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RESEARCH ARTICLE

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Continuous in situ measurements of water stable isotopes in soils, tree trunk and root xylem: Field approval

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Rationale: New methods to measure stable isotopes of soil and tree water directly in the field enable us to increase the temporal resolution of obtained data and advance our knowledge on the dynamics of soil and plant water fluxes. Only few field applications exist. However, these are needed to further improve novel methods and hence exploit their full potential.

Methods: We tested the borehole equilibration method in the field and collected in situ and destructive samples of stable isotopes of soil, trunk and root xylem water over a 2.5-month experiment in a tropical dry forest under natural abundance conditions and following labelled irrigation. Water from destructive samples was extracted using cryogenic vacuum extraction. Isotope ratios were determined with IRIS instruments using cavity ring-down spectroscopy both in the field and in the laboratory.

Results: In general, timelines of both methods agreed well for both soil and xylem samples. Irrigation labelled with heavy hydrogen isotopes clearly impacted the isotope composition of soil water and one of the two studied tree species. Inter-method deviations increased in consequence of labelling, which revealed their different capabilities to cover spatial and temporal heterogeneities.

Conclusions: We applied the novel borehole equilibration method in a remote field location. Our experiment reinforced the potential of this in situ method for measuring xylem water isotopes in both tree trunks and roots and confirmed the reliability of gas permeable soil probes. However, in situ xylem measurements should be further developed to reduce the uncertainty within the range of natural abundance and hence enable their full potential.

1 | INTRODUCTION

Plant transpiration fuels the hydrological cycle by returning 35% to 90% of water from land surfaces to the atmosphere.[1,2] Therefore, transpiration greatly influences our climate and impacts water availability in consequence of land use and climate change.[3,4] However, why is the quantification of this essential water flux so uncertain? This comes down to knowledge gaps in the mechanistic functioning of root water uptake (RWU) as well as plant rooting depth that persist despite its crucial role in predicting the future of one of our most important resources.[5] A major constraint is the practical difficulty in observing below-ground processes. Moreover, the magnitude and location of RWU are the results of multiple influencing factors, such as extent of the root system and its hydraulic properties,

soil water availability as well as water potential gradients, and their complex interactions.[6] In addition, RWU changes dynamically over short periods of time.[7,8]

Water stable isotopes are an essential tool to shed light on hard-to-observe below-ground processes and have been used for decades to trace water fluxes across the soil-plant-atmosphere continuum.[9] They help to investigate which water sources, such as water in different soil depths or groundwater, plants actively use. This has provided valuable insights into the functioning of plants and their impact on overall ecosystem water cycling (e.g., reviewed in Sprenger et al[10] and Rothfuss and Javaux[6]). These tracers are well suited to investigate active RWU because isotope values often vary between ecosystem water pools due to physically well-understood fractionation processes during phase changes as water moves within the hydrological cycle.[11,12] Differences in isotope values, for example, between soil depths, can also be enhanced by the addition of water enriched in heavy isotopes, mostly ^{2}H, applied as surface irrigation[8,13–15] or to specific depths in the soil profile.[16] This can be utilised to investigate the contribution of different water sources, for example, soil depths to plant water use. Furthermore, RWU and water transport in suberised plants are generally believed not to alter the isotope value for most plants[17] except for xerophytic and halophytic species.[18–20] Water transported in the xylem then reflects a weighted mixture of all water (with different isotope values) taken up at a specific point in time.

Lately, this long-standing principle has been questioned repeatedly, and a multitude of factors are discussed that complicate the estimation of RWU depth with water stable isotopes. These factors include, for example, fractionation during RWU,[21,22] time lag from precipitation to RWU to xylem sampling, exchange of xylem water with tree storage water and spatial and temporal heterogeneity in soils and plant xylem.[23,24]

Analysis of water stable isotopes in soil and plant material has traditionally been performed using destructive sampling and subsequent extraction of water in the laboratory, with cryogenic vacuum extraction (CVE) being the most common method.[25] This captures only snapshots in time[26] and has hindered the assessment of the above-mentioned isotope effects, impairing a deeper mechanistic understanding of ecohydrological dynamics.

Following the invention of laser-based isotope spectrometry,[27] efforts were directed at developing new methods to measure water stable isotopes in precipitation,[28] soils[29–31] and plant xylem[32,33] in situ. These methodological advances hold great promise to gain a better understanding of at least some of the just-mentioned issues by enabling more frequent measurements with a lower disturbance of the system monitored. For instance, new in situ techniques have been used to show the strong short-term variations in RWU of an herbaceous species in a soil column experiment.[7]

Several studies have applied in situ soil water isotope measurements in the field[8,34–36]; however, methods are still in the development phase and could benefit from further improvement. Specifically, no uniform protocol regarding sampling setups, calibration as well as data analysis exists,[23] and it would be desirable to identify the simplest approach to get reliable data. Little progress has been made in including in situ analysis of xylem water stable isotopes in field studies, and a systematic comparison to destructive samples over an extended time period is missing. However, this is essential to assess inter-method comparability and identify strength and weaknesses associated with each methodology. The simultaneous in situ measurement of water stable isotopes in soils and plant xylem will allow us to dig deeper into the complexity of ecosystem water fluxes.[23]

In this study we determined water stable isotope values in situ and via destructive sampling and subsequent CVE in both soil water and tree xylem of two tropical dry forest species. For this, we applied the borehole equilibration method[32] in a field experiment for the first time. This additionally allowed to measure the isotopic composition within root xylem repeatedly, which, to the best of the authors' knowledge, has not been performed before. Data were collected over a period of 2.5 months. During this time, we determined natural abundance δ values first and hence followed the systems reaction to labelled irrigation events. In contrast to previous studies, we measured both soil and xylem δ values in situ to obtain an unprecedented temporal resolution of obtained data and compare this with repeated destructive sampling over the entire experimental period.

The specific objectives were to:

1. Compare between in situ and destructive soil and xylem water isotope measurements under natural abundance conditions as well as following labelled irrigation;
2. Identify areas of improvement to further advance in situ measurements of the isotopic composition in tree xylem water;
3. Explore the possibilities of the new borehole equilibration method to investigate isotope variations among different parts of the root system.

2 | MATERIAL AND METHODS

2.1 | Study area

The experiment was conducted at the Estación Experimental Forestal Horizontes (EEFH), which is part of the Área de Conservación Guanacaste, located in the northwest of Costa Rica. The long-term mean annual temperature in the adjacent Santa Rosa sector is 25°C. Mean annual precipitation is 1575 mm but shows strong interannual variability influenced by the El Niño Southern Oscillation; annual sums range from 880 to 3030 mm.[37,38] Precipitation is strongly seasonal; almost all of it occurs between May and November.[38] Data were collected in 2019 from February to the end of May, that is, from the dry season and transitioning into the rainy season. The precipitation total of the preceding 2018 wet season was very close to the long-term average with 1571 mm.[39] Mean temperature in 2018 was 27 ± 4°C (personal communication, Jennifer Powers) and therefore above the long-term mean.

After having been used for cattle grazing, rice production and other agricultural land uses for decades, the area was not managed for more than 30 years and is now a regenerating tropical dry forest.[40] For the experiment, we chose the two native tree species *Sideroxylon capiri* (SC) and *Swietenia macrophylla* (SM) that do not shed their foliage for the entire dry season and hence continue to transpire and take up water from the soil even under the dry conditions during the beginning of our investigation.

Soils at EEFH are of volcanic origin and feature a high clay content of approximately 38%.[38,41] At the specific site of this experiment, the soil classifies as Vertisol. Within the standard textural fraction triplet, it has a clay loam texture. Mean values for sand, silt and clay content are 26 ± 10%, 36 ± 5% and 37 ± 9%, respectively. On average, organic matter is present at a content of 0.6 ± 0.4% and soil pH is 6.75 ± 0.16. Soil properties do not change systematically over the top 100 cm. With decreasing water content during the dry season, soils become increasingly hard, which prevented the installation of soil sensors in soil depths below 150 cm. Despite the high clay content, we did not observe shrinkage cracks. Soils co-developed on saprolite of ignimbritic origin of 2 to 3 million years of age. At around 30 m depth below the ignimbrites lies a Basaltic aquitard of around 8 million years of age.[42]

An automated weather station (HOBO RX3000 weather station; Onset Computer Corporation, Bourne, MA, USA) was installed at the experimental site at the end of February 2019 and recorded air temperature, relative humidity, solar radiation and precipitation depth in half-hourly resolution. Precipitation events occurring in May, at the beginning of the wet season, were sampled in bulk from the weather station rain collector every 5 days and analysed for their isotope values (see next paragraph for details).

2.2 | Measurement and notation of water stable isotope values

The hydrogen and oxygen stable isotope values of water (δ^2H and $\delta^{18}O$) are reported as relative deviation from the VSMOW standard on the δ scale (Eq. 1,[43]):

$$\delta\text{sample} = \left(\frac{R_{\text{sample}}}{R_{\text{VSMOW}}} - 1\right) * 1000\,[‰] \qquad \text{(Eq1)}$$

with R_{sample} and R_{VSMOW} the ratios of heavy isotope (^{18}O and ^{2}H) to light isotope (^{16}O and ^{1}H) in sample and standard water, respectively.

For in situ field measurements, δ^2H and $\delta^{18}O$ values were recorded with a cavity ring-down spectroscopy (CRDS) analyser (L2130-i, Picarro Inc., Santa Clara, CA, USA). Liquid water from standards, precipitation and destructive samples were analysed using an autosampler and different laser spectroscopy analysers (L2130-i, Picarro Inc., for extracted plant and soil water samples and IWA-45EP, LGR, San Jose, CA, USA for water samples).

2.3 | Manipulation of soil water isotope values by irrigating with ^2H labelled water

The experiment started in February under natural and dry conditions. Thereafter, we manipulated the system by applying irrigation events with tap water and water artificially enriched in ^2H, that is, labelled water. Irrigation was distributed evenly using a garden hose within two plots enclosing investigated tree individuals. Their area was 211 and 139 m^2 for SM and SC, respectively. Labelling such large areas has the advantage that label is distributed equally around stems. Due to high costs of label water and issues of practicability, often only small areas[16] or parts of the root system[33] have been labelled in previous studies.

Table 1 lists all irrigation events, with corresponding duration, depths and mean δ values. We aimed at firstly prewetting the soil with tap water (TW1 and TW2), secondly labelling the top most soil compartment (LW1 and LW2) and lastly pushing the label further into the soil (LW3). From LW1 onwards, we applied irrigation water using a tank with a powerful firefighting pump, thus increasing irrigation intensity. Average δ values were calculated over multiple samples collected throughout each irrigation event. Natural precipitation in May had mean values of −9.5 ± 3.1‰ and −64.9 ± 23.9‰ for $\delta^{18}O$ and δ^2H, respectively. Precipitation δ values decreased systematically towards the end of May. Highest values were −3.7‰ and −21.9‰ and lowest values were −12.3‰ and −85.7‰ for $\delta^{18}O$ and δ^2H, respectively.

2.4 | In situ tree xylem isotope measurements: Borehole equilibration

The stem borehole equilibration method[32] was used to measure the stable isotope values of tree xylem water in situ. Three individuals of each species were chosen. Selected individuals (SM1 to 3 and SC1 to

TABLE 1 List of applied irrigation events, including date of application, duration, total volume applied, irrigation depth and average δ values

Irrigation event	Date (dd.mm.yy)	Depth (mm)	Total volume (l)	Total duration (h)	$\delta^{18}O$ (‰)	δ^2H (‰)
1st irrigation tap water (TW1)	29.03.19	5	1750	14	−7.8	−52.0
2nd irrigation tap water (TW2)	31.03.19/01.04.19	10	3500	19	−7.8	−50.0
1st irrigation label (LW1)	05.04.19	15	5250	5.5	−7.8	+152.1
2nd irrigation label (LW2)	09.04.19	15	5250	5.5	−7.7	+540.8
3rd irrigation label (LW3)	23.04.19	15	5250	5.5	−7.7	+39.6

3) were located in close proximity to each other and to soil water isotope profiles (compare Figure S1, supporting information). See the next section for information on soil water measurements. Individuals differed in diameter at breast height (DBH, 1.3 m above ground). For SM1, SM2 and SM3, DBH was 15.8, 26.1 and 21.7 cm, respectively. SC1, SC2 and SC3 had a DBH of 24.1, 11.1 and 12.1 cm. We measured δ values within various tree compartments (trunk, lateral and tap root). For this, we excavated the root system in close proximity to the tree trunk in a radius of approximately 0.5 m while taking care that roots were not harmed unnecessarily.

After we had exposed roots of interest, a hole was drilled through each tree compartment to be investigated (trunk, lateral root or tap root) using an increment borer (core diameter 5.15 mm, Haglöf Sweden AB, Långsele, Sweden). Borehole installation was completed in the period 25 February 2019 to 03 March 2019. Trunks were equipped at 1.2 m height. With three tree compartments and three replicates for the two species investigated, this amounted to a total of 18 boreholes. We used a manual increment borer instead of an electric drill to decrease friction and therefore heating and potential evaporation during drilling into xylem wood. Obtained xylem core samples were collected and used to verify the new method with results from an established method, that is, CVE. To reduce the production of pitch and other organic substances in response to wounding, freshly drilled holes were flushed with acetone as suggested in Marshall et al.[32]

Commercially available stainless-steel connectors (SS-200-1-2, 1/8 in. tube OD × 1/8 in. male NPT, Swagelok, Solon, OH, USA) were used to attach Teflon tubing (PFA, 1/8″ OD, BEMU, Krefeld, Germany) to both sides of each borehole. For this, predrilled holes had to be widened at the outermost centimetre. Where necessary, air tightness around boreholes was increased by applying elastomeric sealant (StopLeak, Lanco & Harris, Alajuela, Costa Rica). Boreholes were regularly checked for air leakages over the course of the experiment (compare Figure 7). Those were sealed tightly with plasticine. All boreholes as well as isotope standards (see below) were combined in one system, which allowed for automatic switching between measurements via solenoid magnetic valves (2-Way Elec. Valve, EC-2-12, Clippard Minimatic, Cincinnati, OH, USA). Switching between measurements was controlled via a custom-made programme that was run on the CRDS analyser. To reduce total tubing length, one manifold with mounted valves was placed in each of the two plots. Both of them had a flushing port connecting the ends of the manifold mounts that enabled clearing of water vapour from the tubing path between manifolds and the CRDS analyser in between measurements and allowed us to detect and remove condensation.

To conduct a measurement of one particular borehole, one pair of valves was opened simultaneously (one valve at the inflow and one valve at the outflow) and a dry air stream, regulated by a mass-flow controller (FC 260, Tylan General TCA GmbH, Eching, Germany), was directed into the borehole at a constant flow rate of 125 mL/min. With contact to liquid xylem water, water vapour was taken up by the air stream and subsequently directed into the CRDS analyser to determine the δ value and water content (water vapour mixing ratio (WVMR), ppmV) of sampled moist air. Each borehole was measured for a time span of 15 to 20 min. The necessary duration to reach stable plateaus of sampled water vapour and δ values was determined for each borehole separately and depended on tubing length and air tightness. Flushing time between measurements was set to 5 min. The amount of air flowing through the system (initially 125 mL/min) exceeded the amount that is taken in by the CRDS analyser (approx. 35 mL/min). To avoid overpressure, excess air was allowed to leak at an open-split close to the CRDS analyser. This excluded the possibility of sample contamination with ambient air even though the system was not completely air-tight. We checked this with a mass flow meter (Serie 358, ANALYT-MTC GmbH, Müllheim, Germany) attached to the end of the open-split tube.

The borehole equilibration method, like other novel in situ methods, is based on measuring δ values of water vapour that is in isotopic equilibrium with liquid water surrounding the borehole. Therefore, the isotopic difference between both phases can be calculated using well-established equations and depends on the temperature (T) at the location of the phase change.[30,44] To approach isotope equilibrium fractionation between liquid xylem water and sampled water vapour, air throughflow must be slow enough (depending also on trunk diameter) for sampled air to be saturated.[32] To decrease the risk of condensation, heating lines (Quintherm ILLw, 10 W/m; Quintex GmbH, Baden-Württemberg, Germany) were attached along all sample lines, from borehole outflows, over manifold mounts and hence to the CRDS analyser. With the high air temperatures at the field site, water content of sampled air was mostly too high to conduct measurements with the CRDS analyser. Therefore, we used a second mass flow controller (Serie 358, ANALYT-MTC GmbH, Müllheim, Germany) to dilute sampled water vapour with dry air.

Type-T thermocouples (copper-constantan) were inserted via a separate—tightly sealed—small access hole to record T directly inside boreholes. Ten measurements were recorded every 15 min, for each investigated tree compartment at one individual per species. Temperature data were stored on a data logger (CR1000X, Campbell Scientific Inc., Logan, UT, USA) and used to derive δ values of liquid water from measured vapour values.

2.5 | In situ soil water isotope probes

The stable isotope ratios of soil water were determined in situ on the basis of direct water vapour equilibration (e.g.,[30,31]). In this experiment, we used a pull-only system (e.g.,[31]) with self-made soil gas probes. In this set-up, water vapour is pulled out from the soil probe by the suction of the CRDS analyser (approx. 35 mL/min). Each probe consisted of two stainless steel capillaries (1/8″ AD × 1/16″ ID, SCP Seitz, Darmstadt, Germany) that were inserted into a gas permeable tube (GPT, Accurel GP V8/2HF, 3 M, Germany; 0.155 cm wall thickness, 0.55 cm i.d., 0.86 cm o.d.) of 10 cm length, which is however impermeable to liquid water. One of the capillaries was used

for sampling and reached almost the end of the GPT. The other was used for pressure compensation, reached only a few centimetres into the GPT and was connected to a container filled with desiccant on the other side. This way, air removed from the probe by the CRDS analyser could be replaced by dry air, hence reducing potential contamination with ambient water vapour. Both ends of the GPT were sealed with two-component glue (Pattex Kraft-Mix, Henkel AG & Co. KGaA, Düsseldorf, Germany). We built probes mid of February and verified that materials used did not affect measured δ values by placing them in ambient air and comparing to measurements without soil probes.

To access different soil depths, a soil pit with dimensions 1 × 3 × 1.5 m (width × length × depth) was located between investigated tree individuals (compare Figure S1, supporting information). It was excavated on 20 February 2019. Soil water isotope probes were installed at the end of February at 5, 10, 20, 50 and 150 cm and 5, 10, 20, 30, 50, 100 and 150 cm soil depth in soil profiles 1 and 2, respectively. Profiles 1 and 2 represented heterogeneous conditions within the experimental plot and featured bare soil and soil with a litter layer, respectively. Probes were not evenly spaced over depth but featured a higher spatial resolution in the soil top because there we expected more pronounced and dynamic changes of δ values in response to soil evaporation, irrigation and precipitation. We inserted soil isotope probes horizontally into the soil and as far away from the soil pit walls as possible. After probe installation, the soil pit was covered. All 12 soil gas probes were connected to a manifold (16-Port Distribution Manifold, A0311, Picarro Inc.), which allowed for automatic switching between measurements. A schematic of the whole set-up to measure δ values in standards, tree and soil water in situ is depicted in Figure 1. In addition, we show some field impressions of those measurements.

From the point where the capillaries protruded from the soil pit walls until they reached the manifold, sample lines were heated (Quintherm ILLw, 10 W/m, Quintex GmbH). Soil water isotope profiles were generally measured during the night; therefore, ambient temperatures were low enough that we did not need to dilute sampled water vapour with dry air. The maximum water vapour concentration of our analyser was 50 000 ppmV. Under standard pressure, this value is surpassed in saturated air over 32.3°C. Sample lines were regularly flushed with dry air to remove possible condensation and water vapour from previous measurements.[23] Each soil depth was sampled for 20 min. Soil water isotopes were sampled at least every 2 days. To convert measured vapour to liquid values, we used soil temperatures from soil sensors (5TM, METER Group, München, Germany and Tensiomark, ecoTech, Bonn, Germany) installed at the same depths in both profiles.

2.6 | Standardisation

In situ soil and xylem water stable isotope measurements were standardised using three water vapour standards that were integrated into the automated system. This allowed us to measure standards in the same phase and with similar preconditions as the soil and xylem δ

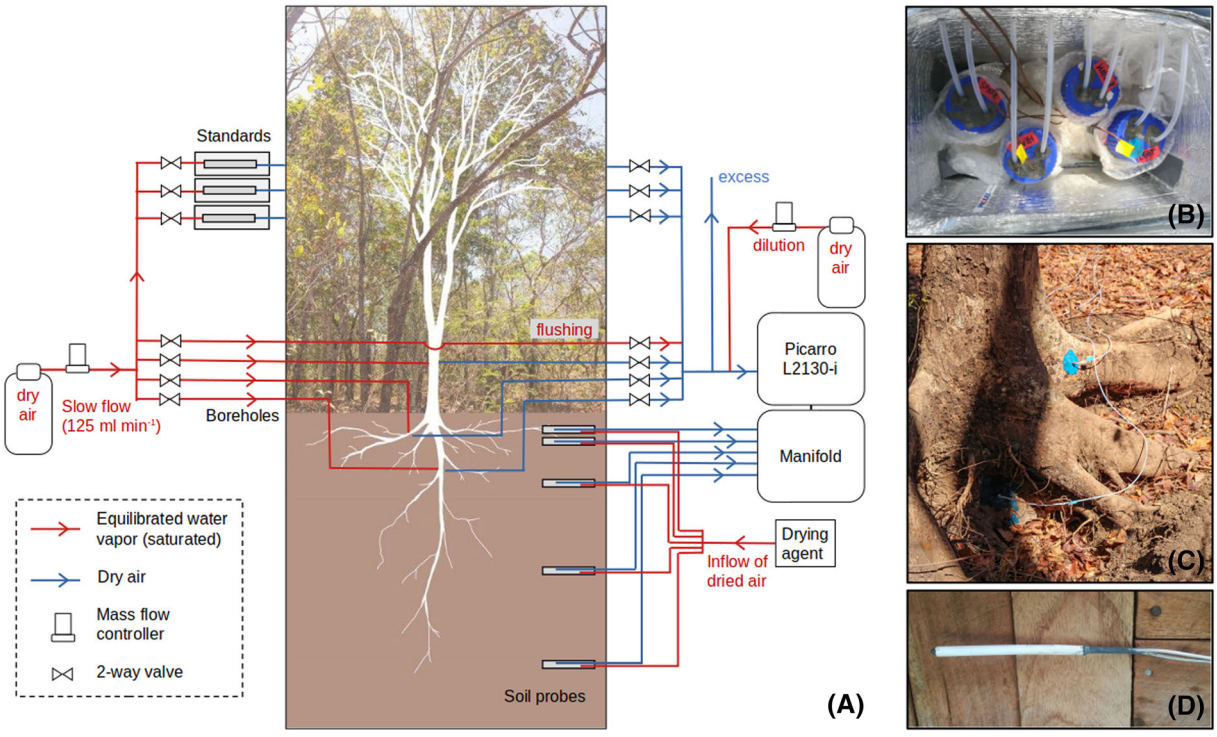

FIGURE 1 Schematics showing the field set-up for measuring water stable isotopes in situ in water vapour standards, tree xylem and across soil depths (A). The pictures show impressions from the field set-up, namely standards within the insulated box (B), root boreholes (C) and a soil probe before installation (D) [Color figure can be viewed at wileyonlinelibrary.com]

values, as emphasised by Beyer et al[23] For this, holes were drilled into the lids of three gastight vessels (DURAN GLS 80, 1000 mL, DWK Life Science, Millville, NJ, USA) to insert one inflow and one sample tube each. The tubes were connected with GPT as used in the construction of soil isotope probes to decrease the risk of liquid water intruding into the system and holes were sealed air-tight. It was confirmed previous to the experiment that the tubing did not affect measured δ values compared to purely sampling bottle headspace. Each of the standard vessels was filled with 200 mL of water with different δ values (S_{light}: $\delta^{18}O = -14.1 \pm 0.1‰$, $\delta^{2}H = -102.0 \pm 0.4‰$, S_{medium}: $\delta^{18}O = -7.2 \pm 0.1‰$, $\delta^{2}H = -48.3 \pm 0.5‰$, S_{heavy}: $\delta^{18}O = 31.6 \pm 0.1‰$, $\delta^{2}H = 136.6 \pm 0.6‰$). Liquid standard water was sampled before and after the experiment, and no systematic enrichment due to the preferential removal of lighter water molecules was detected. All standard vessels were placed in a cooling box to decrease the impact of diurnal temperature fluctuations. Cool packs were added regularly to lower T within the box and further decrease the likelihood of condensation during standard measurements. Standards were measured for 20 min each. T was recorded every 15 min with a thermocouple (Type-T, copper-constantan) directly inside one of the standard vessels. Sample lines were also heated.

2.7 | Data processing of in situ measurements

To obtain final δ values of the liquid phase from the measured vapour phase, all in situ measurements, that are standards, soil and xylem water, were processed in the same way. Shortly, faulty measurements were identified and liquid δ values were derived from the measured vapour composition. Finally, δ values of liquid standard samples, determined in the laboratory, were used to reference both borehole and soil measurements to the VSMOW scale.

The CRDS analyser provided values of air WVMR, $\delta^{18}O$ and $\delta^{2}H$ of water vapour along with other diagnostic variables at a temporal resolution of 1 s. Values for every measurement were derived by combining raw data with valve position information and averaging over the last 3 min before switching, when values were usually most stable and a measurement plateau was reached. In addition to mean values, we calculated the standard deviation (sd) and slope through the averaging window for WVMR and δ values as measures of data quality. To identify inaccurate data points, values for standards and boreholes were first flagged automatically (if WVMR < 3000 ppmV or > 50 000 ppmV, sd $\delta^{18}O$ > 1 or sd $\delta^{2}H$ > 2). We decided for the sd thresholds by plotting all measured δ values against their sd and determining from which sd unrealistic outliers occurred. This was followed by a visual inspection of each individual measurement in the context of adjacent measurements because sd alone was not necessarily a good indicator of unstable plateaus, for example, for samples with low WVMR. Regarding soil water δ values, only measurements with previous flushing were selected due to frequent condensation issues and unstable plateaus observed otherwise. δ values of CRDS instruments are known to depend on the WVMR of sampled air.[7,45] We evaluated this device-specific dependency by conducting a separate lab experiment following the field campaign, where we changed the WVMR and isotopic composition of vapour originating from water with known δ values by changing the sample temperature. We accounted for changing equilibrium fractionation and used the remaining change in δ values to correct for WVMR dependency.

Liquid phase δ values ($\delta^{2}H_{liq}$, $\delta^{18}O_{liq}$) were hence derived from measured vapour values ($\delta^{2}H_{vap}$, $\delta^{18}O_{vap}$) using recorded temperatures (T) and the equations detailed in Rothfuss et al[30] for calculating equilibrium fractionation in a similar in situ system (Eqs. 2 and 3).

$$\delta^{2}H_{liq} = 104.96 - 1.0342 * T + 1.0724 * \delta^{2}H_{vap} \quad (Eq2)$$

$$\delta^{18}O_{liq} = 11.45 - 0.0795 * T + 1.0012 * \delta^{18}O_{vap} \quad (Eq3)$$

Before this, data gaps in temperature timelines, caused by occasional failures of the power supply, were filled. All small data gaps (≤2 h) were linearly interpolated, and bigger data gaps were completed by different approaches depending on the measurement. Within the standard vessel, missing T was estimated from diurnal courses on adjacent days. In soils the highest correlation of a sensor in a different soil depth was used. Missing borehole T was filled by correlating measured T with that of ambient air while determining and accounting for the time lag between the two.

We observed jumps in δ values of two out of three standards at the beginning and the end of the experiment that were not visible in other concurrent measurements. Because we additionally did not see any systematic instrument drift over time, we used standard measurements conducted between end of March and end of April, calculated a linear regression through all data points and applied it to standardise all measurements of the entire experimental period and reference them to the VSMOW scale.

2.8 | Destructive samples for determining water stable isotope ratios

Destructive samples of soil and xylem water were collected throughout the experiment to (a) validate in situ measurements and (b) gain insight into spatial soil water isotope patterns across experimental plots. We collected soil samples during in situ soil probe installation in 5, 10, 20, 30, 50, 100 and 150 cm depth. On five occasions throughout the experiment, following labelled irrigation events, we sampled at three locations within each irrigation plot in 5, 10, 20 and 30 cm soil depth. Overall, this amounted to 252 samples. Xylem was sampled with an increment borer (core diameter 5.15 mm, Haglöf Sweden AB), bark was removed and sapwood transferred into Valco exetainer vials (Labco Ltd., High Wycombe, UK). Over the experiment, a total of 67 and 70 xylem samples were collected for SM and SC, respectively. All samples were stored in gastight sample vials immediately after collection to avoid isotope fractionation due to

evaporation. Soil samples were stored in 50 mL headspace glass vials with PTFE seal (La-Pha-Pack GmbH, Langerwehe, Germany). Water was extracted from all samples at TU Braunschweig, Germany using CVE based on a system as described in Koeniger et al.[25] The set-up consisted of an aluminium block mounted on a heating plate with slots to insert sample vials. First, samples were frozen by submerging them into liquid nitrogen. Sample and extraction vials were connected with a stainless-steel capillary and evacuated (pressure < 0.04 mbar) by inserting a syringe connected to a vacuum pump (TRIVAC T, Leybold GmbH, Köln, Germany) through the septum of the sample vial. Water contained in samples was extracted at 140°C for 25 and 30 min for xylem and soil samples, respectively. Evaporated water was collected in the extraction vial which was submerged in a liquid nitrogen cold-trap and subsequently analysed on a CRDS analyser (L2130-i, Picarro Inc.). After extraction, samples were weighed and then dried at the extraction temperature for 24 h. A comparison of the weights after extraction and after drying allowed us to determine whether water extraction was complete. Samples that still contained water after extraction and consequentially showed systematic offsets in derived δ values were excluded.

2.9 | Data analysis

Data processing and analysis were conducted in R, Version 3.5.2.[46] Values are reported as mean with corresponding sd. We used Shapiro-Wilk to test data sub-samples, for example, δ values in different soil depths or determined with the two methods, for normal distribution. As most groups did not follow normal distribution, we used the non-parametric Mann-Whitney *U*-test to test for statistical difference between groups. Methods differed in temporal coverage across the experimental period. Therefore, we used sub-samples of the time series for overall method comparison (compare Figures 3 to 5). Specifically, we selected in situ data points in a time window of ±2 days around destructive sample time points and excluded destructive samples that did not have associated in situ data points. Due to the limited amount of destructive samples of root xylem, we included the first destructive samples and the first 5 days of in situ measurements for those.

3 | RESULTS

3.1 | Soil water content and δ values

Figure 2 compares soil water δ values across soil depths before the first irrigation (TW1). Displayed are average profiles of in situ soil water isotope measurements (in the time 11 to 19 March 2019) as well as destructive samples collected during soil probe installation (22 to 23 February 2019). Meanwhile, no precipitation events occurred. Both methods produced similar depth profiles for $\delta^{18}O$ and $\delta^{2}H$ ($P > 0.05$ for all soil depths) with exponential shapes and higher δ values in the uppermost soil depth. Differences between mean values were highest in 5 cm soil depth. Here, destructive samples resulted in less negative values with a statistically insignificant difference of 2.5‰ and 13.4‰ compared to in situ measurements for $\delta^{18}O$ and $\delta^{2}H$, respectively.

Over the course of the experiment, evaporation, artificial labelled irrigation and natural precipitation impacted soil water content and δ values. Figure 3 shows the timing and depths of irrigation and

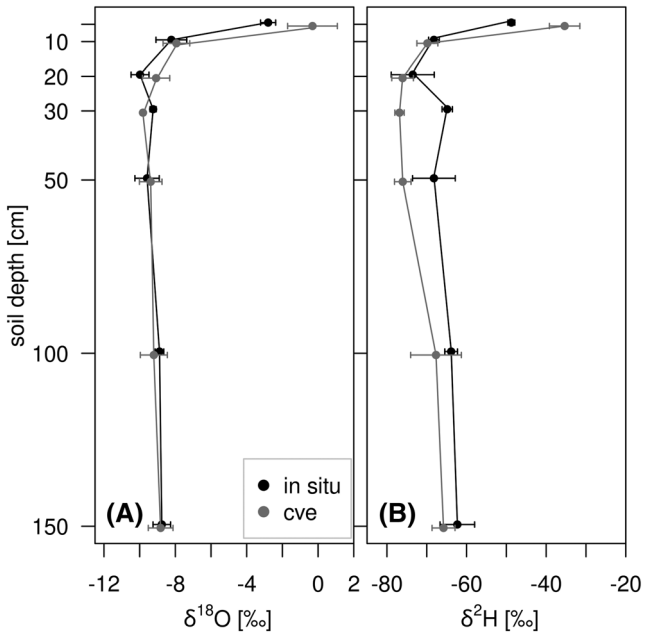

FIGURE 2 Comparison of soil water δ values ($\delta^{18}O$: A, $\delta^{2}H$: B) between in situ measurements before the first irrigation event (11 to 19 March 2019) and destructive samples (CVE) collected during soil probe installation (22 to 23 February 2019)

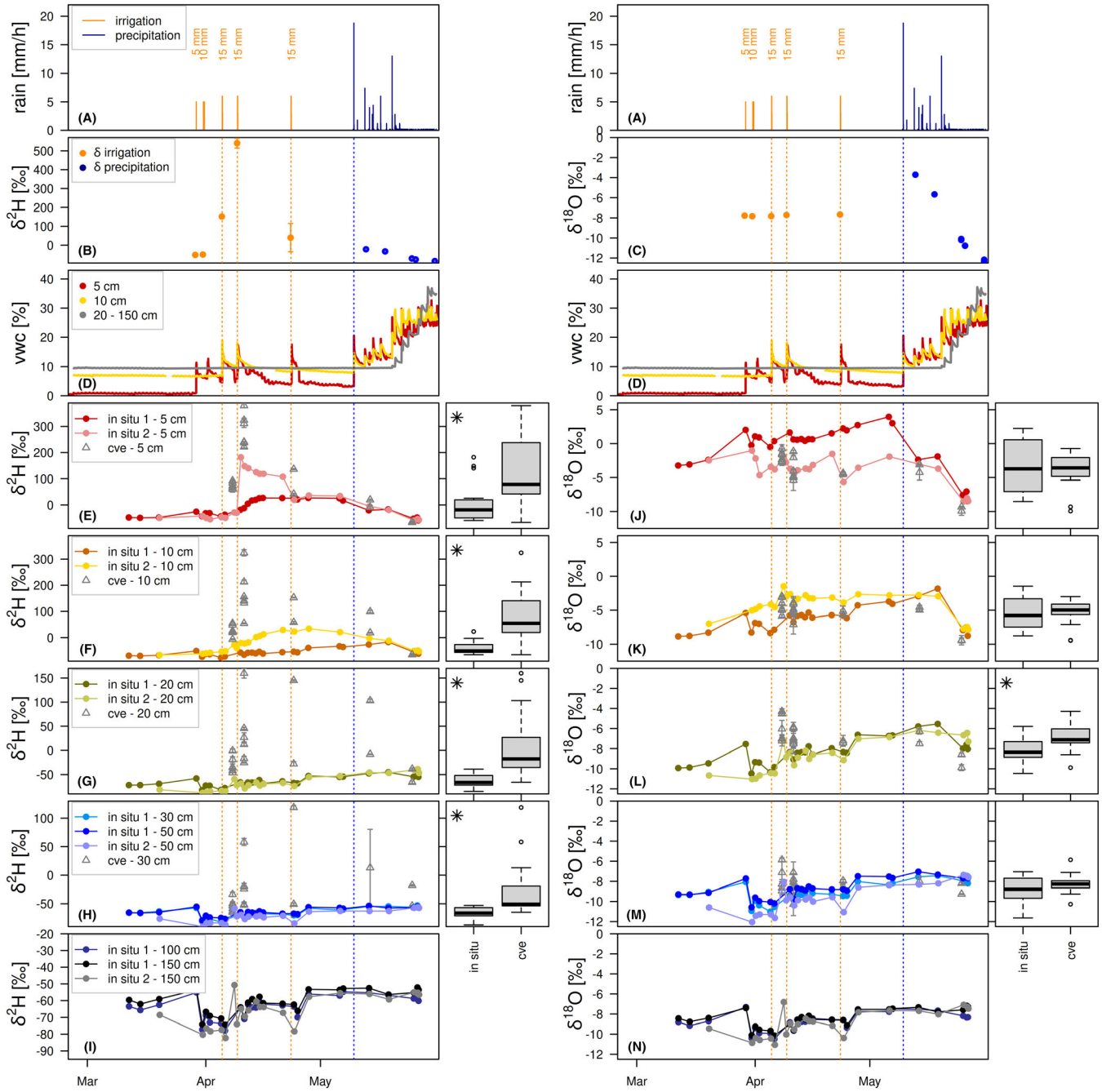

FIGURE 3 Timeline comparing soil water δ values (δ^2H: E-I, δ^{18}O: J-N) determined with in situ and destructive sampling over the experimental period. Data were split by soil depth. Triangles depict destructive samples taken regularly to investigate spatial heterogeneity of label distribution across the experimental plots. To compare the two methods, data are summarised as boxplots next to the timeline. For this, we selected in situ measurements within a period of ±2 days around destructive sampling. The box represents the 25% and 75% quartile. Significant differences between overall mean values are indicated by an asterisk (Mann-Whitney *U*-test: $P < 0.05$). Also displayed are depth (A) and δ values (B, C) of irrigation and precipitation events along with volumetric soil water content (VWC) across the soil profile (D). The timings of labelled irrigation events (orange) as well as the first precipitation event (blue) are displayed as vertical dotted lines [Color figure can be viewed at wileyonlinelibrary.com]

precipitation events along with volumetric soil water content (VWC) across the soil profile. To increase the readability, we calculated a mean VWC value over all soil depths that were not impacted by artificial irrigation, that is, sensors below 10 cm soil depth. Also displayed are the time courses of in situ soil water δ values with values of spatial destructive sampling across different soil depths. Boxplots next to timeline panels compare both methods. For this, in situ measurements within ±2 days around destructive sampling were selected. Average values of δ^2H were statistically different between methods for all soil depths (Mann-Whitney *U*-test: $P < 0.05$). δ^{18}O

values only differed significantly in 20 cm depth. In general, time courses of $\delta^{18}O$ values were more similar between the methods than those of $\delta^{2}H$ values.

Labelled irrigation events influenced the top soil centimetres for both methods and increased the $\delta^{2}H$ variability between the two in situ soil profiles as well as between destructive samples collected within the same day. The impact of labelled water was generally less pronounced for in situ sampling and decreased rapidly with depth. In situ soil probes were able to capture rapid changes in measured $\delta^{2}H$ values with a sharp increase from −29.7‰ to +182.4‰ measured at 5 cm depth within 1 day in profile 2.

Considering all in situ measurements, values for both $\delta^{18}O$ and $\delta^{2}H$ in 5 and 10 cm soil depth were statistically different from each other and from all lower soil depths (Mann-Whitney *U*-test, $P < 0.05$). No statistical difference for both investigated isotope values was found between any of the soil depths below 10 cm. In those lower soil depths, δ values did not change markedly across the experimental period and were on average −8.8 ± 1.2‰ and −65.3 ± 9.8‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. In contrast to δ values derived in situ, destructive samples were statistically different for all soil depths sampled, apart from $\delta^{2}H$ between 5 and 10 cm.

With increasing intensity and frequency of precipitation events, water infiltrated into soil depths >10 cm. Incoming precipitation became increasingly negative from the middle to end of May and had a mean value of −8.5 ± 3.6‰ and −57.3 ± 27.9‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. Latest on 25 May 2019, precipitation equalised the δ values across soil depths. We observed this for both sampling methods. On average across depths, $\delta^{18}O$ was −7.7 ± 0.5‰ and −9.3 ± 0.7‰ for in situ and destructive sampling, respectively. Similarly, $\delta^{2}H$ was −54.1 ± 4.9‰ and −54.7 ± 17.5‰, respectively.

3.2 | Tree xylem water δ values

The timelines of $\delta^{2}H$ measured in situ in different boreholes are compared against CVE samples in Figure 4. An analogous display of the course of $\delta^{18}O$ over time can be found in Figure 5. Significant inter-method differences are labelled by an asterisk. Data selection for the method comparison is detailed in the Methods section. In general, we found similar temporal dynamics of xylem δ values measured in situ and using CVE for both investigated trunk and root xylem. For instance, an increase in $\delta^{2}H$ in destructive samples coincided with $\delta^{2}H$ measured in situ simultaneously.

Leaf area impacts the timing and amount of plant water uptake and consequentially affects possible δ variation in xylem water. Therefore, changes in this regard are shortly summarised before detailing results. In our case, the foliage of two individuals changed throughout the experiment. SM3 reduced its leaf area in the beginning of our experiment and SC1 quickly exchanged its entire foliage in the beginning of April.

On average, destructive sampling in tree trunks yielded lower $\delta^{18}O$ values compared to in situ measurements for all SM individuals ($P < 0.05$). SM1 had significantly lower destructive $\delta^{18}O$ values in all tree compartments investigated. Apart from SC3 lateral root, no statistical inter-method difference in $\delta^{18}O$ was found for SC individuals. SM3 tap root was the only borehole in which higher $\delta^{18}O$ values were determined in destructive samples. For $\delta^{2}H$ measured in tree trunks we found no species-specific pattern, only SC3 and SM2 showed a significant difference between the methods, again with lower values determined from destructive samples. The same was observed for SC2 lateral root and SC3 tap root.

During measurements of natural abundance, that is, before the first labelled irrigation (LW1, first dotted line), and summarising all measured individuals, $\delta^{18}O$ of trunk xylem was significantly higher ($P < 0.05$) for in situ than for CVE with an average of −5.6 ± 1.4‰ and −7.9 ± 1.3‰, respectively. Similarly, trunk xylem $\delta^{2}H$ had mean values of −58.8 ± 13.4‰ and −63.6 ± 6.8‰, respectively. During this period, we detected no statistical difference between the xylem water isotopes of the two investigated species apart from $\delta^{2}H$ measured in situ that was significantly higher for SC than for SM, with averages of −52.9 ± 9.6‰ and −61.9 ± 14.1‰, respectively. SM δ values measured in situ were therefore also closer to values determined using destructive sampling.

Generally, we observed a more pronounced increase in $\delta^{2}H$ in response to labelled irrigation for SM compared to SC individuals. Labelled irrigation affected trunk xylem $\delta^{2}H$ of SM1 and SM2 in a similar way but seemingly did not impact values in SM3 trunk. At the end of April, both SM1 and SM2 (trunk) reached maximum values (mean of five highest values) of 44.6‰ and 49.7‰, respectively. Interestingly, the label signal was much less pronounced in corresponding SM destructive samples, whereas in situ measurements and SM2 root destructive samples showed a clear enrichment of ^{2}H. In contrast, in situ $\delta^{2}H$ values matched very well with CVE samples for SM3 trunk. In lateral roots, labelled irrigation impacted the xylem isotope value earlier, more pronouncedly, and in all three SM individuals investigated. Maximum values were 57.8‰, 121.3‰ and 4.8‰ for SM1, SM2 and SM3, respectively. Within SM individuals, $\delta^{2}H$ changed the least in tap roots, with SM2 posing an exception. For all SM boreholes that featured a clear label signal in response to irrigation, $\delta^{2}H$ also decreased again after the last irrigation event, which only had slightly increased $\delta^{2}H$ values. Except for SC2 (lateral root and trunk), SC individuals did not show a clear change of $\delta^{2}H$ in any tree compartment, considering measurement precision. The increase in $\delta^{2}H$ in SC2 lateral root with a maximum of 19.9‰ decreased again faster compared to SM individuals and was not captured by destructive sampling at all. This was also the only lateral root for which we found a significant difference for $\delta^{2}H$ between in situ and CVE measurements (Mann-Whitney *U*-test: $P < 0.05$, compare Figure 4).

We observed a scatter between single in situ measurements in a particular borehole. The average sd for each borehole before label application was 1.5‰ and 10.9‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. We found the highest precision for SC3 tap root with an sd of 0.5‰ and 2.1‰. The highest scatter in $\delta^{18}O$ was measured in SM1 tap root (4.4‰) and for $\delta^{2}H$ in the lateral root of SM3 (24.8‰).

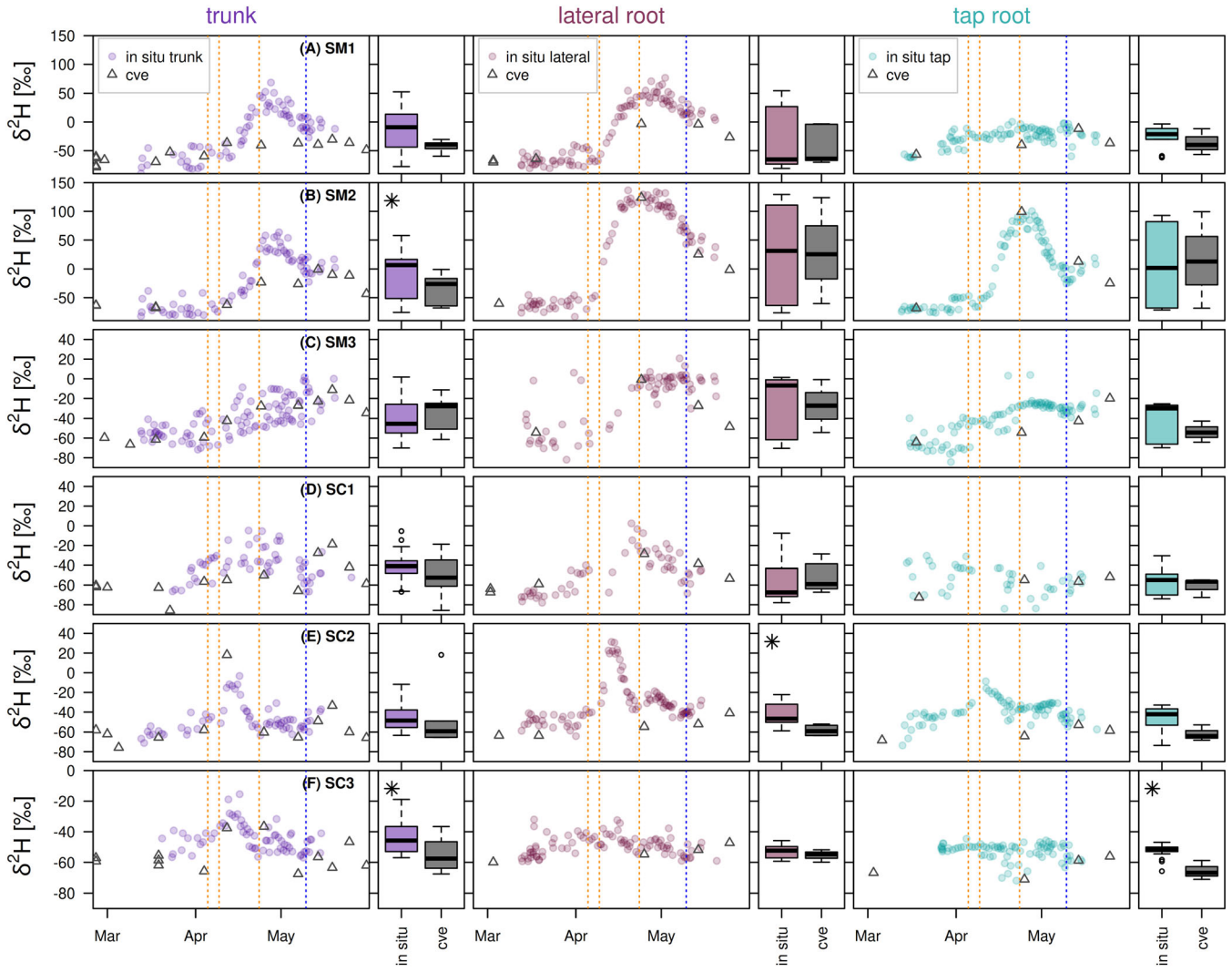

FIGURE 4 Xylem δ^2H measured in *Swietenia macrophylla* (SM, A to C) and *Sideroxylon capiri* (SC, D to F) on three individuals each. For every investigated individual, in situ measurements were conducted in three xylem components, trunk, a lateral and a tap root. Destructive samples are displayed as triangles. To compare the two methods, data are summarised as boxplots next to the timeline. For this, we selected in situ measurements within a period of ±2 days around destructive sampling. The box represents the 25% and 75% quartile. Significant differences between mean values are indicated by an asterisk (Mann-Whitney *U*-test: $P < 0.05$). The timings of labelled irrigation events (orange) as well as the first precipitation event (blue) are displayed as vertical dotted lines [Color figure can be viewed at wileyonlinelibrary.com]

We investigated the influence of time of day on xylem δ values. For this, we combined all measurements before isotopic labelling per tree compartment investigated. δ values were normalised to the respective mean value of every borehole and data were summarised in 3 h bins. The results for both δ^2H and $\delta^{18}O$ are shown in Figure 6. We found a clear diurnal pattern of measured δ^2H with highest δ values around midday and lower values after sunset (amplitude approx. 30‰).

4 | DISCUSSION

We applied the novel borehole equilibration method for the first time in a field experiment,[32] and proved that in situ tree water stable isotope measurements are possible in a high temporal resolution over periods longer than 1 month (compare Marshall et al[32] for a test of around 1 month). This enabled the observation of dynamic changes in tree xylem δ values following labelled irrigation instead of only capturing snapshots in time provided by traditional sampling. In addition, we present one of the first data sets combining in situ field measurements of stable isotopes concurrently in soil and tree xylem water (see Seeger and Weiler[47] for another example). Recently, multiple scientists within the field of ecohydrology have identified this new methodological approach as a crucial step forward in improving our understanding of the complex and dynamic water fluxes across the soil-plant-atmosphere continuum.[23,26,48–53]

An extensive set of destructive samples along the experimental period allowed for a thorough comparison between traditional and novel methods and illustrated their advantages and limitations. Such

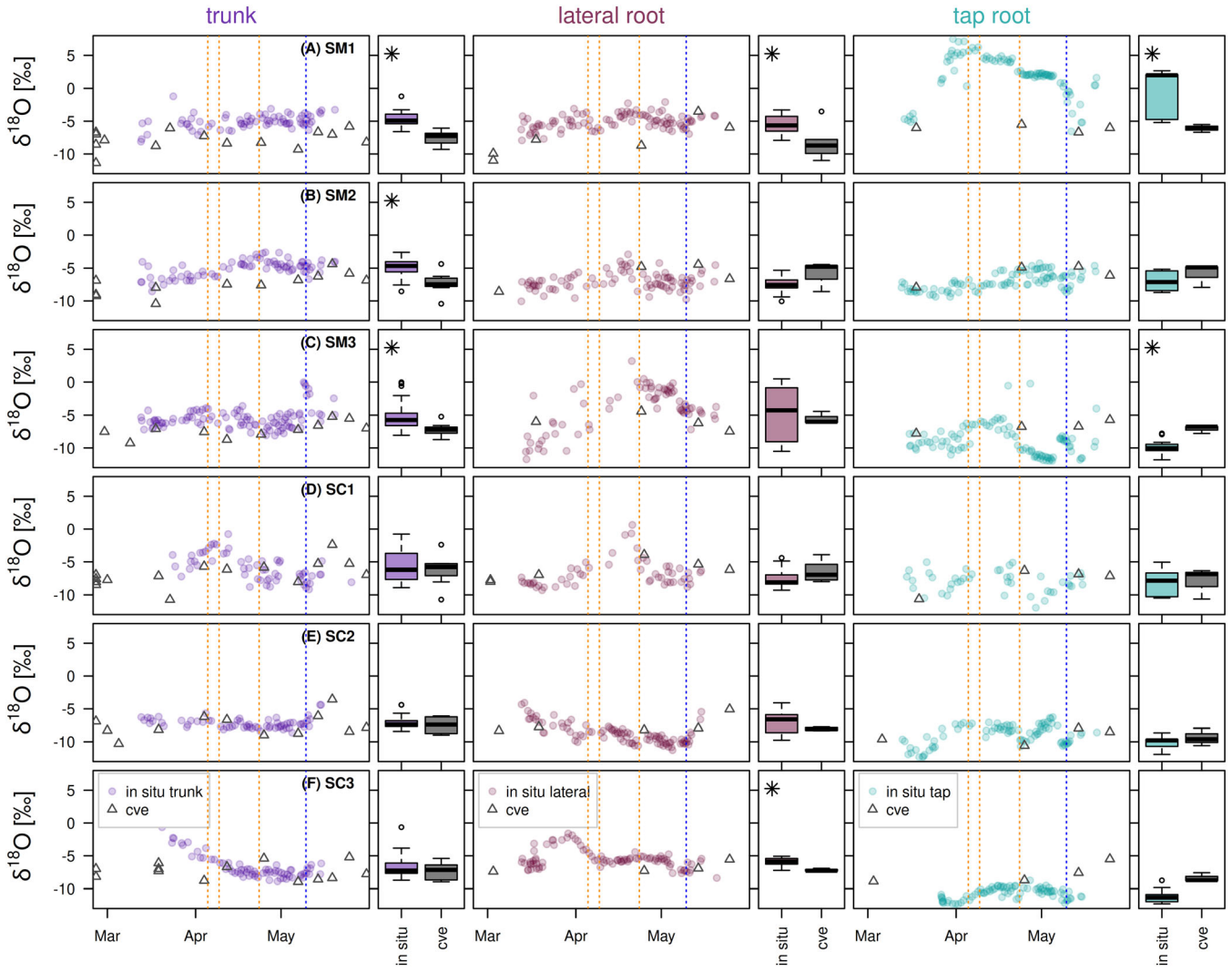

FIGURE 5 Xylem $\delta^{18}O$ measured in *Swietenia macrophylla* (SM, A to C) and *Sideroxylon capiri* (SC, D to F) on three individuals each. For every investigated individual, in situ measurements were conducted in three xylem components, trunk, a lateral and a tap root. Destructive samples are displayed as triangles. To compare the two methods, data are summarised as boxplots next to the timeline. For this, we selected in situ measurements within a period of ±2 days around destructive sampling. The box represents the 25% and 75% quartile. Significant differences between mean values are indicated by an asterisk (Mann-Whitney *U*-test: $P < 0.05$). The timings of labelled irrigation events (orange) as well as the first precipitation event (blue) are displayed as vertical dotted lines [Color figure can be viewed at wileyonlinelibrary.com]

a comparison was necessary to identify how in situ methods could be improved to reach their full potential in depicting isotope dynamics in unprecedented temporal resolution. One should keep in mind that, despite its widespread application over the past decades, recent findings have questioned the reliability of CVE.[54–56] Furthermore, many issues and uncertainties raised lately, for example, representation of natural heterogeneity, influence of the method used and water pools sampled, exchange of xylem water with phloem or tree storage water as well as impact of organic contamination,[23,24] apply to both in situ and destructive sampling. Attempting to enhance novel in situ methods, this discussion focuses on issues related to their application. In addition, methods differ in the water pool they sample[57] and hence inter-method differences likely do not only arise from associated methodological difficulties.

4.1 | Soil water δ values

At natural abundance levels, in situ soil measurements overall agreed well with CVE samples (compare Figure 2). The trend to lower δ values in 5 cm soil depth measured in situ, as compared to CVE, could arise from a bigger influence of ambient air intrusion into the very dry top soil.[7,58]

Noticeable differences between the two methods occurred after the application of labelled irrigation. δ^2H values in CVE samples were much higher than the values measured in situ. A likely explanation arises from the pronounced δ^2H gradient across the soil profile that was created with labelled irrigation. While in situ probes were measuring at a constant soil depth of 5 cm, destructive sampling could have included (due to the nature of the sampling method) soil material above 5 cm with a higher label water quantity. In situ measurements

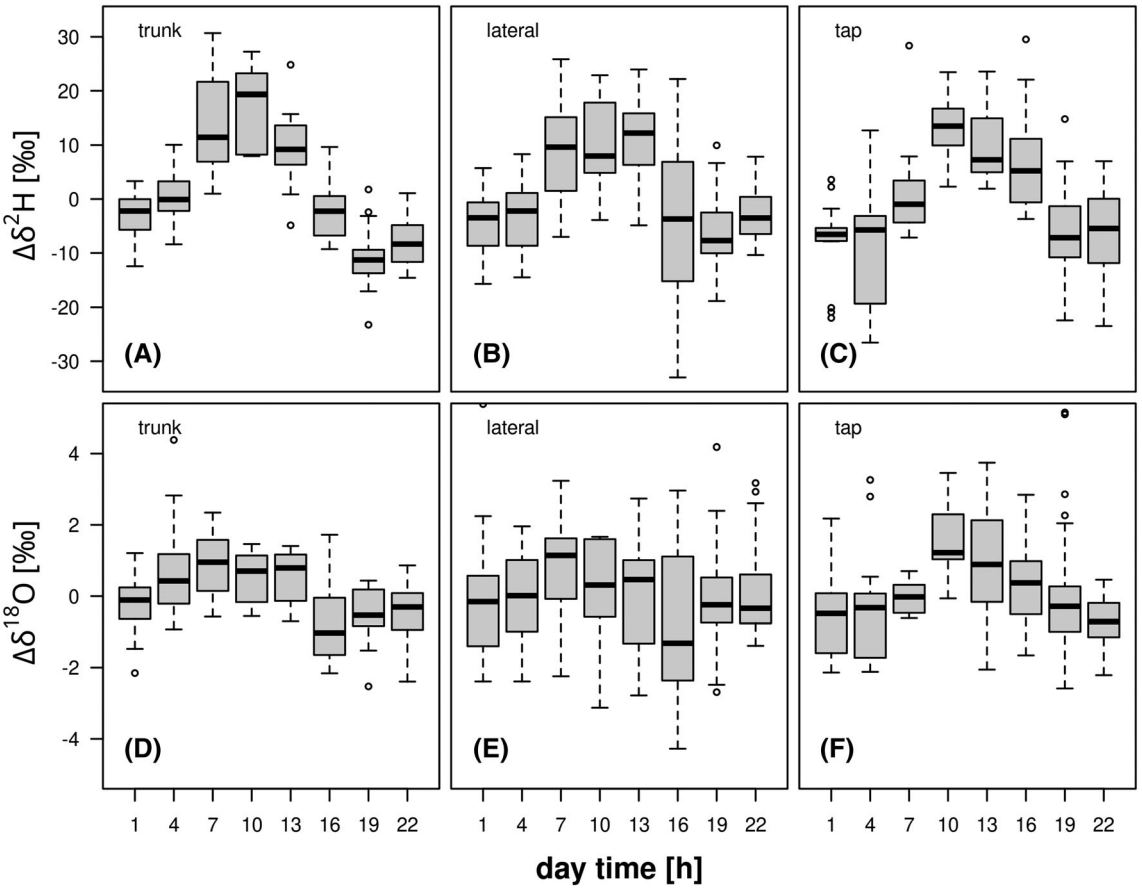

FIGURE 6 Diurnal patterns for both δ^2H (A to C) and δ^{18}O (D to F) per tree compartment investigated. We used in situ data points during natural abundance measurements, that is, before the first labelled irrigation and summarised them in 3 h bins. δ values were normalised to the respective mean value of every borehole

below 10 cm soil depth did not experience changes in response to label application, suggesting that the irrigation water was held near the surface. The higher δ^2H of CVE samples in soil depth >10 cm presumably resulted from a smearing of topsoil containing a strong label signal to subjacent soil depths. In contrast to the results of this experiment, Kübert et al[35] found a higher impact of labelled irrigation on in situ sampling as compared to CVE. The authors discussed different water fractions sampled by different methods as a potential reason but concluded that the most likely cause for the observed discrepancy was that in situ and destructive methods differ in their ability to represent spatial heterogeneity of soil water. In general, spatial isotopic heterogeneity of soil water δ values was often neglected in past studies, even though it can be substantial[59] and is likely the rule rather than the exception.[60] Quade et al[36] estimated for their silt loam site the represented volume for in situ soil probes to be located within 1 to 5 cm distance around the tubing material for wet and dry conditions, respectively. In the presented experiment, it is possible that the clayey soil favoured uneven infiltration due to preferential flow[61] of applied irrigation in larger distance, which in combination with introduced labelled water caused strong spatial isotopic heterogeneity. To account for this, we suggest installing multiple in situ soil probes in the uppermost soil depth, especially when conducting labelling experiments and investigating water stable isotopes in clayey soils. In cases where this is not feasible, spatially distributed destructive samples should be collected, as in the current study.

Spatial heterogeneities also provide an explanation for the different magnitudes of the δ^2H increase in 5 cm between both in situ soil profiles. Although the time course of profile 2 featured a fast and extreme isotopic change and overall higher δ^2H values in 5 cm soil depth, the same soil depth in profile 1 showed a less abrupt response to labelled irrigation. This can be explained by a progressive mixing of soil waters with different δ values, that is, an equalisation of small-scale spatial heterogeneities by diffusion. Although in situ field studies are (so far) often limited in portraying spatial patterns, the presented time series confirms their ability, in contrast to destructive sampling, to enable observations of temporal dynamics in the first place.

Even though sample lines were heated, extreme care and constant checks for condensation were indispensable for both soil and borehole measurements. Diurnal ambient air temperature fluctuations averaged 18.3 ± 3.2°C during the dry season, increasing the likelihood of condensation. Beyer et al[23] provide examples for "good" soil probe data as well as measurements affected by condensation. In consequence, only in situ soil data with previous

flushing was included in the final data set. This decreased temporal resolution of obtained time series by 70% and hence leaves room for method improvement. Condensation was also identified as an important source of error by Beyer et al,[23] Kübert et al,[35] Marshall et al,[32] Oerter and Bowen,[29] Quade et al[36] and Volkmann and Weiler[31] and other coping mechanisms, for example, immediate dilution of sampled water vapour with dry air are discussed herein. Further differences between in situ and destructive soil sampling, as well as criteria for a well-grounded choice of method, for example, cost and time expenses, technical equipment of the field site and required temporal and spatial resolution, can be found in Beyer et al,[23] Kübert et al[35] and Quade et al.[36]

In summary, soil water δ values matched between both methods for destructive samples taken at the location of soil isotope probes, confirming results obtained by novel in situ measurements. Differences between in situ and destructive measurements as well as between the two in situ soil profiles were likely caused by a mixture of the following factors: strong isotopic gradient in the top few centimetres, spatial infiltration heterogeneities, influence of atmospheric intrusion as well as amount of soil volume contributing to measured results.

4.2 | Tree xylem water δ values

SM individuals used the labelled irrigation water in the top soil, although it had little effect on xylem water δ values of SC individuals. Deviating from this species-specific pattern, we observed a lower enrichment and in general slower responses in SM3. This can be explained by its smaller size and the fact that it shed a substantial proportion of its leaves during the dry season and hence presumably had very low water uptake rates.

Trees are living beings and react to wounding by forming physical and chemical borders to restrict a potential expansion of intruding pathogens.[62] This happens whether we take an increment core, install a sap flow sensor or a borehole. Wiedemann et al[63] connected a change of sap flow measurements to progressing wound formation following sensor installation, and it is widely accepted that sap flow sensors should, for this reason, be reinstalled regularly. Similarly, it is expectable that trees would alter boreholes over time. Up until now, we do not know for how long boreholes stay responsive and if, for example, measurements across a vegetation period or a whole year would be impacted by tree wound response. This likely depends on species, ambient conditions and the set-up used and still needs to be investigated systematically. With this in mind, it is not surprising that trees complicated our measurements in unexpected ways even within our rather short experimental period. The following summarises our experiences from the field and provides some thoughts for further developing in situ measurements of xylem δ values.

Borehole measurements were complicated by tree individuals producing liquid that drained into and blocked sample tubing. This happened even though boreholes were initially flushed with acetone in hope to kill cells that produce pitch or other substances in response to wounding, as suggested by Marshall et al.[32] We observed that SC individuals generated a brownish fluid after they ceased transpiration, that is, when SC1 exchanged its entire foliage, and produced latex with increasing stem water content at the beginning of the wet season. Based on this observation, we hypothesised that this is connected to the tension with which water is held within the tree xylem. Potentially, water is transported from cells surrounding xylem vessels to refill embolised vessels[64] at times of low transpiration and hence lowered tension pulling water upwards from the soil to the leaves. This refilling is known to even cause positive sap pressure in spring before bud break in some temperate tree species, for example, birch.[65] The issue of liquid blocking sample tubing could potentially be prevented by installing GPT into tree boreholes, similar to in situ soil probes and probes used in the method test conducted by Volkmann et al.[33] However, it remains to be tested whether produced tree sap could block membrane pores, therefore alter and delay xylem δ values and consequently introduce further uncertainties into measurements. For instance, Seeger and Weiler[47] reported an impact of biofilms on probe heads on measured $\delta^{18}O$ in their experiment with similar duration.

Before the experiment, we explored the effect of dry air stream velocity. For this, we chose a borehole with small diameter and high air tightness and started measurements with low flow rates, that is, just above the intake rate of the analyser but ensuring that excess air was coming out of the open-split. After δ and WVMR values stabilised, we slowly increased the air stream until we reached the desired flow rate. If flow rates were very high and sampled vapour would consequently depart from isotopic equilibrium, WVMR and δ values should decrease. A theoretical examination of the limits of the borehole method is presented in Marshall et al.[32] In post-processing, raw data were screened thoroughly for faulty measurements as well as unstable plateaus. In addition, the system was checked for leaks and freed from condensation daily. A number of other factors influencing δ values and hence causing the observed scatter between single measurements in a particular borehole come into question. They can be categorised into (a) methodological uncertainty and (b) true natural variability[66] and are discussed in this order hereafter. Figure 7 summarises our experiences on how to routinely check the borehole in situ system and identify methodological issues in the field. It also provides useful considerations for post-processing and is intended as a hands-on guide for other field scientists applying the method.

Next to the influence of condensation as described above, derived δ values were impacted by challenges in calculating equilibrium fractionation during phase change. The borehole equilibration method does not allow measurement of δ values in liquid xylem water directly, but values are inferred from vapour phase measurements using temperature recordings. Although we recorded temperature directly within the borehole, some uncertainty about within-trunk and within-borehole temperature gradients as well as on the exact location of phase change and hence isotopic equilibration persists.

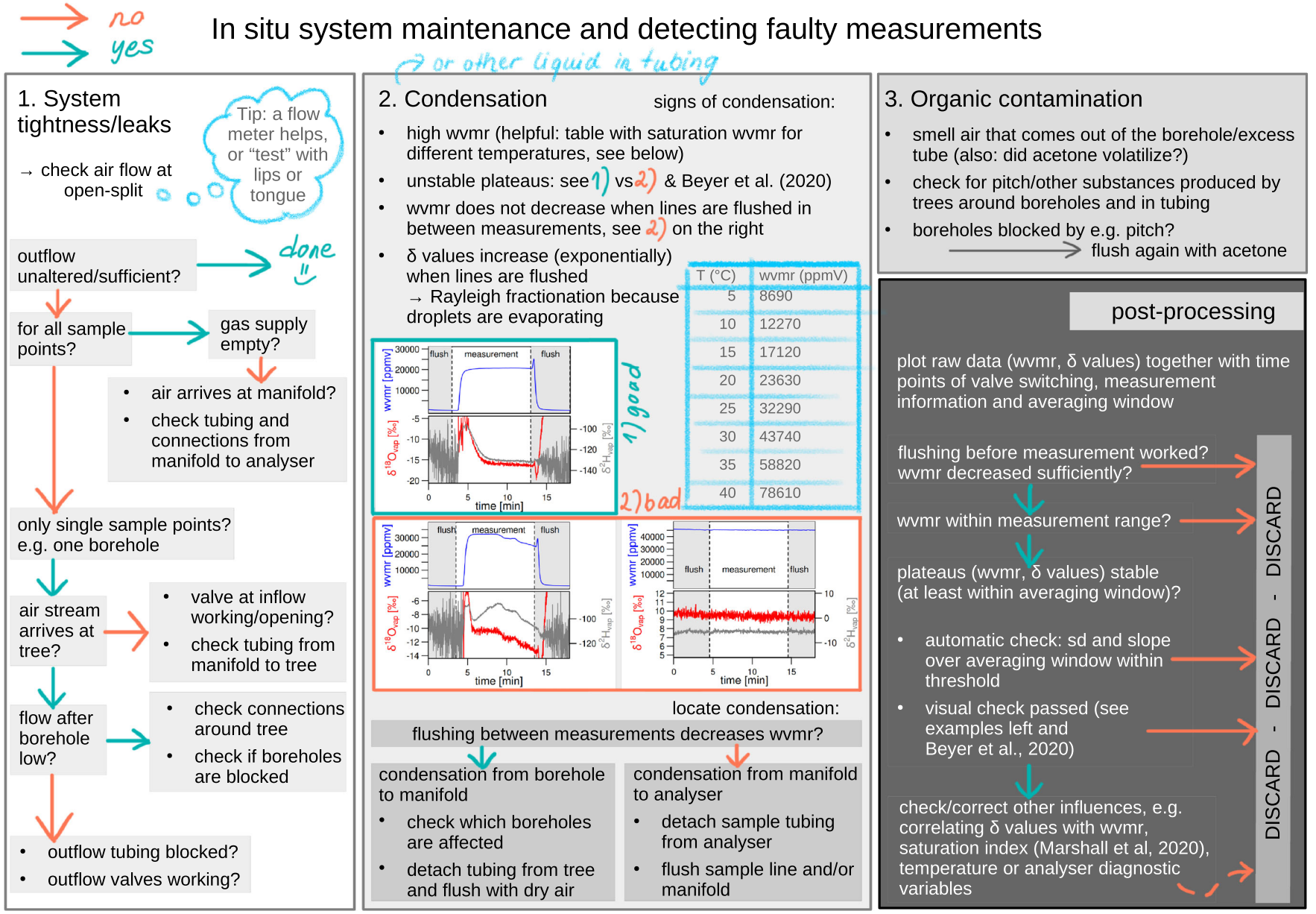

FIGURE 7 Practical guide on how to identify faulty measurements and solve associated issues. The graphic summarises measures that we found useful both in the field and during post-processing and is intended as a starting point for other scientists applying the borehole equilibration and other in situ methods [Color figure can be viewed at wileyonlinelibrary.com]

Organic molecules are known to affect IRIS (isotope-ratio infrared spectroscopy, including CRDS) measurements.[67] Schmidt et al[68] conducted a systematic test comparing liquid water extracted from different plant species and found deviations between IRIS and IRMS (isotope-ratio mass spectrometry) of 2.3‰ and 23‰, for $\delta^{18}O$ and $\delta^{2}H$, respectively. Methanol was found to strongly influence measured δ values of liquid samples (up to 143.0‰ for $\delta^{18}O$ and −1077‰ for $\delta^{2}H$) at a contaminant concentration of 0.1% in the study of Martín-Gómez et al.[69] Up-to-date it remains unclear, whether this applies to the same extent to measurements based on direct water vapour equilibration. We checked for the existence of a relationship between measured δ values and the instrument's diagnostic variable "CH4". We observed negative "CH4" values, which point to the presence of methanol and ethanol in sample air (J. Woźniak, Picarro Inc., personal communication). For natural abundance measurements, we found a significant negative correlation ($P < 0.001$) to $\delta^{18}O$ values. Due to the high variance ($R^2 = 31\%$), we did not correct for the effect. $\delta^{2}H$ and "CH4" were not significantly correlated, which contradicts with recent findings suggesting a higher spectral interference of organic compounds with $\delta^{2}H$ than with $\delta^{18}O$.[68,69] This also points towards other factors underlying the high scatter of $\delta^{2}H$ measurements. An effect of diurnal temperature amplitudes on the IRIS measurements[68] also cannot be excluded. Other considerations potentially affecting in situ borehole measurements, like wall deposits and cavitation, are discussed in Marshall et al.[32]

Apart from methodological difficulties, the observed scatter could also arise from natural variability of xylem δ values. Goldsmith et al[59] observed within tree crown variability of up to 4.2‰ ($\delta^{18}O$) and 25.2‰ ($\delta^{2}H$). De Deurwaerder et al[70] found a similar degree of natural variability: for trees, $\delta^{2}H$ differences of up to 13.1‰ and 18‰ across tree height and within 1 day of intensive sampling, respectively, were reported. As an explanation, the authors proposed diurnal differences in RWU depth in consequence of diurnal plant water potential variations. The resulting differences in δ values of soil water taken up are subsequently propagated along the trunk. Until now, it was simply not feasible to observe temporal short-term variability at one consistent sampling location over an extended time period. Due to the number of sampled boreholes, standard measurements, concurrent soil measurements and the necessity to discard a substantial number of data points during post-processing, we achieved a mean frequency of 1.06 ± 0.22 measurements per day for each borehole (1.67 ± 0.11 before post-processing), restricting the evaluation of diurnal variations of xylem δ values. However, combining measurements before isotopic labelling, we found a clear diurnal pattern of measured $\delta^{2}H$ (compare Figure 6). Even though the observed diurnal course agrees with the theory presented in De Deurwaerder et al,[70] it could also arise from methodological issues, for example, slight and undetected condensation or an incomplete accounting for diurnal temperature fluctuations on isotopic equilibration within boreholes. A methodological cause is supported by the fact that variability of δ xylem values did not noticeably increase after labelling even though the gradient in soil water δ values was strongly enhanced. Next to the just described axial heterogeneity along the tree trunk, radial heterogeneity of xylem water δ values, for example, observed by Volkmann et al[33] could also affect measurements. This also provides a plausible explanation for the weak $\delta^{2}H$ label signal detected in some SM destructive samples.

Such described uncertainties associated with in situ xylem measurements illustrate the need for further systematic investigations (of single influencing factors and under controlled conditions) before they can be utilised to routinely and accurately determine natural abundance δ values at sub-daily resolution. Even though we might have to accept a lower accuracy when using in situ methods,[23] the comparably high number of data points collected undoubtedly represents an advantage. The time point of destructive sampling surely influenced the averages of xylem δ values calculated across the experimental period, which resulted in inter-method differences. In contrast, in situ sampling allowed us to observe the temporal evolution of δ xylem following a label pulse, which might easily be missed with destructive sampling, if the impact was only short-term (compare Figure 4, time series for SC2 lateral root). The higher frequency of collected data points also provided valuable insights into measurement precision and allowed for a robust estimation of associated uncertainties, unlike restricted sample amounts using traditional approaches. Measurements were also accessible in real-time directly in the field, which makes it easier to spot and solve problems. It also provides an opportunity for targeted sampling of plant physiological variables, for example, at the time of label uptake.

Maintenance of the in situ system and post-processing were time consuming. Method development should therefore aim at simplifying the approach to facilitate access to more researchers within the interdisciplinary field of ecohydrology[23] and to ideally achieve unattended, continuous measurements. At this point it cannot be definitely assessed if the latter will ever be possible or will remain an idealised conception. In addition, this dream is based on installing and maintaining rather complicated technical set-ups and deploying measurement devices directly in the field. Where this is not desired or feasible, we could also envision a semi in situ set-up where water vapour is sampled from boreholes in a comparable way as described here but then stored in inert and gastight containers, transported to the laboratory and analysed there for its isotopic composition. Although measurements then cannot be conducted automatically, it would decrease the possibility for leaks and condensation due to shorter tubing lengths and a less complex system.

4.3 | Borehole equilibration as a novel possibility to measure δ values of root xylem water

With this data set, we showed that the borehole equilibration method allows measuring xylem δ values in roots with a minimum borehole length of 5 cm[32] and hence provides new opportunities to investigate plant water use and within plant water transport and mixing. Literature exists on measuring water fluxes in parts of the root system to

disentangle hard-to-observe below-ground processes and the root system's contribution to overall tree water uptake and transpiration.[71] For example, David et al[72] monitored sap flow in the trunk as well as in superficial roots of *Quercus suber* over a period of 1.5 years and used the data to estimate contributions of shallow soil and groundwater to overall tree water uptake with changing seasonality. To the best of the authors' knowledge, time series of water stable isotopes in tree roots have not previously been measured.

As expected, due to their proximity to and presumably high proportion of fine roots in the labelled top soil, δ^2H in lateral roots of SM in general increased faster and to a larger extent than in other compartments in response to labelling events. Meanwhile, δ^2H changed least in tap roots. An exception to this is SM2, where we could not reach the tap root located directly below the trunk and chose a different root that seemed to be extending downwards. However, measured δ values suggested that it rather classified as a lateral root. This clearly shows that we are restricted in predicting the extent and functioning of distinct roots by assessing a small section of the entire root system and shows the possibilities that arise from determining δ values within tree roots. Like for SM, the highest δ^2H was also found in a lateral root for SC (SC2). Surprisingly, for SC water within lateral roots and trunk xylem was not impacted markedly by labelling.

It should be noted that measurements of SM1 tap root yielded unreasonable δ values. This was especially noticeable for $\delta^{18}O$, where values were about 10‰ higher than in tap roots of other individuals (see Figure 5). Concurrently, we also observed higher δ^2H values. The increase was, however, less remarkable due to the higher uncertainty and the potential influence of applied label. Because the pattern observed neither matched with destructive samples nor affected measured trunk values, it is likely a methodological artefact. One possible explanation is that no water was transported through this particular root during the dry season and hence the applied dry air stream enriched the water surrounding the borehole. This would also explain why a decrease to logical δ values occurred with increasing soil water availability at the beginning of the wet season. The presumption of evaporative enrichment is supported by the fact that d-excess was decreased (on average -50.7 ± 20.3‰) during the time of higher $\delta^{18}O$ values.

It would be interesting to combine measurements of δ values within different roots with areal or point labelling as well as with other plant root variables such as transported water volume, that is, sap flow measurements, or transported nutrients. This provides information on the location of water (and nutrient) sources tapped by different parts of the root system and elucidates how they contribute to overall tree water uptake. In view of methodological limitations,[73] we believe that the presented approach could provide an innovative way forward in understanding hidden and hard-to-observe plant below-ground processes and the contribution and functions of different root system compartments. This is particularly important in view of persistent fundamental knowledge gaps on the functioning of different root compartments, in particular deep roots.[74]

5 | CONCLUSION

We collected a unique data set in a semi-arid, tropical dry forest in the northwest of Costa Rica, illustrating both spatial and temporal heterogeneity of water stable isotopes using novel in situ methods and destructive sampling of soil and xylem water. We applied the borehole equilibration method for the first time in a field experiment and proved that in situ tree water stable isotope measurements are possible at a high temporal resolution over a period of several months. Having a consistent sampling point in the tree xylem allowed, for the first time, monitoring of water stable isotopes repeatedly in root xylem, which opens up new possibilities to investigate tree RWU.

Following multiple irrigation events with different levels of ^2H-enrichment, we observed the changes in isotope values as applied water moved through the soil and the trees' roots and trunks. In our case, single irrigation events were not clearly propagated to the isotopic composition of xylem water. Therefore, the high temporal resolution recorded would not have been necessary to depict xylem isotope dynamics. However, it enabled a thorough evaluation of the methods precision when applied in the field, which is not possible to the same extent for traditional destructive sampling.

The time courses of the two methods, that is, in situ and destructive sampling, in general agreed well. For soil water, systematic inter-method differences occurred after labelling and were mainly attributed to the strong isotopic gradient as well as spatial heterogeneity introduced to the top few centimetres of soil. For in situ xylem measurements, a scatter between individual measurements within the same borehole was observed. We evaluated and discussed potential methodological reasons, that is, the impact of condensation, interference of volatile organics, uncertainty in determining the temperature at the location of isotopic equilibration and tree wound responses. These should further be investigated to improve in situ xylem isotope measurements, enabling an accurate determination of δ values at natural abundance levels. Natural within trunk heterogeneity as well as timing of destructive sampling likely also caused inter-method differences. Future efforts should aim at improving and automating the indication of corrupted measurements and establishing post-correction schemes to increase the percentage of reliable measurements. Furthermore, simplifying in situ measurements of xylem δ values would be desirable to allow for a widespread application within the field of ecohydrology and related disciplines investigating tree RWU.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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