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Variability in tree water uptake determined with stable water isotopes in an African tropical montane forest

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Abstract

Ecohydrological processes in tropical rainforests are insufficiently understood, and existing studies yield contradictory results. We investigated relative contributions of different soil depths to tree water uptake of 83 trees and possible species-specific differences in a 50 × 50 m forest plot at four dates in a tropical montane forest in Kenya using stable water isotopes and the Bayesian mixing model framework MixSIAR. We found distinct individual tree differences (e.g. *Drypetes gerrardii* taking 75% of its water from <0.5 m, or a rather large shift in uptake patterns based on the climatic conditions, that is the fourth sampling date), but no consistent species-specific or small-scale spatiotemporal patterns in water uptake and depth contributions. Soil water $\delta^{18}O$ showed a lateral variation of up to 6‰, which was accounted for by a spatial interpolation of soil water isotopes and enabled us to improve allocations of water uptake sources to individual trees. Our results show that ignoring the lateral variability of water isotope signatures in soils complicates the applicability of a mixing model in this context and might be a widespread constraint reducing the validity and comparability of mixing model results. Further research on underlying processes of water fluxes in forest ecosystems is urgently needed and we point out the need for considering large individual differences in water uptake patterns and small-scale variability of soil water isotopic composition despite homogeneous soil characteristics.

KEYWORDS
Bayesian mixing model, deuterium, hydrogen isotopes, montane forest, oxygen isotopes, stable water isotopes, tree water uptake

1 | INTRODUCTION

Tropical forests are known hotspots for biodiversity and endemic species and are therefore of high conservation value (Brooks et al., 2006; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000). However, climate change and anthropogenic disturbance severely threaten remaining forest habitats in the tropics (Foster, 2001; Lewis, Malhi, & Phillips, 2004). Since water plays an important role in the functioning of forest ecosystems, changing temperatures and precipitation regimes can alter species interactions, compositions, and distributions (Clark et al., 2016; Corlett, 2011; Hughes, 2000). For the conservation of these forest ecosystems and their services, it is crucial to better understand the underlying ecohydrological processes and improve our knowledge to be able to

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counteract negative consequences of anthropogenic activities (Bruijnzeel, Mulligan, & Scatena, 2011; Wright et al., 2018). In this study, we will explore the spatiotemporal patterns in water uptake depth by different tree species in a montane forest in Western Kenya. This will be useful to understand why certain species outperform other species under specific conditions, and which species will be particularly vulnerable to change in the climate regime. Such knowledge is specifically missing for data scarce regions like the African tropics (de Wispelaere et al., 2017; Wright et al., 2018).

Stable water isotopes ($\delta^{18}O$, $\delta^{2}H$) have proven themselves to be useful natural tracers to study the relevant ecohydrological processes to fill this knowledge gap. They have been widely used to study ecohydrological processes such as water fluxes at different scales and interactions between plant water use and environmental variables (Asbjornsen et al., 2011; Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002). The stable isotope composition of water transported by the plant xylem reflects the isotopic mixture of the sources of the water taken up through the roots, assuming that no fractionation (i.e. discrimination of a certain isotope) occurs during water uptake (Ehleringer & Dawson, 1992; Ellsworth & Williams, 2007; Lin & Sternberg, 1993; Zhao et al., 2016). Temporal and spatial tree water use patterns can be traced through the ecosystem using these principles (Dawson & Ehleringer, 1998).

Methodological approaches to study tree water use with stable isotope tracers commonly include mixing models to determine water contributions from distinct sources on the basis of isotopic variations in time and space (Evaristo, McDonnell, & Clemens, 2017). The principle to infer source contributions from a comparison between isotopic mixtures in sources and consumers was first applied in food web studies (Haines, 1976). Later, increasingly more advanced mixing models were developed and used in various research fields (Parnell et al., 2013; Phillips & Gregg, 2003). These mixing models present, however, challenges and limitations to partition sources of water uptake (Brett, 2014; Penna et al., 2018; Phillips et al., 2014). These limitations include, for example, the extent of the spatial variation in the soil water isotope signatures (Goldsmith et al., 2018) and the occurrence of xylem water deuterium depletion (Barbeta et al., 2019). Thus, prior to the application of a mixing model in this context, it has to be carefully evaluated, how the model could or should be applied given the specific research questions and site conditions at hand.

The current literature on water uptake depth in tropical climates, as for other regions, has yielded contradictory results. For instance, studies in China and Panama, identified species-specific soil water uptake depth (Liu, Liu, Li, Duan, & Li, 2010; Meinzer et al., 1999), whereas Goldsmith et al. (2012) found that the eight investigated tree species used mostly shallow soil water in both wet and dry conditions in Puerto Rico. Jackson, Cavelier, Goldstein, Meinzer, and Holbrook (1995) showed that evergreen species in a lowland tropical forest in Panama took more water from deeper soil layers than deciduous trees. In some studies, plant traits such as tree height or stem diameter have been found to be correlated with shallower soil water uptake (Meinzer et al., 1999) whereas in other studies these traits explained very little of the variability in water uptake across soil depth (Jackson et al., 1999; Stahl et al., 2013). Others found seasonal variations in water uptake depth, where trees shift their dominant water source to deeper soil layers during drought, although inconsistent patterns are reported as well (Fritzsche et al., 2006; Romero-Saltos, Sternberg, Moreira, & Nepstad, 2005; Schwendenmann, Pendall, Sanchez-Bragado, Kunert, & Hölscher, 2015; Yang, Wen, & Sun, 2015). Presumably, these contradictory findings result from various complex environmental and tree individual specific factors that influence tree water uptake, or from methodological shortcomings. Further clarification of water fluxes in the soil–plant interface is urgently needed.

Hence, the primary objective of this study was to determine spatiotemporal water uptake patterns of all trees in a study plot in a tropical montane forest in Kenya over several months and to identify possible species-specific differences. To do so, we collected throughfall and soil water samples at five locations within the study plot as well as xylem water samples from 83 trees, and analysed their stable water isotope signature (^{2}H and ^{18}O). We interpolated the measured values of the source water (i.e. soil water at different depths) to better represent the small-scale spatial variability and subsequently applied a Bayesian mixing model (BMM) for each tree to infer the contribution of different depths to relative water uptake.

2 | MATERIALS AND METHODS

2.1 | Study area and site description

The study site is located in the South-West Mau region of the Mau Forest Complex in Western Kenya (Figure 1). The Mau Forest is the largest indigenous closed-canopy forest in East Africa with an altitude range between 2000 and 3098 m (Krhoda, 1988; Mutugi & Kiiru, 2015; Olang & Kundu, 2011). Forests in this area are classified as diverse Afromontane moist forests (Beentje, 1994; Kinjanjui, Karachi, & Ondimu, 2013; Kinyanjui, 2011; White, 1983). The western forest edge of the southwest block of the Mau was chosen as study area (35°18′36″E, 0°27′50″S) (Figure 1b) due to relatively low disturbance by human activities (Brandt et al., 2018). The selected forest plot lies close to the mountain ridge at 2000 m elevation, with a slight slope of approximately 4–6°. The soils are deep humic Nitisols with a total clay and silt content of over 82% as well as a high water storage capacity (Krhoda, 1988; Olang & Fürst, 2011; Owuor et al., 2017).

The area is characterized by marked seasonality with long rains between April and July and short rains between September and December (Olang & Fürst, 2011). A dry season typically occurs in the first months of the year with less than 75 mm per month (Jacobs, Breuer, Butterbach-Bahl, Pelster, & Rufino, 2017). The average annual precipitation in the period between 1905 and 2014 was 1988 ± 328 mm (Jacobs et al., 2017). Locally, the temperature varies with altitude (Krhoda, 1988) but has little variation throughout the year (Ekirapa & Shitakha, 1996). Between 1990 and 1996 at 2134 m elevation the mean annual temperature was 15.7°C with average daily minima and maxima of 9.1°C and 22.2°C, respectively (Ekirapa & Shitakha, 1996).

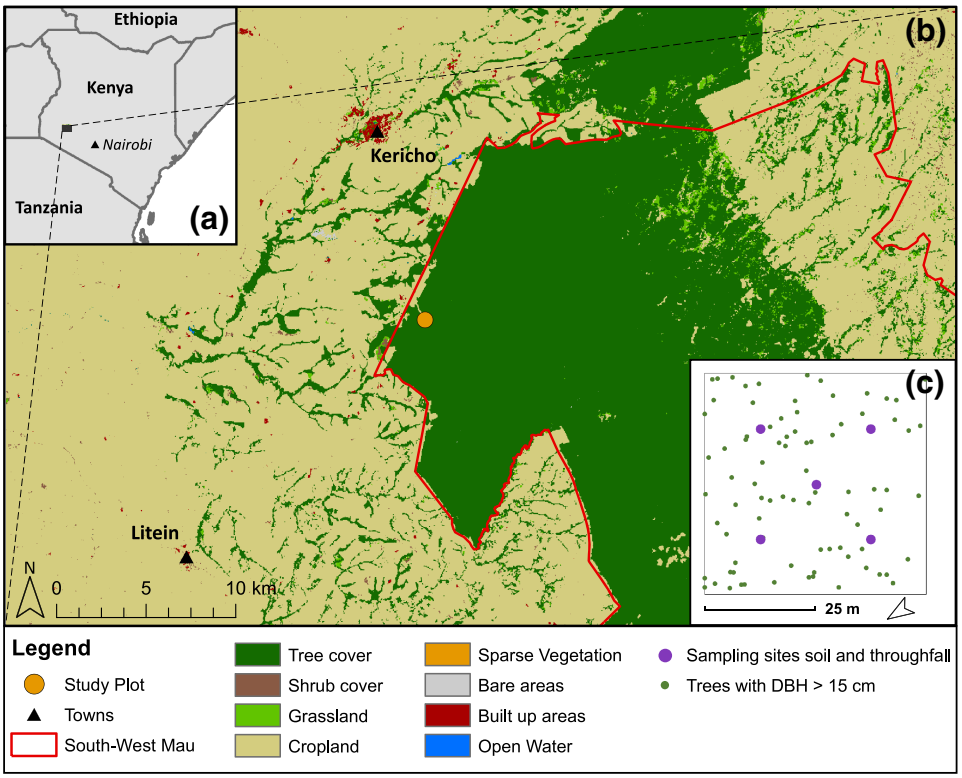

FIGURE 1 Location of the study area in Kenya (a) at the western edge of the southwest Mau Forest (b). Inset (c) shows a sketch of the study plot with locations of the studied trees (DBH = diameter at breast height) and the five soil and throughfall sampling sites. Land cover data comes from RCMRD GeoPortal (2016), administrative boundaries from GADM (2018)

2.2 | Experimental setup and sampling

During the study period from 11 September to 13 December 2017, we took samples from tree stems, soil, and throughfall for stable isotope analysis on a 50 by 50 m forest plot parallel to the hillslope. We sampled stems and soil on 20–21 September, 4 and 18 October, and 13 December. The last date in December at the beginning of the drier season was included to cover possible divergent seasonal patterns. To identify spatial patterns in the isotopic signature of the source water, throughfall samples were collected twice a week at five locations across the plot (see Figure 1c). The installed throughfall collectors were made of 1 L plastic bottles with a circular funnel of 12.1 cm diameter. To prevent evaporative fractionation of the collected water, a table tennis ball was placed into the funnel (Windhorst, Waltz, Timbe, Frede, & Breuer, 2013) and the samplers were isolated with aluminium foil. Throughfall samples for stable isotope analysis were filtered (0.45 µm KX syringe filter, Kinesis Ltd., St. Neods, UK) and stored in airtight 2 mL brown glass vials.

On the four sampling dates, we took soil samples with a soil auger from five different depths (0–0.10, 0.10–0.25, 0.25–0.50, 0.5–1.0, and 1,0–1.5 m) in proximity to each of the five throughfall collectors (n = 25). From each depth, 200–300 g soil were collected and stored in a zip lock aluminum bag (WEBAbag standing bag with zip, 165 × 292 × 102 mm, Weber packaging GmbH, Germany). The bags were evacuated, sealed, and frozen the same day.

On the same sampling dates, xylem samples were collected from all mature trees in the plot with a diameter at breast height (DBH) ≥15 cm (Figure 1c) comprising in total 83 trees of seven different species: *Drypetes gerrardii* Hutch (n = 34), *Neoboutonia macrocalyx* Pax (n = 1), *Pouteria altissima* (A. Chev.) Baehni (n = 12), *Syzygium guineense* (Willd.) DC. (n = 7), *Tabernaemontana stapfiana* Britten (n = 15), *Xymalos monospora* (Harv.) Baill. (n = 7), *Zanthoxylum gilletii* (De Wild.) P.G.Waterman (n = 1), and six unidentified individuals. Tree species were identified using available literature (Beentje, 1994; Dalitz, Dalitz, Musila, & Masinde, 2011; Noad & Birnie, 1994) and with the help of local forest rangers. We took xylem samples from the stem with increment borers (Haglöf 250 mm, two-threaded, 5.15-mm core diameter) approximately at breast height (following Goldsmith et al. (2012), Hervé-Fernández et al. (2016), Muñoz-Villers, Holwerda, Alvarado-Barrientos, Geissert, and Dawson (2018)) and avoided the stem centre. We removed the bark tissue from the sample before storing them in the same manner as the soil samples.

Additionally, we measured volumetric soil moisture content and soil temperature every 15 min with sensors (Decagon Devices GS3 sensors, METER Group, Inc. USA) installed in the middle of the plot at five soils depths (0.05, 0.175, 0.375, 0.75, and 1.25 m) representing the soil sampling depths. Outside the forest (~160 m from the study plot) precipitation was measured with a tipping bucket rain gauge (Theodor Friedrichs, Schenefeld, Germany), which recorded cumulative precipitation (resolution of 0.2 mm per tip) at 10-min intervals. Weekly samples from a precipitation collector (similar to the throughfall collectors) outside the forest were used to determine the isotopic composition of precipitation. The data that support the findings of this study are openly available in Zenodo.org at DOI: 10.5281/zenodo.4409137 (Windhorst, Hahn, & Breuer, 2020).

2.3 | Water extraction and isotope analysis

The water from the stem and soil samples was extracted using cryogenic vacuum distillation as described in Orlowski, Frede, Brüggemann, and Breuer (2013). Soil samples were submerged in heated sand (205°C) whereas xylem water samples were extracted from the tree cores in a hot water bath (90°C), each for 3 h at 0.3 Pa. To check for complete water extraction, all samples were weighed after extraction, then oven dried at 105°C for 48 h and reweighed afterward (Orlowski et al., 2013). The extracted water samples were stored in airtight 2-mL brown glass vials until analysis.

Soil, tree, and throughfall water samples were analysed for $\delta^{18}O$ and $\delta^{2}H$ isotope ratios with cavity ring-down spectroscopy (Picarro L2120-i, CA, US for throughfall; Picarro L2130-i including the microcombustion module, CA, USA, the laboratory at Justus Liebig University Giessen, Germany. In addition, the Picarro software ChemCorrect was used to identify and quantify any remaining organic contamination and therefore further minimize the likelihood of optical interferences. Isotope ratios are expressed as follows:

$$\delta^{18}O \text{ or } \delta^{2}H = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000‰$$

where R is the ratio of the heavy to the light isotope ($^{18}O/^{16}O$ or $^{2}H/H$) of the sample in relation to a standard (i.e. Vienna Standard Mean Ocean Water [VSMOW], Coplen, 1995) (Gat, 2010; McGuire & McDonnell, 2007). The precision of the measurements is given as 0.025‰ $\delta^{18}O$ (0.1‰ δD) for Picarro L2130-i and 0.1‰ $\delta^{18}O$ (0.5‰ δD) for Picarro L2120-i.

2.4 | Data analysis

The source contribution of the five different soil depths to tree water uptake was estimated using a BMM, for which certain prerequisites needed to be addressed (Phillips et al., 2014). Soil water was considered the only relevant water source for the mixing model, since river and groundwater were not accessible by the trees in the study plot due to the limited rooting depth and deep ground water level (Blackie, 1972; Edwards, Blackie, & Eeles, 1976). Despite the homogeneous soil characteristics (i.e. similar soil textures as identified by the finger probe method described in DIN ISO 19682-2:2007-11 (2007) for all five soil pits), we observed considerable variability of soil water isotope signatures across the plot. The importance of incorporating true small-scale spatial variability of soil water isotopes as input data has recently been pointed out by Goldsmith et al. (2018), who reported high sensitivity of two end-member mixing model results to the soil water sampling location in a 1 ha temperate forest plot. To identify the likely source water signatures at each monitored soil layer at the position of each tree, we used an inverse distance weighting (IDW) interpolation of the soil $\delta^{18}O$ and $\delta^{2}H$ values for the trees within the bounds of the five sampling points and extrapolated the closest measured values to those trees outside the bounds. The source water used in the BMM is therefore represented by the interpolated/extrapolated isotopic source data at the location/spatial coordinates of any given tree. This also has the advantage that we indirectly account for the mostly unknown lateral extent of the root systems of the trees, as the IDW interpolation will, for the most part, represent a mean over the surrounding area at any given point.

A mixing model further requires distinguishable isotopic signatures of the sources (Gannes, del Rio, & Koch, 1998). The interpolated isotope values of most soil layers (70–100% of possible combinations per sampling date) showed significant ($P \leq 0.05$, Kruskal–Wallis and Mann–Whitney–U Test) and thus distinguishable differences between layers so that no further aggregation of sources was performed.

Another requirement for the applicability of mixing models is that the isotopic signatures of the consumer (tree) lie within the mixing space of the sources (soil layers) (Phillips et al., 2014). However, the dual isotope space plot (Figure 3) suggested that the xylem $\delta^{2}H$ isotopic values were frequently more depleted than any of the expected water sources. This behaviour has frequently been observed in other studies, but underlying reasons are yet to be resolved (Barbeta et al., 2019; Bertrand et al., 2014; Bowling, Schulze, & Hall, 2017; Brooks, Barnard, Coulombe, & McDonnell, 2010; Evaristo, McDonnell, Scholl, Bruijnzeel, & Chun, 2016; Goldsmith et al., 2012; Muñoz-Villers et al., 2018; Sohel, 2018). Possible explanations comprise hydrogen fractionation during root water uptake or within woody tree tissues (Barbeta et al., 2019; Vargas, Schaffer, Yuhong, & Sternberg, 2017; Zhao et al., 2016), or the concept of ecohydrological separation (i.e. trees use a less mobile pool of older precipitation water) (Bowling et al., 2017; Brooks et al., 2010; Goldsmith et al., 2012; Oerter & Bowen, 2017). In our case, only 10 trees (3%) matched their respective soil water mixing space based on original tree water isotopic values. To address this issue we applied the soil water correction method developed by Barbeta et al. (2019). Following Barbeta et al. (2019) we computed the soil water excess (SW excess) for each tree based on its individual interpolated soil water regression line and corrected the xylem deuterium values by subtracting the respective SW-excess:

$$SW - excess = \delta^{2}H - a_s * \delta^{18}O - b_s$$

where a_s and b_s are the slope and intercept of the soil water line for a given plot and date (see Figure 3), respectively, and $\delta^{2}H$ and $\delta^{18}O$ correspond to the isotopic composition of a xylem water sample collected on that plot at that date (Barbeta et al., 2019).

After the correction, only those trees with corrected deuterium values within the mixing space (convex hull polygon) were considered for further analysis. This applied to 55, 35, 49, and 56 out of 83 trees on the four sampling dates, respectively (see Figure S2). It has to be noted that with the correction also 17.8% of 332 tree samples with positive SW excess (indicating more enriched xylem deuterium values compared to soil) might have received an 'overcorrection', implying a slightly varying (i.e. not only distinctly depleted) extent of $\delta^{2}H$ offset caused by unknown influencing factors. As suggested by Barbeta et al. (2019), special attention needs to be paid in such cases to the reported values. However, our $\delta^{2}H$ offset revealed no systematic

pattern and Barbeta et al. (2019) illustrate for their data that their method produces plausible correlations with environmental variables based on SW-corrected δ^2H values. Even though we achieved a considerable improvement of the on-site representation of the xylem and source water signature for each individual tree (from 3% to 59% of trees falling in the mixing space), the remaining mismatches indicate, that the spatial allocation and the SW correction method still do not capture the full spectrum of processes shaping the actual isotopic signature in the soil and during soil water uptake by the trees. While our current hydrological understanding of the flow processes at the study site (based, e.g. on Jacobs et al., 2018) does not support the presence of any additional water source, it cannot be completely ruled out that a missing water source or small-scale hydrological processes (e.g. due to preferential flow or microtopography), might cause an additional bias.

We applied the latest BMM framework MixSIAR 3.1.10 in R version 3.5.2 (R Core Team, 2018; Stock et al., 2018). The MixSIAR package implemented previous advances in BMM approaches and estimates the proportions of source contributions (soil water depths) to a consumer mixture (tree water) using Markov Chain Monte Carlo (MCMC) methods (Stock & Semmens, 2016). Corrected xylem deuterium values and the measured $\delta^{18}O$ values for each tree on each date were used as consumer input data for the MixSIAR mixing model. The corresponding interpolated soil water values were used as raw source input data. Discrimination factors were set to zero, because no isotopic fractionation during plant water uptake is assumed (Brunel, Walker, & Kennett-Smith, 1995; Dawson & Ehleringer, 1991). In addition, we assessed the possible effect of isotope fractionation during sample preparation by comparing the BMM results for either oxygen or hydrogen, and both isotopes. As both isotopes individually yielded visually very similar results (results not shown), a significant impact of fractionation during sample preparation is unlikely. The model was run separately for each tree data set using only the implemented process error option, and assuming a uniform ('flat') prior distribution (all Dirichlet parameters set to 1). As informative priors, representing, for example the root distribution (see Jackson et al., 1996), tend to mask/overrule possible information in small datasets and therefore bias the outcome, we decided to use a non-informative/uniform prior. In case the root water uptake is well identified by the collected data the choice of prior distributions will have minor effects on posterior inference. The MCMC parameters were set to chain length = 500,000; burn-in = 200,000 (discarded initial iterations); thinning rate = 100 (select only every 100th value to reduce autocorrelation); number of chains = 3. Convergence of the MCMC simulations was confirmed by checking the Gelman and Rubin (Gelman & Rubin, 1992), Geweke (Geweke, 1992), and Heidelberger and Welch (Heidelberger & Welch, 1983) diagnostic tests (Stock & Semmens, 2016).

3 | RESULTS

3.1 | Environmental conditions

Preceding rainfall amounts were highest for the first sampling campaign in September 2017 and lowest before the last sampling

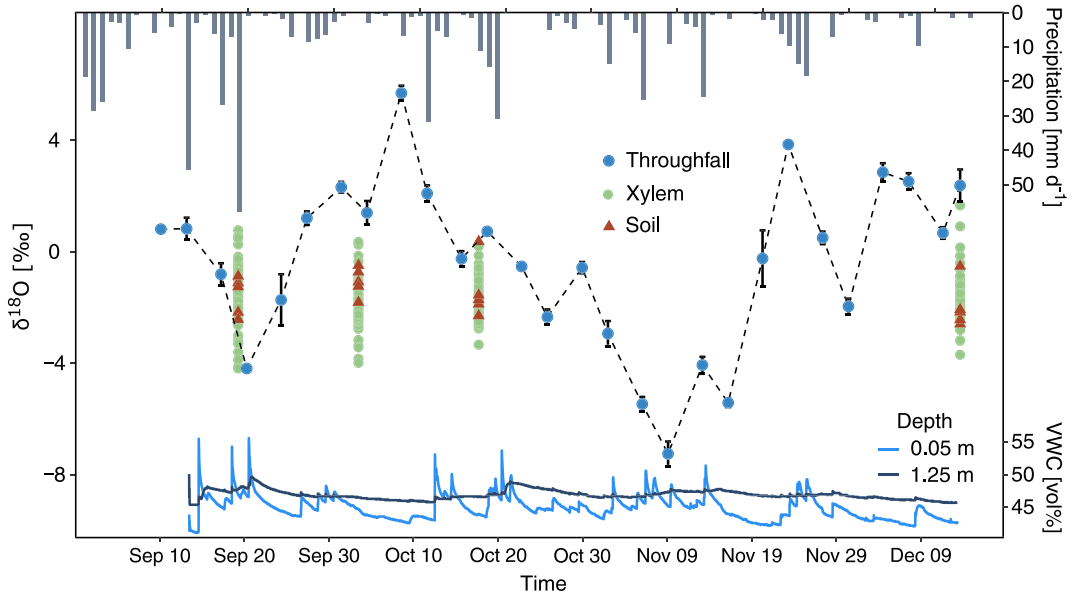

FIGURE 2 Daily precipitation amount, soil volumetric water content (VWC), and $\delta^{18}O$ values of throughfall, soil, and tree xylem between September 2017 and December 2017. October 21 to 26 Oct and November 7 to 9 contain missing values for precipitation amount. Mean throughfall $\delta^{18}O$ values were calculated from five sampling points (error bars representing ±1 SD) in a 0.25 ha tropical montane forest plot. The tree xylem water $\delta^{18}O$ values of 83 trees (at least seven different species) in the plot and mean soil water $\delta^{18}O$ values of five sampling points for five soil depths on the four sampling campaigns are shown

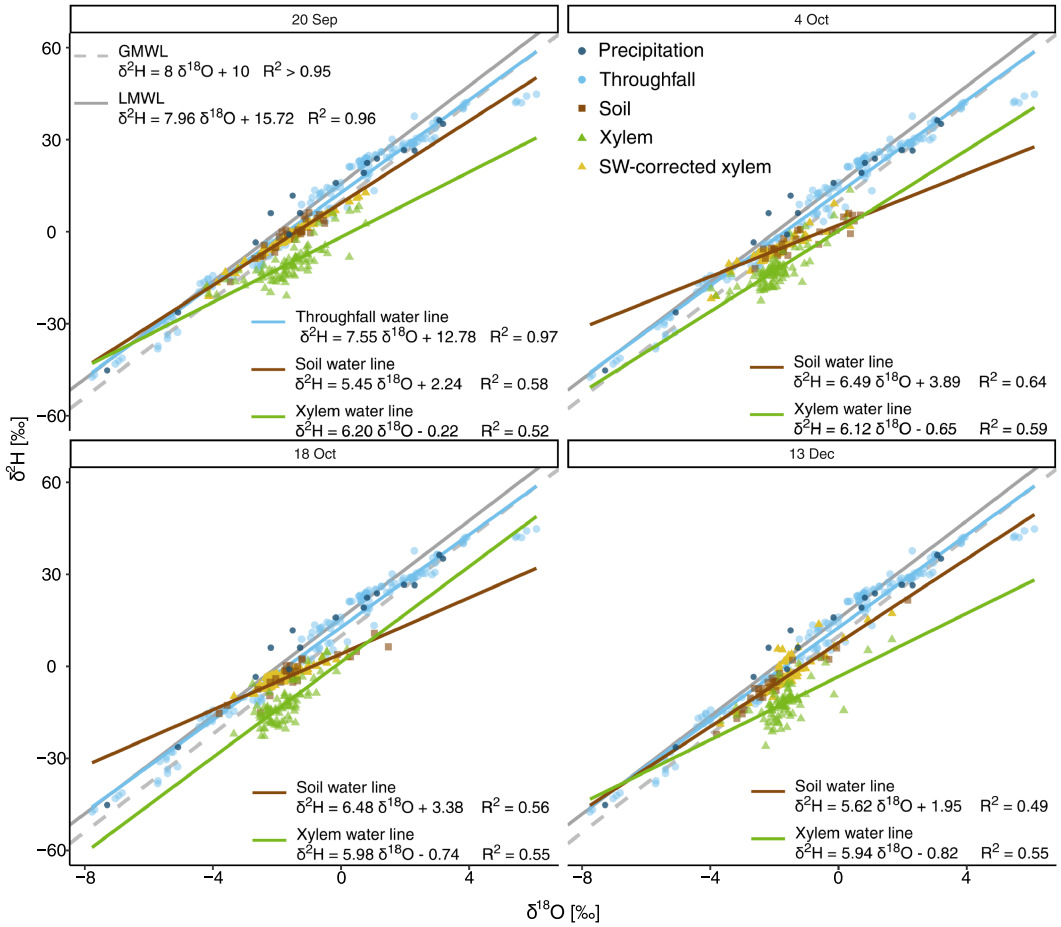

FIGURE 3 Dual isotope (δ^2H and $\delta^{18}O$) plot for four sampling dates between September and December 2017 of precipitation (15 samples during whole study period shown in every panel) throughfall, soil, xylem, and SW-corrected xylem water (see text Section 2.4) in a 0.25 ha tropical montane forest plot. The global meteoric water line (GMWL) follows Craig (1961) and the local meteoric water line (LMWL) is based on weekly precipitation measurements between October 2015 and March 2018 (Jacobs et al., 2018)

campaign in December 2017 (Figure 2). The variations in the soil moisture in the upper three soil layers (0.05, 0.175, and 0.375 m) generally followed the incoming rainfall amounts with a decreasing amplitude in deeper layers (Figure 2, for clarity reasons only values recorded at 0.05 and 1.25 m are shown). The two deepest layers (0.75 and 1.25 m) only showed minor responses to rainfall events. The mean soil moisture (±SD) was highest in the two deepest layers (0.75 and 1.25 m) averaging 46.9 ± 0.7 and 46.9 ± 1.2 vol%, followed by the upmost layer at 0.05 m (45.0 ± 1.9 vol%) with a wide range from 34.9 to 55.6 vol%. The 0.175 and 0.375 m layers had the lowest soil moisture averaging 37.7 ± 1.6 and 36.3 ± 1.5 vol%, respectively. Approaching the onset of the dry season, the variation in and values of soil moisture showed a decreasing trend toward the end of the study period. The mean soil moisture content over all soil layers at the four sampling events before and during xylem sampling procedure (between 00:00 and 13:00 h on each sampling day) was 44.6 ± 4.5, 41.9 ± 4.2, 42.2 ± 4.4, and 40.8 ± 4.8 vol%, respectively. Soil temperature was highest and fairly constant in the deepest soil layer with 15.7 ± 0.1°C. Layers closer to the surface had lower mean temperatures, but increasing variation ranging from 12.5°C to 16.6°C in the upmost layer.

3.2 | Relationships and spatiotemporal variability of water isotope signatures.

The throughfall stable isotope signature was highly variable during the study period (Figure 2) ranging from −7.77‰ to 6.10‰ for $\delta^{18}O$ and −47.36‰ to 44.86‰ for δ^2H. In contrast, the spatial variability within the plot at each sampling day was relatively small averaging 0.77 ± 0.55‰ $\delta^{18}O$ (4.58 ± 3.52‰ δ^2H). Throughfall and precipitation isotope signatures were often similar with a mean difference between precipitation measurements (weekly) and throughfall measurements (mean of the two samplings within the same week) of 0.7‰ $\delta^{18}O$ and 5.0‰ δ^2H, which did not show a trend or pattern in the deviation extent. The maximum deviations between rainfall and throughfall isotope signature per plot were 2.2‰ $\delta^{18}O$ and 18.7‰ δ^2H. The mean soil water isotope signatures for each depth were generally slightly more depleted than the mean throughfall values over the time period and comprised isotopic values between −3.82‰ and 2.15‰ $\delta^{18}O$ and −22.14‰ and 21.64‰ δ^2H. The soil water signatures generally deviated from the preceding throughfall samples. Tree water isotopic signatures were between −4.19‰ and 1.66‰ $\delta^{18}O$ and −25.94‰ and 13.65‰ δ^2H. They also ranged slightly below the mean

throughfall values but rarely overlapped with the preceding throughfall measurements. Overall, temporal patterns for $\delta^{18}O$ and $\delta^{2}H$ were similar.

The isotopic signatures of the throughfall samples were close to the global meteoric water line (GMWL (Craig, 1961) and the local meteoric water line (LMWL) in the dual isotope space (Figure 3). The LMWL, based on weekly precipitation measurements between October 2015 and March 2018 (Jacobs et al., 2018), showed a higher intercept ($P < 0.001$) and a slightly lower slope ($P = 0.664$) than the GMWL, indicating recycled and more enriched precipitation water (Gat, 2000). Precipitation and throughfall isotope signatures were generally synchronized, suggesting little evaporation during the passage through the canopy. The soil isotopic signatures roughly followed the GMWL and fell below the LMWL, with several samples from the upper soil layers enriched in heavy isotopes, indicating fractionation from evaporation processes. The xylem water signatures clustered slightly below the GMWL, showing little overlap with the soil or throughfall samples. After SW correction of the xylem water deuterium values (see Section 2.4), xylem and soil signatures were more closely correlated. Although the correction method introduced by Barbeta et al. (2019) and the spatial interpolation of the soil water signature enabled us to apply the BMM to 59% of the 332 tree samples, there were still 27–48 tree samples per date outside their mixing space. This complicated the comparability of results between the four sampling dates since only 12 trees were within their mixing space at all dates (see Figure S2 in the supporting information). Xylem isotopic composition of *Xymalos monospora* showed significantly more enriched isotopic values compared to all other species over the study period (excluding unidentified individuals and species with fewer than five individuals on the plot; $P < 0.001$ Mann–Whitney–U Test). *Drypetes gerrardii* and *Syzygium guineense* ($P = 0.18$) had most depleted xylem signatures, both differing from *Pouteria altissima* ($P < 0.001$) and *Tabernaemontana stapfiana* ($P < 0.001$). There was no correlation between DBH and xylem $\delta^{18}O$ either for all species or for the most frequently occurring species (*Drypetes gerrardii*) (Pearson's correlation $P > 0.2$).

The isotopic compositions of the five soil depths along the soil profiles behaved differently for the four sampling dates (Figure 4a). On the first sampling date, the upper three soil layers were more enriched in heavy isotopes than the lowest two layers. On the second date, all layers were less differentiated. On the third sampling date, the upmost layer showed more depleted values, and on the fourth date, the layers showed the lowest differentiation. In general, the vertical isotopic soil profiles showed similar and mostly constant isotopic composition of the deepest soil layers (0.75 and 1.25 m), while the upper soil layers (0.05 to 0.375 m) had overall higher variability (Figure 4a). While all soil water isotope values were in the range of incoming throughfall, the temporal course of individual throughfall events was not reflected in different sections of the soil profiles. However, overall higher lateral soil water variability on the second and third soil sampling date (Figure 4a) could have been affected by more variation in the isotopic composition of throughfall within the two weeks before the sampling on these dates (Figure 2). Contradicting our assumption of a homogeneous plot, the soil water isotope signatures showed a notable spatial variation with values ranging from −3.82‰ $\delta^{18}O$ (−22.14‰ $\delta^{2}H$) to 2.15‰ $\delta^{18}O$ (21.64‰ $\delta^{2}H$) with minimum and maximum values occurring both on the last sampling date in the first layer (Figure 4). No specific characteristics of the vegetation cover, topography, or soil characteristics could be observed to further explain the observed spatial stable water isotopes pattern. In addition, an overall trend of depletion in the deeper layers could be observed on the first three sampling dates, while the last sampling date showed a contrasting trend (Figure 4a).

3.3 | The contribution of different depth to water uptake

The interpolated soil water isotopic signatures across the plot used as input values for the mixing model are shown in Figure 4b. The magnitude of vertical variability depended on the date and the location of the soil profile in the plot, which resulted in either fairly distinct or quite similar isotopic compositions between the soil layers. The spatial pattern of soil water isotopes especially in the topsoil was highly variable between the four sampling dates. The mean relative water uptake contributions of the five soil depths (0–0.1, 0.1–0.25, 0.25–0.5, 0.5–1, and 1–1.5 m) for all species and sampling dates was quite similar averaging between 17% (second soil layer) and 23% (lowest soil layer). Contributions for the individual layers ranged between 7.5% and 40.6% for 0–0.1 m, 9.7% and 39.3% for 0.1–0.25 m, 12.5% and 28.1% for 0.25–0.5 m, 9.8% and 40.1% for 0.5–1 m, and 10.8% and 40.7% for 1–1.5 m (Figure S1). The overall patterns show no dominant water source, but rather differences between individual trees and sampling dates (e.g. up to 138% difference for the upmost soil layer on the fourth date). For example, three individuals of *Drypetes gerrardii* standing in the same quadrant took only 25% of their water from layers deeper than 0.5 m on the first sampling date. Their quadrant did not stand out in its soil water isotopic composition. In contrast, all other 22 *D. gerrardii* individual trees took up on average 52% (all >37%) from deeper layers on the same date. Even *Xymalos monospora*, a species that showed significantly more enriched xylem isotopes, did not show a distinct water uptake pattern. In addition, there was no significant correlation between DBH and water uptake depth or measured xylem isotope values.

An overall comparison between aggregated upper (0–0.10, 0.10–0.25, and 0.25–0.50 m) and lower (0.5–1.0 and 1.0–1.5 m) soil layers showed that all species took more water from the upper three soil layers (averaging 58 ± 8.7%) in comparison to the deeper layers (averaging 42 ± 8.7%) (Figure 5). The distinction between upper and deeper soil water contributions to tree water uptake was clearest on the last sampling date, where variations (SD 3.3%) in relative contributions were smallest. There were no consistent species-specific differences in water uptake depths or temporal shifts in soil layer contributions between the sampling dates. Moreover, relative contributions of the layers for trees standing in different quadrants of the plot did not differ.

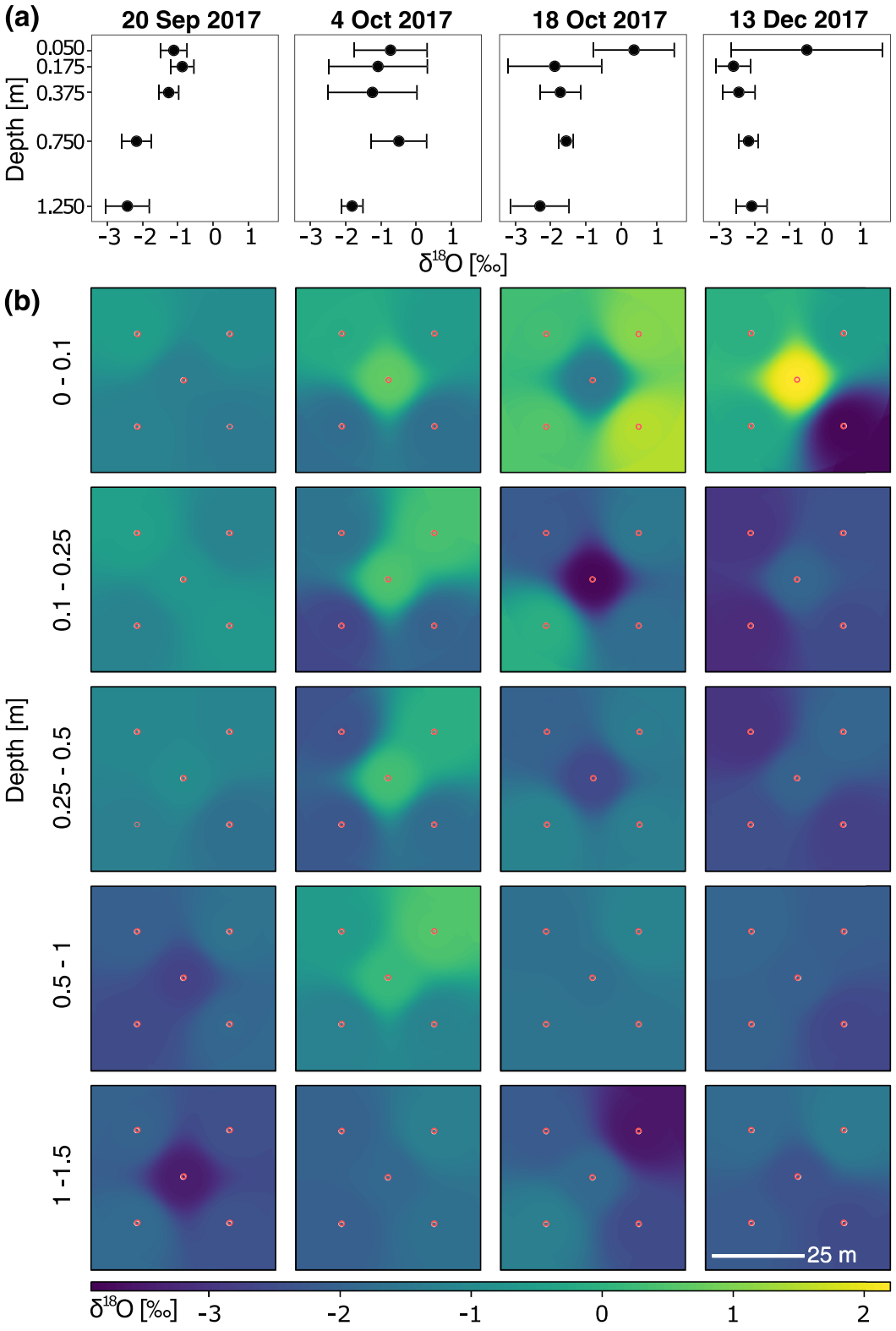

FIGURE 4 Mean $\delta^{18}O$ soil water signatures of five sampling points in five soil depths (error bars representing ±1 SD) in a 0.25 ha tropical montane forest plot at four dates (a) and interpolated soil water $\delta^{18}O$ signatures for five soil layers on four dates based on five sampling points (red circles) (b)

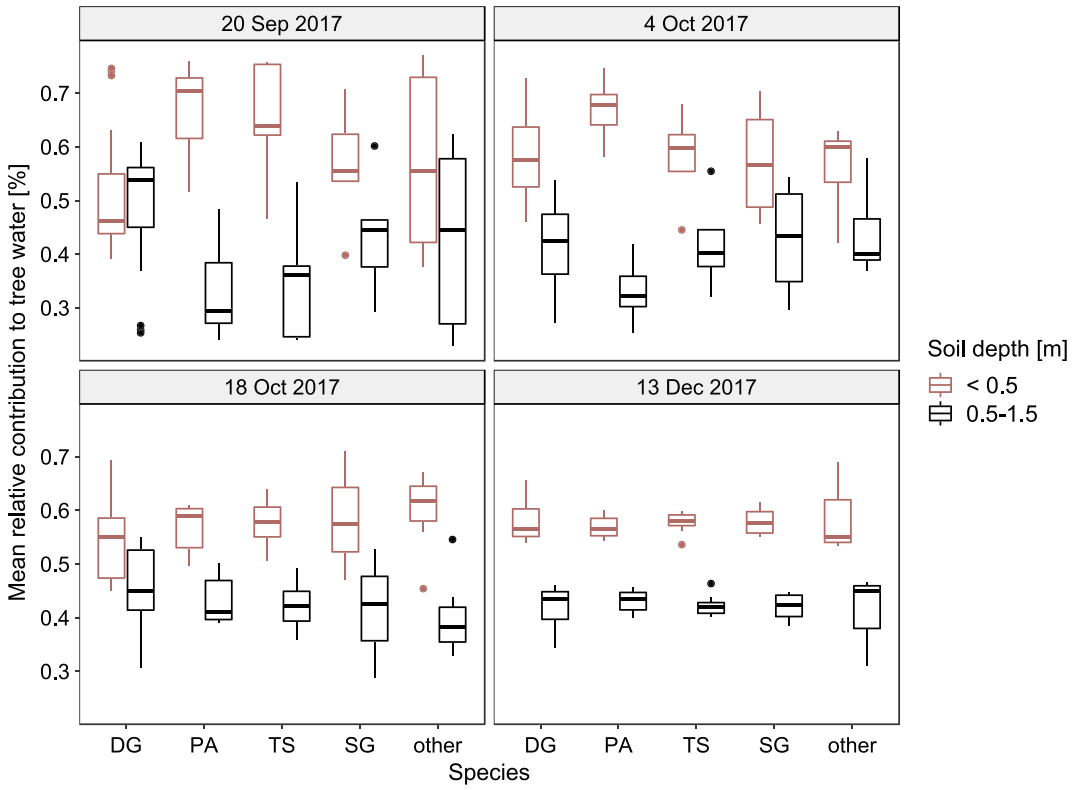

FIGURE 5 Mean contributions to tree water uptake from aggregated upper (0–0.1, 0.1–0.25, and 0.25–0.5 m) and lower (0.5–1 and 1–1.5 m) soil layers at four sampling dates. The most abundant tree species *Drypetes gerrardii* (DG), *Pouteria altissima* (PA), *Tabernaemontana stapfiana* (TS), and *Syzygium guineense* (SG) are displayed separately, all others (<3 individuals available on at least one date and not identified species) are aggregated (including *Xymalos monospora*, *Zanthoxylum gilletii*, and *Neoboutonia macrocalyx*)

4 | DISCUSSION

4.1 | Water uptake depths and species-specific differences

Based on the MixSIAR results we found different uptake proportions for individual trees, but no consistent species-specific patterns. Goldsmith et al. (2018) also report no interspecific water uptake patterns in a tropical montane cloud forest with roughly comparable climate and soils to our study area in the Mau Forest. This contradicts the clear species-specific differences in other (seasonally dry) tropical (Hasselquist, Allen, & Santiago, 2010; Liu et al., 2010; Schwendenmann et al., 2015; Sohel, 2018; Stratton, Goldstein, & Meinzer, 2000), arid (Huang & Zhang, 2015), and temperate climates (Barbeta et al., 2019; Brinkmann, Eugster, Buchmann, & Kahmen, 2019). No general spatial patterns in uptake depth between quadrants and short-term temporal patterns in preferred tree water uptake depth could be observed. This suggests that spatial soil water differences of the source water did not cause the observed tree individual-specific differences in water uptake. However, our study period did not cover the dry season when shifts or more pronounced uptake depth preference could be expected based on other findings in seasonal climates (Brinkmann et al., 2019; Meinzer et al., 1999; Romero-Saltos et al., 2005). Our results show preliminary evidence supporting this behaviour, since the variability in uptake depths was highest when the soil moisture was highest and vice versa. However, as this assessment is solely based on the available soil moisture data in the centre of the study site and the observed rainfall at five locations within the plot, further work is required to gather more robust evidence for this montane forest.

While other studies found relationships between tree characteristics, such as DBH, and xylem water compositions or water uptake depth (Gaines et al., 2016; Goldsmith et al., 2012; Stahl et al., 2013), our results did not reveal such a dependency under the given climatic conditions.

Although the modelling with BMM revealed no overall dominant water source (Figure S1), aggregated soil layers in the first 50 cm made up nearly 60% of uptake proportions. This was to be expected based on the typical root architecture (decreasing root density with depth, which was also observed in the excavated soil pit) of the trees within this forest (Jackson et al., 1996). Furthermore, most nutrients are available in the upper soil layers (see Jobbágy & Jackson, 2001; Quesada et al., 2011), and trees generally prefer shallow soil water, which is easier to access (Adiku, Rose, Braddock, & Ozier-Lafontaine, 2000; Schenk & Jackson, 2002). Due to the moist tropical climate and clayey soil texture, soil moisture (averaging 42.6 vol% over all soil layers) was presumably not limiting during the study period. Hence, the reliance on deeper soil water was not crucial for

the trees in this study, which has been reported for drier conditions (Brinkmann et al., 2019; Romero-Saltos et al., 2005; Yang et al., 2015).

Predominant shallow water use was also observed in other studies for several evergreen species in seasonally dry tropical forests on fine textured soils (Goldsmith et al., 2012; Liu et al., 2010) and in a tropical forest with sandy soils (Sohel, 2018). However, uptake of deeper soil water occurs occasionally in seasonal tropical climates as well (Liu et al., 2010 reported >60 cm; Meinzer et al., 1999 reported >100 cm; Schwendenmann et al., 2015 reported >30 cm; Stahl et al., 2013 reported 100–120 cm). To conclude, the trees in the Mau Forest seem to show a preference for shallower soil water, but at the same time certain tree individuals show a considerable contribution of water uptake from deeper layers. This has not been reported before for a tropical natural forest during the wet season.

4.2 | Spatiotemporal water isotope patterns

Similar to other studies, throughfall isotopes showed distinct temporal variability (Dewalle & Swistock, 1994; Goldsmith et al., 2012; Munksgaard, Wurster, Bass, & Bird, 2012). Both throughfall and precipitation showed a similar pattern but no consistent deviation between the two. Therefore, deviations of the isotopic signature of throughfall from precipitation and lateral variation within the plot probably result from a combination of evaporation and mixing processes in the complex canopy structure (Allen, Keim, Barnard, McDonnell, & Brooks, 2017). Despite the homogeneous soil characteristics, we expected a certain lateral isotopic variability of soil water due to the throughfall variability and other small-scale processes. For instance, tree species or vegetation cover can affect soil water isotopic composition (Schwendenmann et al., 2015; Sprenger, Tetzlaff, & Soulsby, 2017) and roots or animal burrows can lead to preferential flow paths. Compared to a lateral soil water isotopic range of 10.7‰ $\delta^{18}O$ in a 1 ha temperate forest (Goldsmith et al., 2018), our lateral range of up to 6‰ $\delta^{18}O$ was lower, but twice as high as in seemingly homogeneous soils in an Australian tropical forest (Sohel, 2018). Unlike Goldsmith et al. (2018) and Sohel (2018), who only sampled once, we sampled four times. In this way, we revealed a different extent of lateral heterogeneity depending on the date and soil depth, which frequently exceeded the vertical variability and is further discussed in the *Methodological limitations* section.

Although we observed variation in the vertical isotopic soil profiles on the four sampling dates, it did not directly reflect the isotopic composition of the previous throughfall samples. This was also reported by Goldsmith et al. (2018). Only the shallow soil water isotopic composition on dates 1 and 3 roughly coincided with previous throughfall isotopes, but the topsoil water was in general slightly or not at all enriched compared to previous throughfall samples. This is probably because the evaporative fractionation is low, owing to the moist conditions and dense vegetation cover (Sprenger, Leistert, Gimbel, & Weiler, 2016). Soil water therefore presumably consisted of a mixture of various earlier throughfall events coupled with negligible evaporation effects in the soil (Benettin et al., 2018).

4.3 | Methodological limitations

The extent of observed spatial variability of soil water isotopes and the δ^2H offset of xylem water isotopes highlighted some limitations regarding the applicability of mixing models to identify uptake depth in the way it is conventionally calculated. By sampling five soil profiles in the plot on every date, we observed a considerable lateral variability in soil water isotopes up to 6‰ $\delta^{18}O$ depending on layer and date (Figure 4). To date, the small-scale variability of soil water isotopes has not been sufficiently accounted for in mixing model applications (Goldsmith et al., 2018), and we strongly advice to (a) verify if the assumption of a low spatial variability holds, or (b) to thoroughly asses the spatial variability in advance. Even though the lateral interpolation of soil water isotopic signatures enabled us to incorporate this small-scale variability into the BMM, the possibilities within the selected sampling design to verify the selected procedure are limited. These limitations mostly resulted from the a priori assumption of a mostly homogenous plant cover and underlying soil conditions within the study plot. Given the a posteriori information of the observed spatial variability, the number of sampling points could have been adjusted, or instead of a simple interpolation algorithm (e.g. IDW, Sohel, 2018), a more sophisticated model (e.g. ordinary kriging, Goldsmith et al., 2018) could have been applied.

Nevertheless, by accounting for the small-scale variability, we notably improved the allocation of water sources to the individual trees compared to relying on only one sample (Yang et al., 2015), random replicates (Lion et al., 2017), or sampling one soil profile per quadrant, which would have been less realistic for the trees in the middle of the plot (Figure 4).

In our study, lateral soil water isotope variability was frequently in the same order of magnitude as the vertical variability. Moreover, on two sampling dates the maximum lateral range was higher than the maximum observed vertical range of any sampling point (see Figure 4). In such cases mixing models, as they are currently applied, potentially reach their limits. Firstly, since tree roots spread laterally wider in the upper soil layers, it is likely that they take water from various locations in the already more variable topsoil (Bonal et al., 2000; Goldsmith et al., 2018; Sternberg, Moreira, & Nepstad, 2002). When applying BMM to infer tree water uptake depth, it is therefore important that vertical soil water variability is distinctly higher than lateral variability, or to explicitly account for the lateral variability within BMMs. Secondly, relatively weak vertically differentiated soil water sources at some locations in our plot, similar to observations elsewhere (Moreira, Sternberg, & Nepstad, 2000; Weltzin & McPherson, 1997), complicated the application of a BMM. Sources that are hard to differentiate lead to small source mixing space polygons and a BMM result bias towards the flat prior distributions, that is similar contributions for every source (Brett, 2014). This could be the case in our study for those tree individuals with less differentiated soil profiles. Similar results have been presented by Zhao, Tang, Zhao, and Tang (2018) and Evaristo et al. (2016), who also report weakly differentiated source water isotopes.

Another common problem for tree water uptake studies with stable water isotopes are the depleted xylem δ^2H values deviating from their corresponding sources. Possible reasons for this isotopic offset have been discussed in detail by Barbeta et al. (2020). In general, it can be noted, that a better understanding of the underlying water isotope interactions in the soil–plant-atmosphere interface would further improve the application of natural tracer methods and BMM to investigate tree water uptake, which is an important field of further research.

5 | CONCLUSION

The study offers new insights into the spatial and temporal variability in water uptake depth by trees in a remote tropical natural forest in Kenya. By considering lateral and vertical soil water isotopic variability and comparing four points in time, we revealed distinct differences in soil water contributions between individual trees but found no species-specific differences. More work is needed to better understand the ecohydrological processes in forest ecosystems and our findings are another step in this direction and for the first time show how tree water uptake is controlled by spatiotemporal drivers in the selected study area. Our results indicate that the interpolation of soil water isotopes can improve the spatial allocation of source water isotopes to the trees. However, high lateral variability in soil water isotopes can fundamentally complicate the applicability of a BMM to infer tree water uptake contributions from different soil depths and we advise future studies to assess the lateral and vertical variation in advance. Therefore, mixing models are no universal tool in this context, but rather depend on the data quality and on site conditions.

Our findings offer important methodological observations for future ecohydrological research and point out the need for respecting the complexity and highly individual patterns of tree water uptake processes in natural tropical forests, as well as the possibly high spatiotemporal variability of underlying stable water isotope relations. It should be noted that our work by itself does not serve as a blue print for future studies, but rather highlights the importance to account for and incorporate the spatial and temporal variability in similar studies in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo.org at DOI: 10.5281/zenodo.4409137.

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SUPPORTING INFORMATION

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