has several critical limitations (Freyberg et al., 2020; Penna et al., 2018). One disadvantage of this method is that the natural abundance of soil water $\delta^{18}$O and $\delta^2$H gradients often does not extend deep into the soil (e.g., Kahmen et al., 2013) and therefore does not allow detecting the water uptake depth from deeper soil layers. This is problematic as deeper soil layers might constitute hydrologically and physiologically relevant water sources (Brinkmann et al., 2018; Dawson et al., 2020; Meißner et al., 2012). Even in the shallow soil, natural abundance soil water isotope profiles can at times not be sufficiently distinct to allow the assignment of specific soil water uptake depths (Prechsl et al., 2015; Stahl et al., 2013). In addition, several recent studies have suggested that water in the soil is not well mixed and that different water pools possible exist that are differently available for water uptake by the roots of trees (e.g., Berry et al., 2017; Brooks et al., 2010). The methods that are typically used for extracting water out of the soil for stable isotope analysis can, however, not distinguish between different soil water pools and/or may extract soil water that is isotopically not perfectly identical to the water taken up by plants (Newberry, Nelson, et al., 2017; Orłowski et al., 2016; Penna et al., 2018). Doubt has also been raised about the assumption that no fractionation occurs during soil water uptake by trees and during the transport of water within the trees and/or that plant-internal isotope effects modify xylem water $\delta^{18}$O and/or $\delta^2$H values (Barbeta et al., 2019; Barbeta et al., 2020, Chen et al., 2020; Ellsworth & Williams, 2007; Zhao et al., 2016). While possible $^{18}$O and/or $^2$H fractionations during soil water uptake or in the plant itself are often small, they might yet affect the estimation of soil water uptake depth, in particular in the deeper part of the soil water profile, where small errors in xylem water $\delta^{18}$O and $\delta^2$H values can result in large errors of estimated soil water uptake depth (Barbeta et al., 2019). In view of these limitations and uncertainties of the natural abundance method, complementary approaches should be tested for determining soil water uptake depth of plants.

The application of artificially $^2$H- or $^{18}$O-enriched water as an isotope label could be such a complementary approach. Several studies have already utilized the application $^2$H- or $^{18}$O-labelled water to determine the soil water uptake depths of individual tree species or the complementary soil water uptake depths of different tree species (Bishop & Dambrine, 1995; Grossiord et al., 2014; Plamboeck et al., 1999). Other studies have used $^2$H- or $^{18}$O-labelled water to determine the use of shallow water originating from defined precipitation events by different plant species in the Colorado Plateau (Schwinning et al., 2002), the uptake of deep soil water and the subsequent hydraulic re-distribution of the water in the shallow soil by the European Pinus nigra (Penuelas & Filella, 2003), or for investigating the rooting depths of deep-rooted plants in arid African ecosystems (Beyer et al., 2016).

In the study that we present here, we employed a $^2$H-labelled approach to assess the infiltration and mixing of precipitation in the soil and to assess species-specific soil water uptake depths of trees in a mature temperate forest. While previous labelling studies have typically applied $^2$H- or $^{18}$O-enriched water to statically label a specific soil depth, mostly in the shallow soil, we tested a dynamic labelling approach. Specifically, we applied the $^2$H-labelled water as a single pulse to the top soil and then allowed it to infiltrate into deeper soil layers by washing it through the soil column with a sequence of
simulated non-²H-labelled irrigation and precipitation events. We then followed this ²H-enriched dynamic irrigation pulse over a period of 81 days to determine (1) the fate of a simulated precipitation event as it moved through the ecohydrological system and (2) differences in the soil water uptake depths of mature Fagus sylvatica, Quercus petraea, Picea abies and Pinus sylvestris trees.

2 | MATERIAL AND METHODS

2.1 | Site description

For our experiment, we chose a 100- to 150-year-old near-natural mixed temperate forest stand located south of Basel in Hofstetten, Switzerland (47°46′N, 7°50′E) at an elevation of 550 m a.s.l. The majority of mature trees at the site was approximately 120 years old and 30–40 m tall at the time of the experiment. The site is situated on a north-exposed slope belonging to a calcareous mountain range with a local maximum elevation of 837 m a.s.l. The soil is of a silty–loamy Rendzina type sitting on calcareous bedrock at around 1 m below-ground. The region has a humid temperate climate with comparatively mild winters and moderately warm summers and a mean annual precipitation of 990 mm two thirds of which fall during the growing season from May to October (Pepin & Körner, 2002). A total of 10 evergreen and deciduous tree species occur at the site of which we selected a total of 14 mature individuals of the four predominant species F. sylvatica (n = 4), Q. petraea (n = 3), P. abies (n = 4) and Pinus sylvestris (n = 3) (see Figure S1).

The Swiss Canopy Crane (SCC), a 40-m freestanding tower crane, equipped with a 30-m jib and a gondola allowed to access the forest canopy (Pepin & Körner, 2002). Throughout the experiment, we quantified precipitation and temperature with a weather station on top of the canopy crane (Davis Vantage Pro 2, Scientific Sales Inc., Lawrenceville, NJ, USA) at 10-min intervals. Soil water potential was recorded at a depth of −0.2 m throughout the growing season by 12 dielectric matrix potential sensors (MPS-2, Decagon Devices, Pullman, WA, USA) with the same sampling frequency. The burial depth of the MPS-2 sensors was chosen according the root distribution of temperate forest trees (Bello et al., 2019; Schenk & Jackson, 2002). To put the irrigation amount into a long-term perspective, we also analysed precipitation data of a nearby located weather station at Basel-Binningen (MeteoSwiss, Zurich, Switzerland) from 1901 to 2015.

2.2 | Irrigation and labelling

We conducted a large-scale labelling on the experimental site using ²H-enriched water. In the early morning of 26 June 2016 (day 1 of the experiment), we irrigated the forest below the canopy with on average 13.2 ± 1.6 mm, (mean ± SE, n = 13) of ²H-enriched water (δ²H = 2461.1 ± 166.5‰, mean ± SE, n = 4). The labelled water was obtained by mixing of local tap water with a highly ²H-enriched solution using a dosage pump (D45RE15, Dosatron International S.A.S., Tresse, France). The labelled water was applied to the experimental site by an irrigation system consisting of 13 lawn sprinklers (Viereckregner Comfort Aquazoom 250/2, Gardena manufacturing GmbH, Ulm, Germany) installed 1 m above the ground on wooden poles. Sprinklers were arranged in three lines crossing the slope of the plot from East to West and were connected to an automatic control box with an irrigation valve (Bewässerungsventil, Gardena manufacturing GmbH, Ulm, Germany) via water pipes resulting in three independently available irrigation lines (for details of the experimental plot layout, see Figure S1). Each sprinkler was adjusted to cover around 80 m² of rectangular area of soil allowing a uniform irrigation of the site. To ensure consistent water pressure on the irrigation system, a water pump was installed downstream of a 1-m³ water tank the filling level of which was controlled by a floating switch. During the labelling event, the three individual irrigation lines were activated alternately for around 25 min with the upper line being activated first, followed by the lines further downslope. To avoid contamination of samples and potential direct water uptake of trees through bark, trunks were wrapped in plastic foil up to a stem height of 3–5 m during the application of the ²H-enriched water.

To allow the ²H label to travel to deeper soil layers, we irrigated the forest with approximately 10-mm local tap water (δ²H = −59.3‰) several times after the application of the ²H label with the same sprinkler system that we used to label the forest. Irrigation started immediately following the application of the ²H label and continued throughout the 81 days of the experiment. Irrigation events took place during the night from 12 pm to 7 am. A total of 26 irrigation events were performed (Figures 1 and 2). Irrigation frequency was highest at the start of the experiment and was then gradually reduced. Starting in mid-July, no irrigation was conducted for 23 days. Due to an observed decrease in soil moisture in this period, the irrigation frequency was increased again at the beginning of August.

Quantity, uniformity and isotopic composition of labelled water, irrigation water and precipitation (i.e., throughfall) water was monitored using 13 custom-built rain samplers (Prechsli et al., 2014) that were evenly distributed over the experimental site (see Figure S1). To allow for a uniform application of the ²H-labelled and irrigation water, some of the undergrowth (young Acer platanoides, F. sylvatica and Tilia platyphyllos) was cut and removed from the site.

2.3 | Sample collection

Soil and xylem samples were collected a day before the application of the label and frequently thereafter (Figure 1). Sampling frequency was highest until 12 days after labelling and was then consecutively reduced with time (Figure 1).

To sample soil water, soil cores were collected using a soil corer (Nutstange, UP GmbH, Ibbenbüren, Germany). Samples were taken at five different depths (−0.1, −0.2, −0.3, −0.5 and −0.8 m). At each sampling event, five soil cores were taken at randomly chosen
positions across the experimental site with restriction to some areas where collection of soil cores turned out to be impossible due to the rocky composition of the soil. The locations from which the individual soil cores had been collected were marked on the experimental site to avoid multiple sampling of the very same location and were recorded on a map. Soil samples were transferred into airtight Exetainers (Labco, Lampeter Credington, UK) and stored in a freezer at $-20\,\text{C}$ until further processing.

**FIGURE 1** Overview of the irrigation and sampling events throughout the experiment that ran from 25 June 2016 (i.e., day 0) to 15 September 2016 (i.e., day 81 after labelling). Circles indicate collection dates of tree samples for all investigated trees at the respective heights. For soil (squares) at each sampling event, samples from five soil depths ($-0.1$, $-0.2$, $-0.3$, $-0.5$ and $-0.8$ m) were collected from five independent locations scattered randomly across the site ($n = 5$). Triangles represent irrigation events that took place during the night preceding the respective days.

**FIGURE 2** (a) Cumulative mean irrigation and throughfall during the sampling period (25 June to 16 September 2016), measured at the forest ground with 13 irrigation collectors as well as mean cumulative precipitation for the same time period; time averaged for the last 100 years before the experiment (mean ± SD) calculated with data from a nearby weather station (Basel-Binningen). Day 0 is defined as 25 June 2016, the day before the $^2$H label application. (b) $\delta^{2}$H values (mean ± SE) of the applied $^2$H label as well as irrigation and throughfall water collected at forest ground level with 13 irrigation collectors. Closed circles indicate water collected after an irrigation event, open circles mark water from natural rain events. (c) Soil water potential measured continuously at a depth of $-0.2$ m in direct proximity of the trees (mean ± SE, $n = 12$ sensors). (d) Daily mean vapour pressure deficit (VPD) calculated from data measured continuously by a weather station on the canopy crane above the canopy.
Xylem samples were collected from a total of 14 mature trees of the species *F. sylvatica* (*n* = 4), *Q. petraea* (*n* = 3), *P. abies* (*n* = 4) and *P. sylvestris* (*n* = 3) that grew within the irrigated area. Each tree was sampled at the stem base (0 m) and at crown level (c. 35 m) at Lampeter Credington, UK and stored in a freezer. Samples from the canopy were collected by cutting branches to minimize possible influences due to disturbances in sap flow like cavitation in the xylem caused by the wounding of trees. Samples from the canopy were collected by cutting branches in a defined sun-exposed area of the respective tree crowns. All samples were sealed in airtight exotainers (Labco, Lampeter Credington, UK) and stored in a freezer (−20°C) until further processing.

### 2.4 Water extraction and sample processing

Soil and xylem water were extracted using the cryogenic vacuum distillation method following the procedure described in detail in Newberry, Nelson, et al. (2017). Soil and xylem samples were heated in a water bath (90°C) for 3 h, while a vacuum of 0.03 hPa was applied. Evaporating water was condensed and collected in U-tubes submerged in a bath of liquid nitrogen. After extraction was complete, U-tubes were removed and sealed with rubber plugs. After complete thawing of the water samples, a syringe with a filter tip was used to transfer samples to airtight 1.5-ml GC vials (Macherey-Nagel GmbH, Düren, Germany) for isotopic analysis. Several studies have reported potential artefacts associated with the cryogenic extraction methods that could affect the oxygen but in particular the hydrogen stable isotope composition of extracted water from soils or plant material (Chen et al., 2020; Newberry, Prechsl, et al., 2017; Orlowski et al., 2016). The artefacts reported in these studies range around 10% for δ²H values. While certainly relevant for the interpretation of stable water isotope at natural abundances, these artefacts are small compared to the label that we used in this study (2461.1 ± 166.5‰) and are thus not of concern.

### 2.5 Isotopic analysis

Samples were analysed on a thermal conversion/elemental analyser (TC/EA) coupled to a Delta V Plus continuous-flow isotope ratio mass spectrometer (IRMS) via a Conflo IV interface (Thermo Fisher Scientific, Bremen, Germany). Samples were decomposed by pyrolysis at 1420°C to H₂ and CO in the presence of glassy carbon chips. H₂ and CO gases were separated on a Molecular sieve 5A GC column. For each sample, aliquots of 100 nl were injected and analysed in five replications. The first injection was always discarded, and the remaining four injections were averaged to account for memory effects. Every 10 samples (i.e., 50 injections), two calibrated in-house lab standards were used to account for drift and to standardize the data. Another in-house lab standard was analysed in triplicate throughout the sequence as a quality control. Hydrogen stable isotope data were expressed in the delta notation in ‰, relative to VSMOW-SLAP on a scale that δ²H (SLAP2) = −427.5‰ as in:

\[
\delta^2H = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1,000,
\]

where R is the ratio of the heavy to light isotope (²H/¹H). To further minimize the possibility of memory effects in the analysis due to highly enriched samples, batches were analysed in the order of increasing expected δ²H values. All analyses were conducted by the Stable Isotope Ecology Lab at the Department of Environmental Sciences, University of Basel, Switzerland.

### 2.6 Calculation of water uptake depths

We employed two methods to estimate the water uptake depth of the tree species. In a first approach, we utilized a simple linear interpolation model as described in Prechsl et al. (2015). This approach calculates distinct water uptake depths for the respective trees, ignoring possible ranges of uptake depths or multiple sources. Data from a subset of selected days were used (*n* = 7) (Figure 3). Criteria for the selection of these data were (1) samples obtained at least 3 days after the label was applied so that the label was able to arrive in the trees xylem and properly distribute within the soil and (2) evident differences in δ²H values among investigated soil layers resulting in distinct gradients between the shallow and deep soil. Based on these criteria, all xylem and soil profile δ²H values collected between Days 4 and 16 after label application except Days 8 and 12 were used in the analysis (Figure 3). For the interpolation model, it was assumed that the respective soil water uptake depth can be calculated by direct comparison of stem base xylem water δ²H values of the different trees with δ²H values of soil water along the soil profile. Thus, the water uptake depth is defined as the soil depth where δ²Hₓylem equals δ²Hₒil. To obtain continuous soil water δ²H profiles, we linearly interpolated in between the measured values from the five investigated soil depths (−0.1, −0.2, −0.3, −0.5 and −0.8 m [*n* = 5]). Using this method resulted in a distinct value for soil water uptake depth, these values can be interpreted as an indicator of the mean or median soil water uptake depth of a tree, but it ignores possible depth ranges from which the water sources were obtained. Soil water uptake depth was calculated for each of the selected sampling events (*n* = 7), and the respective means were calculated from these values for the different tree species.

In a second approach, we used a Bayesian mixing model to calculate the probability of soil water uptake from different depths (Rothfuss & Javaux, 2017). We used the package ‘Stable Isotope Analysis in R’ (SIAR), developed by Parnell et al. (2010) and Parnell and Jackson (2015) which applies a Markov chain Monte Carlo (MCMC)
approach. In the model, the water of different soil depths was treated as ‘sources’, and the xylem water of the different trees was set as the ‘consumer’. Model parameters were trophic enrichment factor (TEF) = 0 and iterations = 500,000 with 50,000 of those iterations being discarded initially (‘burning of’). The model works best when differences among values of different sources are large. To maximize differences among individual soil depths, we restricted our inputs to three distinct depths (i.e., –0.1, –0.3 and –0.8 m). To account for the slightly inconsistent results for each run due to the MCMC algorithm of the model, we bootstrapped the outputs of the model 100 times by conducting ordinary resampling (Brinkmann et al., 2019), which provided uncertainty estimates of the model (standard error and 95% confidence interval). The modes of probability of water uptake from the three different soil depths were calculated independently for each tree species at each of the selected sampling events (see above, n = 7) and averaged per species.

2.7 | Statistical analyses

For an easier comparison of soil and xylem water δ²H values at different soil depths or in the different species over time, we normalized mean δ²H values to the corresponding maxima at each respective depth or in each respective species with the following formula:

$$r(x_i) = \frac{\delta^2H(x_i) - \min(\delta^2H(x_j))}{\max(\delta^2H(x_j) - \min(\delta^2H(x_j)), ..., (\delta^2H(x_z) - \min(\delta^2H(x_z))))}$$

where $r(x_i)$ is the relative value for this respective measurement and $x_i$ is the respective time of measurement for the respective soil depth or tree species. To analyse changes in relative soil and xylem water δ²H values over time, we used nonlinear least squares approach (nls) function of the stats package; R Core Team, 2015) with two different functions. A linear function (Equation 1) for the increase of relative
$\delta^2$H values right until the maximum was reached, and a logarithmic (Equation 2) function for decreasing relative $\delta^2$H values after the maximum had been reached. The formulae were

$$y = a_1 \cdot x,$$

(1)

$$y = a - b \cdot \log(x),$$

(2)

with x representing day after labelling (DAL) in hours and y representing relative $\delta^2$H values. The parameters $a$, $b$, and $a_1$ determine the curvature and the inclination of the function. Values of parameters for respective soil depths and species are listed in Table 1. For testing the fit of the above functions (1, 2), these were solved for parameter x with the given $\delta^2$H values, and a linear regression ($\text{lm}$) was applied to compare the obtained values for DAL with the actual DAL values from the measured data. We extracted the coefficient of determination ($r^2$). For all statistical tests, a significance level of $p < 0.05$ was assumed. For analysing differences in rooting depths calculated by the linear model, a post hoc Tukey’s honest significance test was applied.

All statistical analyses and visualizations of the data were done in R, version 3.4.1 (R Core Team, 2015) with its packages ggplot2 (Wickham, 2009), scales (Wickham, 2015), readr (Wickham & Francois, 2015), plyr (Wickham, 2015) and gridExtra (Auguie, 2015).

3 | RESULTS

3.1 | Environmental conditions and irrigation

During the experimental period from 26 June to 16 September 2016, we recorded a total water input of 297 mm (irrigation water + natural throughfall) at the forest ground, while the weather station above the canopy registered a total precipitation of 61 mm during the same period. Since the year of our experiment was rather dry, total experimental water input at the site (incl. throughfall) was only 50 mm higher during the experimental period than the long-term mean precipitation in the region for this period (Figure 2a). $\delta^2$H values of the irrigation water after labelling were similar compared to the $\delta^2$H values of the naturally occurring precipitation during this period (mean values: $-59.3\%$ and $-47.3\%$, respectively; Figure 2b).

Soil water potential at $-0.2$ m depth ranged from a daily mean minimum of $-0.24$ MPa to a maximum of $-0.009$ MPa and showed a slight decrease until the beginning of August (Figure 2c). Vapour pressure deficit (VPD) was constantly high with few highs and lows exhibiting a mean of 0.918 ± 0.46 kPa (mean ± SE, Figure 2d).

3.2 | $^2$H label infiltration in the soil

Before the $^2$H-labelled water was applied, the forest soil showed a gradient in soil water $\delta^2$H values with relatively more enriched water in top soil layers ($-0.1$ m: $-59.3 \pm 3.2\%$, mean ± SE, $n = 5$) and more $^2$H-depleted water in deeper soil layers ($-0.8$ m: $-71.5 \pm 1.7\%$, mean ± SE, $n = 5$). After the application of the $^2$H-labelled water, a significant rise in $\delta^2$H values of water of all soil layers down to a depth of $-0.5$ m was observed on the first day after label application (Figures 3 and 4). At a depth of $-0.8$ m, the mean soil water $\delta^2$H values differed significantly from the pre-labelling conditions only on the fifth day after label application. Maximum $^2$H enrichment was observed on the second day after label application at $-0.1$ m ($234.1 \pm 113.1\%$, mean ± SE, $n = 5$), $-0.2$ m ($213.5 \pm 66.8\%$, mean ± SE, $n = 5$) and $-0.3$ m ($183.5 \pm 94.5\%$, mean ± SE, $n = 5$). The maximum $^2$H enrichment at $-0.5$ m was observed on the fifth day after label application ($164.1 \pm 58.6\%$, mean ± SE, $n = 5$) and at $-0.8$ m on the 19th day after $^2$H-labelling ($206.2 \pm 22.3\%$, mean ± SE, $n = 5$) (Figures 3 and 4).

The temporal decline of standardized relative soil water $\delta^2$H values after the maximum value had been reached was best described by a logarithmic function (Equation 2), which indicated that the rates

| TABLE 1 | Values for parameters used to describe the rise (linear function [Function 1]) and decline (logarithmic function [Function 2]) of standardized relative $\delta^2$H values over time after $^2$H label application |
| Function 1 | Function 2 |
| $a_1$ | Multiple $R^2$ | $a$ | $b$ | Multiple $R^2$ |
| Soil depth | | | | |
| $-0.1$ m | 0.0163 | 0.5576 | 1.7714 | 0.2456 | 0.7913 |
| $-0.2$ m | 0.0175 | 0.5763 | 1.6672 | 0.2217 | 0.8512 |
| $-0.3$ m | 0.015 | 0.852 | 1.5002 | 0.1936 | 0.7669 |
| $-0.5$ m | 0.0043 | 0.1612 | 0.5493 | 0.0428 | 0.3217 |
| $-0.8$ m | 0.0019 | 0.2258 | 1.9783 | 0.1759 | 0.437 |
| Species | | | | |
| $F. sylvatica$ | 0.0096 | 0.7419 | 1.8466 | 0.2314 | 0.9614 |
| $Q. petraea$ | 0.0086 | 0.6833 | 0.8286 | 0.0412 | 1.10E-05 |
| $P. abies$ | 0.0086 | 0.7908 | 1.8064 | 0.2248 | 0.9647 |
| $P. sylvestris$ | 0.0033 | 0.3873 | 1.7956 | 0.2366 | 0.8929 |

Note: Multiple $R^2$ values describe the fit of the respective functions to the measured data. Figures of standardized relative $\delta^2$H values with the respectively modelled data can be found in Figure 4 for soil and in Figure 5 for xylem water data.
of decline differed among soil depths (Figure 4, Table 1). We observed a faster decline in water Δ2H values over time in the upper soil compared to deeper soil layers. At Day 67, mean Δ2H values at 0.1 m did not differ from pre-labelling conditions any more (p = 0.459), while for depths of 0.2, 0.3 and 0.5 m, this was only the case on day 81 after labelling (Figure 4). Mean Δ2H values measured at a depth of 0.8 m 81 days after labelling still showed 43% of the maximum values measured.

### 3.3 Arrival and decline of the 2H label in xylem water

Mean xylem water Δ2H values at stem base (0 m) measured the day before labelling (25 June 2016, Day 0) ranged from −69.3 ± 1.7‰ to −79.3 ± 1.8‰ (mean ± SE; n = 3 or 4, depending on species) among the four investigated species (Figure 5). After the 2H-labelled water was applied, the xylem water Δ2H values of all species increased quickly at the stem base, with maximum xylem water Δ2H values occurring within 8 days after label application for all species except Q. petraea, where maximum xylem water Δ2H values were reached 16 days after label application (Figure 5). Maximum mean Δ2H values of xylem water at 0 m stem height were highest for P. abies (86.6 ± 54.5‰, mean ± SE) and lowest for Q. petraea (−2.6 ± 39.1‰, mean ± SE), whereas in F. sylvatica and P. sylvestris, Δ2H values of 52.5 ± 16.2‰, mean ± SE and 28.9 ± 14.5‰, mean ± SE, respectively, were observed. In contrast to the base of the stem, we found no clear differences in the arrival time of the 2H-labelled water in the canopy xylem among the different species (Figure 5). Canopy xylem water Δ2H values remained higher than the respective values at the stem base for all species except P. abies between about 20 days after label application until the end of our survey.

The temporal decline of standardized relative Δ2H values in xylem water at the stem base after maximum values were reached differed among species (Figure 5). Q. petraea showed a much slower decline compared to the other species (Figure 5, Table 1). At the end of our
3.4 | Species-specific soil water uptake depths

Across all species and considered days, linearly inferred soil water uptake depth ranged from \(-0.121\) to \(-0.765\) m soil depth and distinct differences in mean uptake depth were found among species (Figure 6). On average, *P. abies* took up water from the shallowest soil layers \((-0.36 \pm 0.05\) m, mean \(\pm SE)\) and *Q. petraea* from deeper down \((-0.69 \pm 0.04\) m, mean \(\pm SE)\), while for *F. sylvatica* \((-0.49 \pm 0.08\) m, mean \(\pm SE)\) and *P. sylvestris* \((-0.57 \pm 0.02\) m, mean \(\pm SE)\), the linear approach suggested intermediate soil water uptake depths.

**FIGURE 5** (a) $\delta^2$H values of xylem water at 0 m stem height and crown level (approx. 35 m above the ground) for *F. sylvatica* *(n = 4)*, *P. abies* *(n = 4)*, *P. sylvestris* *(n = 3)* and *Q. petraea* *(n = 3)*. Labelling was conducted on 26 June 2016, i.e., day 1 of the experiment. Sample collection started on day 0 (i.e., 1 day before the label application) and commenced until day 81 of the experiment. Error bars represent standard errors of respective means. (b) Xylem water $\delta^2$H values at stem base, normalized to the maximum $\delta^2$H values reached throughout the experiment for a respective tree species. Two different functions were fitted to the data (black lines). A linear function was fitted to the data starting on day 0 until xylem water reached a maximum $\delta^2$H value (equation 1) and a logarithmic function for the data starting at the time when xylem water reached a maximum $\delta^2$H value until the end of sampling period (equation 2). The parameters of the functions as well as multiple $R^2$ for different tree species are listed in Table 1.

**FIGURE 6** Linear estimation of the mean water uptake depths of the four investigated tree species. Differences among species were tested with a Tukey’s honest significance test, where significance levels were defined as $p < 0.05$.
The probabilities of water uptake from different soil depths (–0.1, –0.3 and –0.8 m), calculated with the Bayesian mixing model, differed among species (Figure 7). The probability of soil water uptake from –0.1 and –0.3 m was highest for P. abies, followed by P. sylvestris and F. sylvatica and lowest for Q. petraea (Figure 7a). For –0.8 m soil depth, the probability of soil water uptake was highest for Q. petraea followed by P. sylvestris and F. sylvatica and lowest for P. abies (Figure 7a). When assessing the probability of different soil water uptake depths within a species, it became evident that for Q. petraea, P. sylvestris and F. sylvatica, the highest probability for soil water uptake was from –0.8 m and the lowest from –0.1 m (Figure 7b). This pattern towards a higher probability of soil water uptake depth from –0.8 m compared to –0.1 m was most strongly expressed in Q. petraea. P. abies, in contrast, showed the highest probability for soil water uptake from –0.1 and –0.3 m and the lowest probability from –0.8 m soil depth (Figure 7b).

4 | DISCUSSION

Our study revealed that a dynamic \(^3\)H pulse label quickly infiltrated the soil down to –0.8 m after its application to the forest floor. After maximum soil water \(\delta\)\(^2\)H values had been reached, the \(^3\)H label exponentially declined over time, and this decline was faster in shallow and slower at deep soil layers. Shortly after the application of the \(^3\)H label, we recovered the label in the xylem water at stem base of all four tree species. The maximum \(\delta\)\(^2\)H values that were reached differed, however, strongly among species. The decline of the \(^3\)H label in the trees over time also differed among species, with particularly Q. petraea showing a slower decline compared to the other species. We calculated the soil water uptake depths and found that these differed significantly among the four investigated species with P. abies taking up water mostly from the shallow soil, P. sylvestris and F. sylvatica from intermediate soil depths and Q. petraea from deeper soil layers. From our data, we conclude that the dynamic \(^3\)H pulse label that we applied in our experiment is a valid approach to assess the infiltration of a precipitation event into a mature forest ecosystem and to assess differences in soil water uptake depth among co-occurring tree species. In the following, we will discuss the experimental approach and individual results obtained from our study in detail.

4.1 | Label infiltration into the soil

Following the application of the \(^3\)H-labelled water, we frequently irrigated the forest floor with water that had natural abundance \(\delta\)\(^2\)H values (\(\delta\)\(^2\)H = –59.3‰). The purpose of initiating these irrigation measures was to allow the label to infiltrate deeper soil layers and to wash the label out of the shallow soil in the course of the experiment. The amount of water used for irrigation during the experiment was in the range of the long-term precipitation record (Figure 2). The irrigation of the forest did therefore not expose the trees to an unusual amount of ‘precipitation’.

A significant increase in \(\delta\)\(^2\)H values compared to pre-labelling conditions was observed at all soil depths within 1–5 days after the application of the \(^3\)H label. This confirms that the label strength chosen for this experiment was sufficient to label the entire soil water column up to 80 cm depth. The fast infiltration of the \(^3\)H-labelled water highlights the important role of preferential flow for water when infiltrating the soil. Preferential flow describes the phenomenon where substantial amounts of precipitation reach deeper soil layers by flowing through macropores in the soil, such as cracks and large pores created by soil structure, soil organism activity and plant roots (Beven & Germann, 1982; Beven & Germann, 2013; Gazis & Feng, 2004). Flury et al. (1994) showed the importance of preferential flow for the infiltration of water for different soil types with

**FIGURE 7** Probability of water uptake from three soil depths (–0.1, –0.3 and –0.8 m) calculated using the Bayesian SIAR approach with \(\delta\)\(^2\)H values of soil water from three soil depths and xylem water of different tree species (F. sylvatica \(n = 4\), P. abies \(n = 4\), P. sylvestris \(n = 3\), Q. petraea \(n = 3\)) extracted at the stem base. (a) Species differences in mean probabilities of water uptake from a given soil depth. (b) Distribution of uptake probabilities at –0.1, –0.3 and –0.8 m for a given species. Differences were tested with a Tukey’s honest significance
structured soils being more prone to preferential flow. For Rendzina type soils, such as at the site of our experiment, they reported an infiltration rate of up to 0.8 m per day. This value agrees well with the infiltration rates that we observed in our experiment, where the 2H-labelled water appeared at ~0.5 m soil depth within a day and at ~0.8 m soil depth within 5 days after the application of the 2H label (Figure 3).

Despite the fast infiltration, the intensity of the labelling pulse declined with increasing soil depth. Compared to the maximum δ2H values measured in water extracted from ~0.1 m soil depth, maximum mean soil water δ2H values measured at depths of ~0.2, ~0.3, ~0.5 and ~0.8 m accounted for only 91%, 78%, 70% and 6% of the value, respectively (Figure 4). Progressive mixing of the 2H-labelled water with soil water at natural abundance δ2H values as it infiltrates into deeper soil layers can explain the progressive dilution of the label with increasing soil depth. In addition, temperate forests can transpire up to 4–5 mm of water per day (Larcher, 2003). The uptake of 2H-labelled soil water by the vegetation and the consequential removal of 2H-labelled water from the soil is thus another explanation for the observed decrease in δ2H values with increasing soil depth.

Soil layers from ~0.1 to ~0.5 m all showed their respective maxima in δ2H within the first 5 days after labelling, while at ~0.8 m, the label was found to be strongest 19 days after labelling. After maximum values occurred, a gradual decline of δ2H values in the soil water at the respective depths was observed. The rates of decline differed, however, among soil layers (Figure 4). The decrease was faster in upper soil layers, resulting in a gradient with lower soil water δ2H values in upper soil layers and highest soil water δ2H values at ~0.5- or ~0.8-m depth, respectively, after Day 16 of the experiment. This pattern can, to some extent, be a consequence of continuing infiltration of 2H-enriched water from the upper soil into deeper layers. Considering preferential flow, as described above, different pore diameters can lead to different infiltration rates and therefore to a diffuse vertical labelling pulse front, especially at greater depths. Inversely, the more rapid decline of the 2H label in the shallow soil suggests that continuous mixing with non-2H-enriched precipitation water and preferential water removal by water uptake of trees in these layers. Such a faster turnover of water in shallow soil layers is in line with studies showing increasing mean residence time of water with increasing soil depth (Asano et al., 2002; Brinkmann et al., 2018; Heidbüchel et al., 2013; Sprenger et al., 2016; Sprenger et al., 2019).

The data that we describe above illustrate that a dynamic 2H pulse label, where a label is applied to the top soil and then washed into deeper soil layers by a sequence of irrigation pulses, is a useful approach for labelling the soil column with 2H-enriched water. The temporally dynamic behaviour of the label, i.e., the appearance and the decline of the 2H label at a given soil depth, allows to assess the fate of individual precipitation pulses including their turnover and utilization by the vegetation, as discussed below. The combination of this dynamic labelling approach with emerging analytical technology, such as in situ high-resolution optical isotope measurements as described by Volkmann et al. (2016) and Kübert et al. (2020), provides great new opportunities to assess the dynamics of ecohydrological processes in terrestrial ecosystems in the future.

### 4.2 Soil water uptake by roots

The rapid appearance of the 2H label in the xylem at the stem base of all tree species suggests that all investigated species take up soil water from the upper, shallow soil. Indeed, we found that all species have a probability of water uptake from the shallow soil (i.e., ~0.1 m) that is larger than 0.35, suggesting that about one third of the water utilized by trees originates from the shallow soil (Figure 7b). Previous studies that have compared water uptake depths of temperate tree species have also shown that different temperate species are all able to access shallow soil water (e.g., Bello et al., 2019; Brinkmann et al., 2019). Water uptake from shallow soil layers across species is also in agreement with literature data showing the highest density of fine roots in temperate forests to be found in top soil layers between ~0.1 and ~0.3 m (Bello et al., 2019; Canadell et al., 1996; Jackson et al., 1996; Meißner et al., 2012; Schenk & Jackson, 2002; Yeste et al., 2021). Considering the concentration of nutrients that are cycling in the upper soil layers (Jobbágy & Jackson, 2001), it is indicative that trees, even though they have deep roots, are yet present with their roots also in the shallow soil in order to compete in these soil layers for nutrients and water (Bello et al., 2019; Kreuzwieser & Gessler, 2010; Yeste et al., 2021).

We observed distinct differences in the absolute δ2H values that were reached in the different species throughout the experiment (Figure 5). This finding can be explained by species specific soil water uptake patterns along the soil profile (Figure 6). Although we found in our analysis that all species were able to take up water from the shallow soil, the fraction of soil water taken up from the different soil depths differed significantly among species (Figure 7): P. abies took up water to a larger extent from shallow than from deep soil layers, while Q. petraea followed the opposite strategy and took up water to a larger extent from deep than from shallow soil layers. P. sylvestris and F. sylvatica also took up water to a larger extent from deep than from shallow soil layers but less so than Q. petraea (Figures 6 and 7). The species specific patterns for soil water uptake depths we found are well in line with previous observations. F. sylvatica was shown to take up water from a depth of ~0.3 to ~0.5 m (Meißner et al., 2012), whereas P. abies was found to have more shallowly distributed fine roots in a mixed stand (Bolte & Villanueva, 2006). For P. sylvestris a soil water uptake depth of ~0.08 to ~0.17 cm was shown (Bishop & Dambrine, 1995). The ability of Q. petraea to access deeper soil layers to meet its water demand agrees with studies that have determined a maximum rooting depth for Q. petraea of 2 m (Brédá et al., 1993; Hruska et al., 1999). In a recent study, Barbeta et al. (2019) have compared the soil water uptake depth of F. sylvatica and Q. robur (which is closely related to Q. petraea). They found that F. sylvatica and Q. robur both used a mix of shallow and deep soil water over the season, with Q. robur using a larger fraction of deep soil water than F. sylvatica.
Also, in mixed stands of *P. sylvestris* and *Q. petraea*, oaks utilized water from deeper sources in the soil than the pine trees (Bello et al., 2019).

Compared to other studies that investigated tree water uptake depths using the natural abundance of $\delta^{18}$O and/or $\delta^2$H values in soil and xylem water, our study in general shows deeper uptake depths for the different species (cf. Brinkmann et al., 2019; Meißner et al., 2012). Likely this is because we applied a labelling approach, which resulted in deeper soil water isotope gradients to natural abundance studies. This allowed for a more precise and realistic assessment of soil water uptake depths, in particular at deeper soil layers. Another advantage of the dynamic labelling approach that we introduce here is that the appearance and decline of the label can be observed in trees over time, which provides additional information. For example, we found that the label, once it was washed into deeper soil layers, was still detectable in significant amounts in *Q. petraea* but almost disappeared from the other three species towards the end of the experiment (Figure 5). In agreement with our other assessments the dynamic labelling approach can thus demonstrate that *Q. petraea* can access water from soil depths exceeding $-$0.8 m where soil water $\delta^2$H values were highest at the end of our investigations and from where soil water is less accessible for the other three species.

The clear differences in the ability of co-occurring temperate tree species to access water from different soil layers that we show in our study have implications for the wider field of plant ecophysiology and ecohydrology. The ability of trees to access deep soil layers is a key trait believed to enhance the drought resistance of a tree (Körner, 2019; Nardini et al., 2015). In fact, it has been shown in several investigations that the same tree species that were investigated here differ in their sensitivity to drought, with *P. abies* being most sensitive, *Q. petraea* most resistant and *P. sylvestris* and *F. sylvatica* in between (e.g., Backes & Leuschner, 2000; Brinkmann et al., 2016; Leuzinger et al., 2005; Scherrer et al., 2011; Zweifel et al., 2009). Interestingly, the different drought sensitivities of these species reported in the literature correspond well to their ability to access deeper water sources as shown in our experiment (Figures 6 and 7). At a larger scale, our study helps to constrain the origin of water that is transpired by trees to the atmosphere and has thus implications for understanding vegetation-atmosphere vapour fluxes at the ecosystem and global scale (Dawson et al., 2020; Evaristo et al., 2015; Javou et al., 2013; Werner & Dubbert, 2016).

### 4.3 Vertical distribution and retention of the labelling signal in trees

As indicated by the vertical transport of the label in tree stems, our findings indicate for a faster sap flow velocity in *Q. petraea* compared to the other species, which is in line with higher reported sap flow velocity for ring-porous species as *Q. petraea* compared to diffuse-porous (*F. sylvatica*) and coniferous species (*P. abies*, *P. sylvestris*) (Larcher, 2003). Water $\delta^2$H values at crown level 81 days after labelling differed in all species from pre-labelling conditions, whereas at stem base, this was only the case for *Q. petraea* and *P. abies*. These findings either suggest an enrichment in deuterium of xylem water during the transport towards the upper canopy or a retention of labelled water in the wood as shown by James et al. (2003) for tropical trees. We therefore expect the crown water $\delta^2$H value to be elevated compared to values at stem base due to a slow water transport and retention and storage dynamics of water in the elastic tissue of tree bark (i.e., in cambium, parenchymal and phloem; Zweifel et al., 2000). The presence of the labelling pulse in water extracted from the upper canopy almost 3 months after it was applied to the forest ground was remarkable, and this time lag will have implications on tree ring studies through the isotopic imprint of water into formed woody tissue (Gessler et al., 2014).

### 4.4 Implications for the ‘two water worlds’ discussion

We found a rapid appearance of the $^2$H label in the xylem at the stem base of all tree species. Several studies have suggested a separation of mobile and non-mobile soil water pools in the soil matrix for a wide array of ecosystems, with trees taking up water from the tightly bound water pool rather than from mobile water infiltrating the soil and recharging groundwater (Brooks et al., 2010; Dubbert et al., 2019; Evaristo et al., 2015; Goldsmith et al., 2012). These findings resulted in the ‘two water worlds hypothesis’ (Brooks et al., 2010) and reviewed in (Berry et al., 2017). However, recent studies challenge this hypothesis suggesting fractionation processes in water infiltrating the soil (Oshun et al., 2016), an exchange of bound water with the mobile water phase (Newberry, Nelson, et al., 2017; Vargas et al., 2017), or even no distinct isotopic patterns in mobile and stationary phases (McCutcheon et al., 2017). Our experiment clearly supports the concept of exchange processes between bound and mobile water pools in the soil and shows in particular that the mobile water phase, which carries the $^2$H label, is readily accessible to trees.

### 5 Conclusions

Our experiment revealed a fast infiltration of a dynamically applied $^2$H pulse label into the soil of a temperate forest ecosystem. We found that all tree species have the ability to utilize shallow soil water. Nevertheless, the investigated tree species differed distinctly in their ability to access deeper soil water with *P. abies* relying mostly on shallow, *P. sylvestris* and *F. sylvatica* on intermediate and *Q. petraea* mostly on deep soil layers for water uptake. Interestingly, the ability to access deep soil water corresponds with the drought resistance of the tree species reported in the literature. In all tree species, the labelling signal in the xylem water at crown level was shown to persist surprisingly long, suggesting a substantial storage and exchange of transported water with tree tissues along the xylem. In summary, our study demonstrated that using a dynamic $^2$H-labelled irrigation pulse that is allowed to infiltrate into the vertical soil column and is chased through...
the hydraulic system of a temperate forest over time is a useful approach (1) to assess how water from a precipitation event infiltrates into the ecosystem and (2) to reliably assess species-specific water uptake strategies among co-occurring tree species.

**DATA AVAILABILITY STATEMENT**

The data from this study are available on request from the authors.

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