Xylem water in riparian Willow trees (*Salix alba*) reveals shallow sources of root water uptake by *in situ* monitoring of stable water isotopes

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Abstract

Root water uptake (RWU) is an important critical zone process, as plants can tap various water sources and transpire these back into the atmosphere. However, knowledge about the spatial and temporal dynamics of RWU ~~root water uptake~~ and associated water sources at both high temporal resolution (e.g. daily) and over longer time periods (e.g. seasonal) is still limited. We used cavity ring-down spectroscopy (CRDS) for continuous *in-situ* monitoring of stable water isotopes in soil and xylem water for two riparian willow (*Salix alba*) trees over the growing season (May to October) of 2020. This was complemented by isotopic sampling of local precipitation, groundwater and stream water in order to help constrain the potential sources of root water uptake. A local eddy flux tower, together with sap flow monitoring, soil moisture measurements and dendrometry were also used to provide the hydroclimatic and ecohydrological contexts for *in-situ* isotope monitoring. In addition, respective bulk and twig samples of soil water and xylem water were collected to corroborate the continuous *in situ* data. ~~In terms of the soil the cryogenic extracted data showed good comparability with *in situ* results while for xylem data the results are only moderate or not comparable.~~ The monitoring period was characterised by frequent inputs of precipitation, interspersed by warm dry periods, which resulted in variable moisture storage in the upper 20 cm of the soil profile and dynamic isotope signatures. This

variability was greatly damped at~~in~~ 40 cm and the isotopic composition of the sub-soil and groundwater was relatively stable. The isotopic composition and dynamics of xylem water was very similar to that of the upper soil and analysis using a Bayesian mixing model inferred that overall ~ 90% of RWU ~~root water uptake~~ was derived from the upper soil profile. However, while for the soil water signatures, the direct equilibrium method ~~cryogenic extractions~~ showed good comparability with *in situ* results; for xylem water~~ ~~ the cryogenic extractions signatures were only moderately or not at all comparable. Sap flow and dendrometry data indicated that soil water availability did not seriously limit transpiration during the study period, though ~~there was a suggestion~~it seemed that deeper (>40 cm) soil water ~~might~~ provided a higher proportion of RWU ~~root water uptake~~ (~30%) in a drier period in the late summer. The study demonstrates the utility of prolonged real time monitoring of natural stable isotope abundance in soil-vegetation systems, which has great potential for further understanding of ecohydrological partitioning under changing hydroclimatic conditions.

Key words: isotopes, xylem water, soil waters, mixing model

1. Introduction

Plants - as the interface between atmospheric and soil water – have an important influence on the water cycle. In terrestrial ecosystems, transpiration from plants accounts for up to 90% of evapotranspiration (Jasechko et al., 2013) and up to 70% of incoming precipitation during the growing season (Kozii et al., 2020). Furthermore, vegetation intercepts and redistributes precipitation via canopy evaporation, throughfall and stemflow (Friesen and Van Stan, 2019). Subsequent interactions between soil particles and RWU ~~root water uptake~~ influence soil moisture and soil hydraulic conductivity which in turn affect infiltration and runoff (Thompson et al., 2010). As tree roots take up water, different species can have contrasting preferences in their source water pools (Jackson et al., 1995), while individuals of varying size and age are capable of ~~up~~taking up water from different soil depths (Kühnhammer et al., 2020). However, RWU ~~root water uptake~~ cannot necessarily be simply correlated with root distribution as it also depends on moisture and nutrient availability at different

times of the year (Ehleringer and Dawson, 1992). Consequently, there is still limited knowledge on the temporal dynamics of RWU root water uptake and associated water sources both at high temporal resolution (e.g. daily) and over longer time (e.g. seasonal) periods (Berry et al., 2018; Beyer et al., 2020).

Stable isotopes of water are convenient natural tracers commonly used for the estimation of sources and ages of runoff, (McDonnell et al., 2010; Sprenger et al., 2019), partitioning of evapotranspiration (Williams et al., 2004; Rothfuss et al., 2010; Dubbert et al., 2014), and RWU root water uptake depth distribution (Dawson and Ehleringer, 1991; Goldsmith et al., 2019; Beyer et al., 2018). With the development of compact laser spectrometry systems, *in situ* monitoring of stable water isotopes in soil and tree xylem is now facilitating higher temporal and spatial resolution assessment to advance well-established destructive sampling methods (Herbstritt et al., 2012; Rothfuss et al., 2013; Volkmann and Weiler, 2014; Oerter and Bowen, 2017). However, destructive sampling followed by cryogenic vacuum extraction, is still widely used for stable water isotope analyses in plants (West et al., 2006; Yang et al., 2015; Orlowski et al., 2016; Sohel et al. 2021) and is usually required to corroborate *in situ* measurements (see Mennekes et al., 2021). Despite the complexity, daily maintenance and resource demands, the application of *in situ* methods in different compartments of the critical zone including soil, trees (Kübert et al., 2020; Beyer et al., 2020; Marshall et al., 2020) and the atmospheric-interface (Braden-Behrens et al., 2019) is increasing. Unfortunately, no general, widely-tested setup for such *in situ* measurements has been established and agreed upon yet (Beyer et al., 2020; Marshall et al., 2020).

Despite limitations, destructive sampling in previous studies revealed important process-based insights into plant-soil water interactions. For example, New Zealand riparian willows were analysed monthly over the course of seven months showing that water sources fluctuated seasonally with root water uptake (RWU) from the near-stream aquifer during summer and from groundwater during

winter (Marttila et al., 2017). A labelling experiment of two small willow trees (*Salix viminalis*) in a lysimeter by Nehemy et al. (2021) also showed RWU to be variable and linked to soil water potential and tree water deficits, shifting deeper as the upper soil dried. *In situ* monitoring has also revealed other subtleties: sub-daily investigations of xylem water from several tree and shrub species in French Guiana, China and Germany haves found morning RWU (when transpiration is low) to be sustained by deeper soil layers, while daytime RWU was concentrated in shallow soil layers (De Deurwaerder et al., 2020).

For xylem water sampling in particular, destructive methods still dominate the research field. However, it has been shown that the cryogenic extraction method can affect the results due to volatile organic compounds (like alcohols) which can be mixed into the extracted liquid sample (Martín-Gómez et al., 2015) as well as extraction of water held in cell walls (Barbeta et al., 2020). These effects on xylem water samples are only linked to cryogenic extraction and seem absent in *in-situ* experiments. For example, Volkmann et al. (2016a) installed a probe horizontally inside the sap wood of a stem borehole to sample xylem water *in-situ.* This method, while delivering a new approach for *in-situ* measurements in plants, showed none of the above-mentioned effects but an unexplained ^{18}O offset. Marshall et al. (2020) suggested that this offset might be due to non-equilibrium conditions, leading to their new method of stem borehole equilibration, where the borehole went through the complete stem. This approach was tested to simplify the measuring method and allows for continuous evaporation of liquid xylem water into a flowing airstream that passes the borehole (Marshall et al., 2020). The model description of flow is based on a central core of moving air flow that passes a volume of still air allowing water vapor to diffuse from and to the borehole wall or the moving air stream (Marshall et al., 2020). The method was successful for a cut stem, as well as a live, Scots Pine (*Pinus sylvestris*) (Marshall et al., 2020). It has not been tested for angiosperms yet.

Despite the advantages, *in-situ* xylem isotope monitoring in the field continues to be rare due to methodological and logistical challenges including root distribution, soil heterogeneities and ambiguous water sources. A recent *in-situ* labelling experiment by Seeger and Weiler (2021) using the same type of stem borehole probes as Volkmann et al. (2016a) inside European beech (*Fagus sylvatica*) trees over the course of 12 weeks found that xylem water differed from actual RWU. This led to the conclusion that xylem water isotopic composition does not necessarily represent simple ~~root water uptake~~RWU, but rather an integration over certain fractions of ~~root water uptake~~RWU from different sources in the past (Seeger and Weiler, 2021). They suggested sub-daily monitoring of tree xylem water isotopes might not be suitable to investigate short term RWU dynamics and instead recommended to focus more on spatial heterogeneity in soil and xylem composition (Seeger and Weiler ,2021). Another labelling experiment by Mennekes et al. (2021) investigated plot-scale xylem water isotopes in three different trees of various species (*Pinus pinea*, *Alnus incana* and *Quercus suber*) at two different heights (15 and 150 cm) over the course of 10 weeks using similar tree probes described by Volkmann et al. (2016a). They sampled xylem water isotopes at ~5-hourly intervals and showed that *in-situ* measurements delivered more consistent results compared to destructive samples. Another experiment from May to September 2018 applied an *in-situ* system investigating $\delta^{18}O$ in soil and xylem water of three beeches with an ~2 hourly interval in Switzerland (Gessler et al. 2021). ~~The setup from Gessler et al. (2021) was described to be analogue to Volkmann and Weiler (2014) and Volkmann et al. (2016a, b).~~ With a Bayesian isotope mixing model they ~~Gessler et al. (2021)~~ showed that the beeches did not ~~quantitatively~~ compensate~~d~~ for restricted top soil water by deeper uptake, though the trees recovered rapidly after the rewetting. Recently, a study by Kühnhammer et al. (2022) tested the novel *in-situ* borehole equilibration method ~~invented by Marshall et al. (2020)~~ for xylem and roots of tropical trees, together with *in-situ* soil measurements similar to Volkmann et al. (2014). Over four months, they measured δ^2H and $\delta^{18}O$ in xylem, roots, and soil (only at night and at least every second day) and conducted labelled irrigation events ~~(Kühnhammer et al. 2022)~~. In general, irrigation events were

~~observable~~measurable, ~~–~~though single ones ~~were~~~~showed~~ not clearly distinguishable in xylem water isotopic compositions. ~~(Kühnhammer et al. 2022).~~

The integration of high-frequency measurements of water stable isotopes with other plant-physiological variables like sap flux and stem size variation can also increase our understanding of sub-daily and seasonal variations in xylem isotopic composition, and improve the comparison of isotope data from different individual plants or trees (De Deurwaerder et al., 2020; Nehemy et al., 2021). Stem size variation is~~ ~~caused by the imbalance between canopy transpiration and ~~root water uptake~~RWU or its incremental increase (growth) (cf. Zweifel, 2016). ~~This~~Diurnal patterns can show~~ imbalance causes a~~ swelling (water uptake) or shrinking (transpiration) response of the tree reflecting its stem water tension (Zweifel et al., 2005)~~linked to three co-occurring and opposing processes, which are incremental increase (or growth), swelling (from water uptake), and shrinking of the stem (Zweifel, 2016). The latter can be linked to tree water deficit and occurs during imbalances between transpiration and RWU (Zweifel et al., 2005)~~ or differences in osmotic water potentials depending on the sugar content of the phloem (Mencuccini et al., 2013). Sap flow monitoring, thus, also provides a continuous proxy of transpiration rates (Paloschi et al., 2021). Although ~~usually ~~reflecting the atmospheric vapor pressure deficit (Butz et al., 2018) and canopy conductance, sap flow rates may react to soil water availability as well, but these responses are highly species-specific (Brinkmann et al., 2016) and may depend on the distribution of individual water sources, or access to groundwater (Süßel and Brüggemann, 2021). In general, low soil water content combined with high air temperatures may lead to moisture stress resulting in ~~decreased sap flow, ~~lower leaf water potential and turgor, a decline in photosynthesis, stomata closing, decreased sap flow, reduction in cell enlargement and growth (Joshi et al., 2016).

To identify water sources used by plants from isotopic composition, linear end member-mixing models like IsoSource (Phillips and Gregg, 2003) have been widely used (Barbeta and Penuelas, 2017). More recent approaches with Bayesian frameworks like SIAR (Parnell et al., 2010) or MixSIAR (Stock et al., 2018) also provide statistical uncertainty assessments (Rothfuss and Javaux, 2017). These models are based on the basic assumption that the end members include all potential sources of xylem water and are isotopically distinct (von Freyberg et al., 2020). Furthermore, all end members of a mixture should be identified and uncertainties of the sampling/monitoring method should be taken into account. Recent findings suggest combining Bayesian mixing models with ecohydrological information like soil properties and climate to improve their results (Rothfuss and Javaux, 2017; Kühnhammer et al., 2020). Von Freyberg et al. (2020) stated that *in-situ* monitoring of stable water isotopes with high temporal resolution may help detect isotopic anomalies (like fractionation or new water inputs) and thus, result in more reliable water source attribution.

Here, we used continuous *in-situ* monitoring of the natural abundance of stable water isotopes in soil and xylem water of riparian willow trees (*Salix alba*) under field conditions over a period of five (soil) and three (trees) months. Our overarching research question was: Can we generate new insights into fluxes across the soil-plant-atmosphere-continuum from combined *in-situ* isotope variation and conventional ecohydrological monitoring (e.g. sap flow, biomass accumulation, soil moisture) to assess water sources tapped by the willow trees? In order to understand uptake of potential water sources, we compared the isotopic composition of xylem water with soil water as well as precipitation, surface water and groundwater from liquid sampling. We also used destructive bulk soil water and xylem sampling for comparison with *in-situ* measurements. Furthermore, we also monitored soil moisture, sap flow velocity, stem size variation and eddy flux covariance on site. Finally, we used this data in the Bayesian approach mixing model SIAR to calculate the likely distribution water sources used by the trees. Our specific research question was: Can we generate new insights into fluxes across the soil-plant-atmosphere-continuum from combined *in situ* isotope variation and conventional

2. Study site

The study site is located in the SE of Berlin, Germany (Fig. 1a). The climate is continental temperate with long-term (1981 – 2010) mean annual rainfall of ~570 mm (Deutscher Wetterdienst (DWD), 2020a) and temperature of 9.3 – 10.0 °C (DWD, 2020a). Berlin is situated in the North European Plain which was formed during the Weichselian glaciation. (Deutsche stratigraphische Kommission, 2016). A glacier tongue stretching from east to west formed a bowl-shaped depression which today contains the largest lake of the city, the Lake Müggelsee at an altitude of 32m. The lake's inlet and outlet is the river Spree. As a result of groundwater pumping since 1905, lake water can infiltrate into the adjacent groundwater aquifer (Driescher et al., 1993). The upper unconfined aquifer at the Müggelsee consists of sandy and gravel sediments and is underlain by an aquitard of silt and till at about sea level (Driescher et al., 1993).

The Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) is located (Fig. 1a) on the Northern shore of the Lake Müggelsee. In its grounds, an area of roughly 480 m² was chosen as our study site and focused on two willow trees (*Salix alba*). To the North of the trees is a small wetland and, in the East, a small woodland separated by a small stream (Fig. 1b). The stream is fed by artificial fish ponds which are constantly sustained by pumped lake water. The two willow trees stand relatively isolated and were chosen to replicate the same species and have similar ages (14 years). The northern willow (height ~18 m, later referred to as Northern Willow) had a stem-diameter of 398 mm and the southern willow (height ~19 m, later referred to as Southern Willow) of 353 mm (16.07.2020). Willow trees were described by Nehemy et al. (2021) as having a fast resource acquisition strategy and are known to have large water demands due to typically high transpiration rates (~30 – 100 l/day cf.

Schaeffer et al., 2000). Hence, it could be expected that the trees respond rapidly to variations in water availability. In addition, willows are tolerant of waterlogged conditions and can source water from groundwater, so they potentially have flexible strategies of ~~root water uptake~~RWU depending on soil moisture conditions (Marttila et al., 2017). At our site th~~at also~~is includes the lake-fed stream to the east of the trees.

In addition to the willows and a few other trees fringing the stream and wetland, the area is covered by grass and moss. There is also a well which we used to monitor groundwater level and isotopes. Lake Müggelsee is roughly 50 m away from the study site. Despite developing Brown Earth characteristics, the soils comprise relatively homogenous silty sands, with the upper soil layer having a higher organic content. Those soils developed on backfilled ground (following construction work at IGB), which results in the top soil being classified as an Anthrosol (Rossiter, 2007).

3. Data and methods

3.1 Hydroclimatic and hydrometric monitoring

The study was conducted over most of the growing season in 2020 (from May 20th to October 11th). Climate conditions were monitored using a weather station integrated into a portable ~~E~~eddy ~~C~~covariance system (Li-cor Biosciences, Lincoln, NE, USA with LI 7500DS open path analyser; wind measurements via Gill Windmaster pro and a Smart Flux 3 system, frequency 10-20 Hz (Burba, 2013)) (Fig. 1~~C~~c). Automatically calculated mean values were logged over 30 min. Measured air temperature, wind (speed and direction), vapor pressure deficit, solar radiation, ~~gas fluxes,~~ precipitation, top soil heat flux, and soil moisture were used in this study. To calculate soil heat flux and moisture, soil water potential was measured with ThetaSondes ML2 (Delta-T Devices, Burwell, Cambridge, UK; accuracy ±2 to 5%) at a depth of 5 cm. The Eddy Flux system automatically calculated evapotranspiration from the latent heat flux (Burba, 2013). However, as the Eddy Flux tower was not above the tree canopy, ~~had~~

~~not a full undisturbed footprint we used the~~ evapotranspiration data were used mainly for~~as~~ qualitive assessment~~s~~ of dynamics ~~in evapotranspiration~~ rather than the absolute values.~~the Eddy Covariance results for evapotranspiration are considered as an indicator of dynamics or variability rather than absolute values~~. Further, the ~~all~~ measurements ~~results~~ of the Eddy Covariance were considered to reflect the general environment of the willows and not the specific site. In some cases (e.g. power outage) occasional data gaps occurred which were infilled with data from the IGB weather station, ca. 15 m away at the rooftop of the IGB building. The precipitation data of both stations were compared with the open access precipitation data from the German Weather Service (DWD) of the "Berlin-Marzahn" station (DWD, 2020b).

Soil moisture monitoring took place in two soil pits (Pit A and Pit B) at three depths: 10, 40, and 100 cm. Pit A was located next to the southern willow tree (Southern Willow) and partially covered by its outer branches while Pit B was further south and not covered (Fig. 1c ~~C~~). Soil moisture and temperature were measured at the three depths with water content reflectometers CS616 (Campbell Scientific, Inc. Logan, UT USA; accuracy ±2.5 % for volumetric water content (VWC)) and BetaTherm 100K6A1IA Thermistors T107 (Campbell Scientific, Inc. Logan, USA; tolerance ±0.2°C (over 0° to 50°C)), respectively with a CR800 Datalogger and Multiplexer (Campbell Scientific, Inc. Logan, USA) logging every 10 min. A delay in completion of Pit A was related to Covid19 lockdowns and resulted in a shorter period of record.

Groundwater levels nearby were monitored with an automatic datalogger (groundwater level probe) at an interval of 15 min (see location in Fig. 1b ~~B~~). For comparison, the groundwater level was also measured manually once a week with a water level meter. The average groundwater level is around 2.2 m below ground level, and relatively stable with seasonal variations < 10 cm.

3.2 Ecohydrological monitoring

We continually monitored sap flow and variation in the stem circumference of the willow trees. Sap flow was measured at 15 min intervals using the heat ratio method (Burgess et al., 2001) with four sap flow meters (SFM1 instrument, ICT International, Australia), from which two per tree were installed on the North and South side of the trunk. All sensors were installed at breast height. The measured increase in sapwood temperature following the release of a heat pulse downstream and upstream of the heater is calculated into heat pulse velocity (V_h) after Marshall (1958) as

$$V_h = \frac{k}{x} \ln\left(\frac{v_1}{v_2}\right) 3600 \underline{\hspace{6cm}} (1),$$

where k is the thermal diffusivity of fresh wood, x is the distance between the heater and either temperature probe, and v_1 and v_2 are the increases in temperature at equidistant points downstream and upstream, respectively. Further information on the theory may be found in Burgess et al. (2001).

Variation in stem circumference were observed with two Dendrometers (DR Radius Dendrometer, Ecomatik, Dachau, Germany), one per tree at the northern stem side, at a height of 95 cm measured from the soil surface. Data were logged at a 15 min interval with a CR300 Datalogger (Campbell Scientific, Inc. Logan, USA).

3.3 Stable water isotope monitoring

Stable isotopes of water were monitored in precipitation, groundwater, lake and stream water, bulk soil water, soil water vapor and xylem water vapor. Precipitation water was collected with an autosampler ISCO 3700 (Teledyne Isco, Lincoln, USA) (see Fig. 1c C). The autosampler bottles were filled with a paraffin oil layer > 0.5 cm in thickness (after IAEA/GNIP, 2014) to avoid evaporative effects. Samples were collected at an interval of 4 hours. Lake and stream water samples were collected weekly via grab sampling. A similar interval was used for groundwater, which was accessed with a submersible

pump (COMET-Pumpen Systemtechnik GmbH & Co. KG, Pfaffschwende, Germany). For a period (~~16.09.~~ ~~– 14.11.~~16 September to 14 November 2020) the artificial fish ponds were also sampled as a potential water source for ~~root water uptake~~RWU. All liquid samples were extracted with a canula equipped syringe, filtered with a cellulose acetate filter (0.2 μm pores) into glass vials and stored in a fridge until analysis and ~~. All liquid samples were~~ analysed via CRDS (Picarro L2130-i, Picarro Inc., Santa Clara, CA, USA) at the IGB laboratory.

Destructive sampling of bulk soil water was conducted monthly from June (after the first COVID-19 lockdown) to October 2020. Samples were collected at two locations: an open space with grass cover and a 2nd location close to ~~~~Northern Willow~~the willow further north (later referred to as Northern Willow)~~. Replicate samples were collected with a hand auger (diameter: 2 cm) at $0-10$, $10-20$, $20-40$, $40-70$, and $70-100$ cm depth. Samples were filled in metalized bags and analysed with the direct-equilibrium method from Wassenaar et al. (2008). Details of sample preparation can be found in Kleine et al. (2020). The samples were equilibrated for roughly 48 h before analysis. For correction, nine 10 ml standard water samples δ^{18}O (-10.3, -7.68, 2.91 or 1.53‰) and δ^2H (-72.81, -56.70, 0.78 or 16.74‰) were used during every measuring routine. Soil water vapor analysis from the bags was conducted with a Los Gatos Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS) triple water-vapor isotope analyser (TWIA-45-EP, Los Gatos Research, Inc., San Jose, CA, USA). In this paper, we will refer to these samples measured with the direct-equilibrium method as "bulk soil water".

Further, destructive sampling of xylem water was also conducted monthly from July to October 2020 together with the bulk soil water sampling. Each time three sun-exposed branches were collected on Northern and Southern Willow, respectively. Only twigs with intact bark were collected and had their bark and phloem removed to prevent interference. Next, the twigs were cut inside 12 ml vials from Exetainer® and immediately sealed with a septum cap. In the field, the vials were stored in a

polystyrene container and frozen after reaching the lab until cryogenic vacuum extraction. The cryogenic vacuum extraction was done according to Koeniger et al. (2011), but with 60 – 90 min of extraction time per sample. Before the extraction an empty vial is frozen with liquid nitrogen and evacuated by a vacuum pump. Next, the sample is frozen and connected to the evacuated vial via a stainless-steel capillary tube (Koeniger et al., 2011). Afterwards, to extract the water, the sample vial was placed in a 105°C heated custom-built isolated aluminium block (Gaj et al., 2016). After extraction the xylem water samples were measured using CRDS (Picarro L2130-i). We will further refer to the xylem water extracted by cryogenic vacuum extraction as "cryo-xylem water".

Stable water isotopes in soil water vapor were monitored via CRDS (Picarro L2130-i) in the two soil pits (Pit A and Pit B) at the three depths (Fig. 2). For vapor extraction in the soil, 7 cm long polypropylene membranes (0.2 µm pores) (Kübert et al., 2020) were used. In this paper, we will refer to the samples of the *in--situ* soil pits as "*in situ* soil water".

Xylem water vapor was monitored with the same vapor sampling device from the two willows (Northern and Southern Willow) inside two stem boreholes each (Fig. 2). Xylem sampling was also delayed due to restrictions resulting from COVIDovid-19 lockdowns. Consequently, measurements of Southern Willow started at 22.06.2020 and Northern Willow at 10.07.2020. At the beginning of the monitoring, both willows showed wounding effects resulting in unrealistically high isotopic compositions declining exponentially over time. Hence, these time periods were excluded from further analysis. The boreholes were at 30 cm (further referred to as "lower") and 170 cm (referred to as "upper") height above the soil surface. As in the soil, polypropylene membranes were inserted into the boreholes passing the stem horizontally (stem borehole equilibration). We will refer to all xylem water samples by this method as "*in situ* xylem water". The method of stem borehole equilibration was only tested before on tree logs and in a greenhouse experiment (Marshall et al. 2020) and a

tropical forest in stems and roots of tropical trees (Kühnhammer et al. 2022). Therefore, the borehole equilibration method ~~on gymnosperms and~~ resembles a new design of *in situ* xylem water vapor ~~extraction as tested and suggested by Marshall et al. (2020)~~. In the description by Marshall et al. (2020)~~ir method~~, the carrier gas was provided from one end while an extraction tube of an isotope ratio infrared spectrometer (IRIS) for suction was set on the other side of the borehole allowing for linear flow and equilibrium conditions (Marshall et al., 2020). Volkmann et al. (2016a) already used microporous polypropylene membranes. Their probe was embedded inside the sap wood and sealed for the inner and outer end of the borehole to prevent contamination with air water vapor (Volkmann et al., 2016a). Inside a mixing chamber of the probe, a gas-water-mixture was sucked out by an extraction tube into an IRIS.

However, our new integration of the membrane approach from Volkmann et al. (2016a) into the stem borehole equilibration method of Marshall et al. (2020), which itself is novel, has the advantages ~~of preventing liquid water from entering the membrane (condensation inside the membrane or tubing would still be possible), drying out of the borehole was inhibited since only water vapor (and therefore smaller amounts of water) can pass the membrane pores and~~ ~~that we could~~of us~~ing~~e dried ambient air as input flow for our boreholes with a reduced risk of fungal or bacterial infections of the tree ~~were prevented~~ due to the very small (0.2 µm) pores. ~~Using dried ambient air as carrier gas had the advantage of using~~Further, less artificial air is required that usually would have been needed~~acquired~~ to be carried into the field, hence making this setup easier compared to those needing ~~and more field-ready compared to setups that need~~ constant gas supply from a bottle. Finally~~urther~~, using the same probe type (membrane inserted inside a matrix) makes the comparability of soil vs xylem data easier. The boreholes had an inner diameter of 8 – 10 mm. Inside the membranes, fine PFA-sealed resistance thermometers (HSRTD, Omega Engineering, Norwalk, USA; tolerance: ±0.15° − 0.35°C (over 0° to 100°C)) were installed to measure the borehole temperature. The thermometers logged with the

CR800 Data Logger and Multiplexer. Daily mean temperature of the boreholes (required to convert vapor measurements into liquid isotopic composition) was similar to daily mean air temperature.

All soil and plant borehole tubes were attached to a bottle filled with desiccant (Drierite W. A. Hammond DRIERITE Co. LTD, Xenia, OH, USA) to dry incoming air at one end and attached to the laser spectrometer (Picarro L2130-i) at the other end of the membrane. The boreholes were further sealed with waterproof glue (ORCA, Aquarium Münster Pahlsmeier GmbH, Telgte, Germany). *In situ* soil and xylem water was sampled successively for each point, and each sample was measured for 10 min in total at ~ 2h intervals.

To avoid tube condensation, heating cables (ILLw.CT/Qx, Quintex GmbH, Lauda-Königshofen, Germany) were installed and wrapped with the tube in aluminium foil for insulation. The cables were controlled via an automatic multi socket (Gembird EG-PMS2, Gembird Software Ltd., Almere, The Netherlands) to prevent overheating in summer. To minimise condensation effects, the measurements were checked daily and the system (Manifold with attached tubes and membranes) was flushed for 10 minutes per probe to remove any water (Beyer et al., 2020). In the cases when condensation inside the system was identified the respective data was discarded. We only used daily mean data to exclude sub-daily variance and producing a more reliable dataset, because the focus of our study is on seasonal variability of the willow's behaviour and environment. Daily means were calculated after all corrections and discarding of potential condensation affected data was complete.

3.4 Calibration and data analysis

Calibration of the *in situ* soil and xylem water system was achieved by a standard delivery module (Picarro A0101 Standards Delivery Module, Picarro, Inc., Santa Clara, CA, USA) using standards of known isotopic composition in δ^{18}O (-10.41, -7.66 or 1.45‰) and δ^2H (-72.83, -55.86 or 16.74‰),

respectively. These lab-standards and all values of isotopic composition in this study are relative to the Vienna Standard Mean Ocean Water (VSMOW). To correct for isotopic offsets and vapor concentration dependency we used a similar approach to Schmidt et al. (2010) applying a linear regression of vapor concentration dependency slopes for δ^{2}H and δ^{18}O, and of the slopes on the δ-values for δ^{2}H and δ^{18}O isotopic offset correction. We only used measured water vapor concentrations and added linear regressions of temperature dependency slopes. However, the bulk soil sample results suggested that stronger water concentration dependencies were not fully corrected with this approach, hence, we applied polynomial regressions on measured isotopic compositions for different temperatures. Thus, we were able to correct highly variable water concentrations by using the soil pit temperatures of the specific depths.

To check for CO_2 contamination, we used the linewidth variable ('h2o_vy') of the raw data from the Picarro instrument as suggested by Gralher et al. (2016). We found a strong correlation between this variable and the water concentration measured by the laser spectrometer, which indicated that no significant CO_2 contamination was detected.

Furthermore, we assumed the liquid water source to be in equilibrium with the measured water vapor allowing us to calculate the liquid-vapor fractionation by a model of type 1 (Majoube, 1971):

$$\alpha = \exp \frac{a\left(\frac{10^6}{T_k^2}\right) + b\left(\frac{10^3}{T_k}\right) + c}{1000} \tag{2}$$

where α is the isotopic fractionation factor, T_k is the temperature (in K), and a, b, and c are empirical parameters that vary depending on the isotopologue.

The deviation of δ^{2}H and δ^{18}O composition in the sample from the global precipitation (or global meteoric water line, GMWL) is presented as deuterium excess (short d-excess) by Dansgaard (1964):

$$d - excess = \delta\,^2H - 8 \cdot \delta^{18}O \tag{3}$$

The d-excess indicates fractionation processes of the sample compared to the global precipitation. To investigate the local evaporative effects of the samples water, isotopologues were used to calculate the line-conditioned excess (short lc-excess) (cf. Landwehr and Coplen, 2006). The lc-excess describes the deviation of the sample from the local meteoric water line (LMWL):

$$lc - excess = \delta\,^2H - a \cdot \delta\,^{18}O - b \hspace{3cm} (4)$$

where *a* is the slope and *b* the intercept of the weighted isotopic composition of the local precipitation (IGB: a = 7.4, b = 3.7). The LMWL was calculated by linear regression of the precipitation stable water isotopes (plotting $\delta^{18}O$ against δ^2H) measured at IGB from May 2020 to January 2021 (R^2 = 0.98).

The similarity of soil water isotopic compositions measured with *in situ* and destructive sampling was ~~accomplished~~assessed by calculating the Euclidean distance to the 1:1 line of $\delta^{18}O$ *in situ* vs $\delta^{18}O$ direct equilibrium ("bulk") or cryogenic extracted and δ^2H *in situ* vs δ^2H direct equilibrium or cryogenic extracted for soil and xylem signatures~~results~~, respectively.

3.6 Mixing model

To quantify sources of *in situ* xylem water from soil water of different depths, we used the Bayesian isotopic mixing model, SIAR (Parnell et al., 2010). Following an initial assessment of potential water sources (see below), the soil isotopes from 10, 40, and 100 cm from pits A and B were used. These soil depths likely represent the majority of the root distribution of willows due to high near surface rooting densities (Cunniff et al., 2015; Marttila et al., 2017). To establish if the source changes through the growing season, soil and xylem isotopes were divided into weekly bins beginning June 21st 2020, and SIAR was run independently for each weekly group of sources and *in situ* xylem water. To constrain the mixing model, δ^2H, $\delta^{18}O$, and d-excess (d-excess = $\delta^2H - 8*\delta^{18}O$) were simultaneously used for the soil and vegetation. To optimize the soil source water to *in situ* xylem water, Markov Chain Monte Carlo approaches were utilized with 500,000 simulations, using the first 50,000 as burn-in results (which were then discarded). No prior information was provided to the model (uniform a-priori distribution)

for each soil depth. For Southern Willow, soil isotopes for each depth were averaged for Pit A and B as the relative similarities between Pit A and B would lead to limited identification of soil source between sites. To evaluate the efficiency of the SIAR model for identifying the proportions of source waters, the mean absolute error (MAE) was evaluated for each weekly bin using proportion weighted soil isotopes and vegetation isotopes. Proportion weighted soil isotopes were estimated as:

$$\delta_{mix} = P1 * \delta_{10cm} + P2 * \delta_{40cm} + P3 * \delta_{100cm}$$

where P1, P2, and P3 are the proportion of water from 10 cm, 40 cm, and 100 cm, respectively, as estimated by SIAR.

4. Results

4.1 Hydroclimatic conditions

The study period was characterised by eleven precipitation events with > 10 mm/d (Fig. 3a). The largest event was in late August while the longest dry period occurred in September. Daily mean air temperatures (T) gradually increased until mid-August and decreased afterwards. Vapor pressure deficit (VPD) as a driver of transpiration correlated with T, but also depended on water vapor pressure (high T and low water vapor pressures causes high VPD). Maxima occurred in the beginning of June and mid-August, in late August the VPD declines alongside T. Evapotranspiration (ET) had minima in times of low precipitation amount and high T, and this was reflected in sap flow rates (Fig. 4a). ET increased after large precipitation events when moisture availability increased. At the end of the measuring period, as T and VPD decreased, ET decreased as well.

The volumetric water content (VWC) of the soils at 10 cm depths strongly responded to precipitation events (Fig. 3e and f). Despite the different time series, it became clear that this variability was more distinct in Pit B (min: 5.7%, max: 21.0%, Fig. 3e) than in A (min: 4.0%, max: 11.4%, Fig. 3f). Generally, Pit A (beneath the tree canopy) was much drier than B. A precipitation event on 13th June resulted in

a significant increase of soil moisture in Pit A, with rapid increases in VMC even at 40 cm. VWC responses to precipitation at 100 cm depth were very damped with little changes detected in both pits (Pit A min: 12.6%, max: 14.2%; Pit B min: 17.6%, max: 20.7%) even after the August event.

4.2 Vegetation growth dynamics and transpiration

Daily sap flow ranged from 5000 – 71000 cm³/d (5 – 71 l/d) for Northern Willow ~~(height ~18 m)~~ and 4000 – 86000 cm³/d (4 – 86 l/d) for Southern Willow ~~(height ~19 m)~~ (Fig. 4a). This compares ~~-~~ well, in terms of order of magnitude for other willows shown to vary between 30 and 100 l per day during the growing season (Schaeffer et al., 2020). Sap flux in Northern Willow increased from the beginning of the measuring period until June 30th after which it dropped. Sap flux in Southern Willow increased until August 13th~~;~~ and then decreased until 4 September ~~4th~~ followed by an increase until 24 September ~~24th~~. High~~er~~ ET values (Fig. 4a) were generally reflected in higher sap flow rates, though ET (recorded below the tree canopy) was low at a time of high sap flow in August, reflecting low ET from grassland soils~~-~~. Both trees grew consistently with daily stem-radius incrementation following leaf-out until the end of the growing season at ~15th September (Fig. 4b). Sub-daily shrinking and swelling of the stem occurred, though ~~it~~this does not show ~~is not visual~~ in the averaged daily data set presented in this study. The main-growing phase started around June and total growth during the measuring period was 12.8~~13.6~~ mm for Northern Willow ~~(diameter 398 mm)~~ and 16.4~~17.4~~ mm for Southern Willow ~~(diameter 353 mm)~~.

4.3 Dynamics in stable water isotopes

~~4.3.1 Temporal dynamics of *in situ* isotopes~~

The daily average of the *in situ* isotope values showed strong links between larger (> 10 mm/d) precipitation events and soil water δ^2H data (Fig. 5~~7~~a and b, $\delta^{18}O$ is shown in S Fig. 1~~3~~). The highest fluctuations occurred in the upper soil layer, while the isotopic composition at 40 and 100 cm both

showed very damped responses to precipitation. At 40 cm, responses were more marked in canopy covered Pit A than Pit B (Fig.~~s. 6 and~~ 5~~7~~) probably.~~ This was~~ caused by the relatively smaller water stored~~amount present~~ in the soil of Pit A compared to Pit B~~,~~.~~ The difference of soil water availability results from the~~ due to the canopy cover and ~~hence~~more interception ~~occurring~~ at Pit A. Due to these generally smaller water amounts, incoming precipitation ~~varied~~affected the isotopic composition of the top soil water of Pit A more ~~intense~~ than ~~it did~~ at Pit B.

In contrast, the *in situ* xylem water composition did not show immediate response to precipitation events, though the shorter run of data makes it difficult to interpret longer-term variations, especially for the Northern Willow. However, the longer time series of the Southern Willow suggests that the xylem composition, like the soil at 10 cm, became more depleted after summer rain in July, before slowly increasing again through August and September (Fig. 5b~~7d and e~~). Comparing the *in situ* xylem water of the two probes on each tree (upper and lower) they were mostly very similar to one another.

In situ soil water lc-excess was most negative with again higher variability in canopy covered Pit A than in Pit B at a soil depth of 10 cm, (Fig. 6~~8~~). In Pit A, lc-~~-~~excess exhibited variability that was consistent with cycles of wetting and evaporative drying causing negative values (Fig. 6~~8~~b). A similar, though highly damped trend was apparent at 40 cm, while at 100 cm the lc-excess remained close to zero, with a suggestion of more fractionated water in the latter part of the study from late September. Variations were more damped in Pit B, and at 10 cm could become positive following rainfall (Fig. 6~~8~~c). By late summer, lc-excess at 40 and 100 cm was gradually becoming more negative, consistent with the percolation of more fractionated water from the upper soil with successive wetting fronts in response to rainfall inputs.

In situ xylem water lc-excess in Southern Willow varied around -15‰ until mid-August where the lc-excess increased constantly up to 0.2‰. In the Northern Willow, *in situ* xylem water lc-excess also enriched from the end of August to October.

In the dual isotope plot (Fig. 75), all water compartments, except precipitation, mostly lie below the LMWL (local meteoric water line). ~~is~~ was found in precipitation, while the variability of groundwater and stream water ~~is~~ was restricted. This plot also nicely ~~clearly~~ illustrates the substantial overlap between the isotopic compositions of xylem and near-surface soil water measured *in situ*.

The range of stable isotope values for all measured water compartments (precipitation, soil water, groundwater, lake and stream water) ~~are~~ is also summarised in Fig. 86 and Table 1. These show ~~more quantitatively~~again the large variability of precipitation, and the very narrow range of stream, lake and groundwater samples (-53.2 to -42.8‰ for δ^2H and -7.1 to -5.2‰ for $\delta^{18}O$). The lc-excess (Fig. 86c) of precipitation varied roughly between -10 and 10‰, while mean values of stream and lake water were ≈ -7‰, reflecting the influence of fractionation. Groundwater lc-excess was mostly slightly positive. The uppermost *in situ* soil water of both Pit A and B was most enriched (up to -10.4‰ for δ^2H and 0.0‰ for $\delta^{18}O$) and showed larger variabilities compared with deeper *in situ* soil waters ~~(Fig. 86)~~. This was generally similar to the bulk soil water (S Fig. 21). The upper *in situ* soil water of the drier Pit A ~~(as Pit A being covered by canopy and closer to the "Canopy Cover" site)~~ was more variable and ~~more~~ enriched during the measuring period than in Pit B. At 100 cm depth, *in situ* soil water of both pits showed a very narrow range of isotopic compositions (Table 1) with water from Pit A being roughly 10‰ more enriched in δ^2H (~1‰ in $\delta^{18}O$) compared to Pit B. Like the bulk soil samples, the *in situ* results were more depleted in deeper layers (Table 1). The *in situ* soil water lc-excess of the top layer

of the canopy covered Pit A was in general most negative, while water from open spaced Pit B at 10 cm even reached positive lc-excess values (Fig. 86).

Detailed bulk soil water composition is shown in heat maps of $\delta^{18}O$, δ^2H and lc-excess from the "Canopy Covered" (NW of Northern Willow) and "Open Space" (south of Pit B) sample sites (S Fig. 32). Both locations showed variation with depth and in time in response to rainfall inputs and changing ET. Usually, the bulk water at 10 cm was most enriched in $\delta^{18}O$ and δ^2H, while at 40 – 70 cm it was most depleted. "Canopy Covered" bulk soil water was, in general, more enriched in $\delta^{18}O$ than at "Open Space" resulting in differences in lc-excess. The mean Euclidean distance as a measure of similarity between Comparing the bulk soil results withand in situ data we gain the mean Euclidean distance ofshowed ~0.7 ‰ for $\delta^{18}O$ and ~4.6 ‰ for δ^2H (see Table 2).

The in situ xylem water showed most similarities with the top soil signatures (Figs. 75 and 86). Like the upper in situ soil water, the in situ xylem water varied over wider ranges (e.g., -29.2 to -1.9‰ for δ^2H and -5.4 to -0.9‰ $\delta^{18}O$ of Northern Willow upper) with their its distribution mostly being in the range of the signatures at canopy covered Pit A 10 cm. The in situ xylem water lc-excess values also showed a wide range (-28 to -0.2‰), still the values mostly fit inside the total lc-excess range of in situ soil water from Pit A 10 cm. The xylem water isotopic composition showed diurnal fluctuations that were also affected by sap flow activity of the willows (high sap flow resulted in heavier isotopic compositions, but also higher water concentrations in the measurement). (convert vapor measurements into)

Cryogenically determined xylem water isotopic composition from the destructive sampling (S Figs. 4 and 5) was more depleted compared to the in situ xylem water (Table 1). The lc-excess of the cryo-xylem water was also more enriched compared to the in situ xylem water (S Fig. 4). Like the in situ

xylem water, the cryo-xylem water isotopic composition was comparable to soil 10 cm isotopic composition, but indicated less evaporated conditions. After correcting the cryo-xylem water δH^2 data by adding 8.1 ‰ (Chen et al. 2020) the cryo-xylem δH^2 results ~~are~~ were more comparable to the *in situ* ones. However, after this correction the lc-excess values drifted further apart from the *in situ* xylem water (S Fig. 5). The mean Euclidean distance as a measure of similarity between ~~Comparing the~~ cyro-xylem ~~results with the ones from~~ and *in situ* xylem water ~~we gain a mean Euclidean distance of~~ showed ~1.6 ‰ for δ^{18}O and ~12.1 ‰ for δ^{2}H (or ~6.8 ‰ δ^{2}H after correction by Chen et al. 2020) (see Table 2).

4.4 Determining potential xylem water sources

The SIAR Bayesian mixing model estimated the possible sources of ~~root water uptake~~RWU (i.e. integrated in the composition of xylem) of the willows from isotope measurements at specific soil water depths (Fig. 9). The mixing model indicated that the xylem composition in both willow trees could be explained by uptake of water almost solely from the top soil (10 cm) for most of the study period, regardless of soil moisture variations and with low uncertainty (as indicated by the standard deviations in the plot). This is consistent with the qualitative overlap of upper soil water and *in situ* xylem water in the dual isotope space in Fig. 7~~5~~. However, ~~from a quantitative perspective (~~comparing the cumulative tree transpiration and rainfall to soil water storage~~)~~ it would have been also possible for the willows to solely ~~up~~take water up from the top 40 cm. ~~By using stable water isotopes as tracers in~~Through the soil and xylem water signatures, the model was able to predict ~~the uptake~~RWU as ~~of~~ mainly water from the top 10 cm of the soil. ~~The uptake~~RWU from the top soil ~~water~~ by the willows probably lead~~s~~ to the observed dry conditions in the top soil even though ET ~~is~~was not very high (max. 2.9 mm) and the site ~~is~~was often shaded. ~~A~~at the end of September and in October, there was a suggestion that deeper water sources might have become more important, though results became increasingly uncertain. By this time, only 60 to ~~-~~95% could be accounted for by uptake from the top soil, while 0 to ~~-~~10% and 0 to ~~-~~40% were taken from 40 and 100 cm depth, respectively, in Northern

Willow. In Southern Willow, the inferred increased uptake of soil water from 40 and 100 cm occurred one week later with ~70%, ~25% and ~5% taken from 10 cm, ~~25% from~~ 40 cm and ~~~5% from~~ 100 cm, respectively. Overall, mean absolute errors for the prediction of *in situ* xylem water composition by the SIAR model were small with being 6.5‰ and 8.8‰ for δ^2H and 1.8 and 1.3‰ for Southern Willow and Northern Willow respectively (S Fig. 6).

5 DISCUSSION

5.1 Dynamics of sources of root water uptake

Both qualitative assessment, ~~,~~ and quantitative analysis of the isotope data with the Bayesian mixing model, ~~,~~ infer that RWU~~root water uptake~~ by the willows was predominantly sourced from the upper soil horizons during the period when xylem water was analysed *in situ*. This water tended to be more enriched in heavier isotopes ~~and~~ show~~ing~~ the effects of evaporative fractionation. There was nothing to indicate that deeper, more depleted groundwater or stream water sources were ~~a~~ major components of ~~root water uptake~~RWU during the study period. There was some evidence from the mixing analysis that trees used deeper soil water to supplement shallow sources later in the monitoring period, which coincided with a drier spell in September, though soil moisture availability in the upper soil profile of the pits~~-~~ did not differ significantly compared to hotter summer months like July or August~~was not less than earlier in the summer~~. Although replication was limited, the two soil pits showed marked spatial differences in both soil moisture content and the variability in soil water isotopes, though similar changes with depth were apparent. This likely reflects the effects of interception losses below the tree canopy greatly reducing the moisture content of Pit A. This low storage volume, in turn, result~~ed~~s in more marked effects of both evaporative fractionation and mixing of infiltrating rainfall on the soil water isotope signals. Interestingly, individual r~~R~~ainfall events had no significant effects on xylem water isotopic composition.

These findings are consistent with other studies that have shown that willows mainly utilise water from the near surface soil horizons (Matilla et al., 2017), which is where nutrient availability is highest (Goldsmith et al., 2012) and most tree species adapt their greatest fine root densities to water and nutrient supply (Hertel et al., 2013). Similarly, Nehemy et al. (2021) found that willows use shallower water sources until drier conditions force water uptake from deeper soil layers. In the current study, it is not totally clear ~~if the~~why the results of the mixing model in September showed a ~~-~~shift from top soil to deeper soil layers even though the soil moisture of the top soil started to rewet again.~~similar effect of drier surface soils in September.~~ This shift comes at the end of the growing season, shortly after stem incremental growth ceases, and may be indicative of physiological changes at the onset of autumn. As~~Like it~~ was the case for ~~the~~ beeches ~~from~~in Gessler et al. (2021) it is possible that the total amount of water taken up from 10 cm soil ~~by the willows~~ was reduced at the end of the growing season shifting the relative contributions of deeper soil waters. The decline in sap flow in October (Fig. 4a) also fits such a scenario. However, it should be noted that the summer of 2020 was not characterised by a prolonged period of water stress, such as the well-documented drought summer of 2018 (Kleine et al., 2020; ~~and~~ Gessler et al., 2021). As shown by their constant growth, high sap flow and relatively low ET (Fig. 4), the willows did not experience water stress during our field experiment. Under drought ~~such~~ conditions a very different water uptake strategy of the willows might be evident, underlining the need for multi-year ecohydrology studies that capture hydroclimatic variability. However, other studies have shown that soils and not groundwater are the most likely sources of ~~root water uptake~~RWU, even when the latter might be expected (Brooks et al., 2010; Evaristo et al., 2015). Furthermore, recent isotopic mixing studies ~~like the one from~~(e.g. Gessler et al., ~~(~~2021) showed that some tree species (beech) are not able to compensate drought reduced top soil water availability by taking up more water from deeper soil layers emphasising the importance to investigate different species individually. ~~The~~A labelling field experiment by Kühnhammer et al. (2022) showed a general response of xylem water isotopic composition to irrigation events, but no direct response to single

events which is similar to our findings. ~~that the willows xylem water isotopic composition did not change after single precipitation events.~~

It should be stressed that the mixing model used here is relatively simplistic and coarse as it does not fully characterise the potential heterogeneity in soil properties and root distribution (cf. Sprenger and Allen, 2020). For example, o~~O~~ne of the conditions for the Bayesian mixing model assumes all endmembers are known. Since we conducted a field experiment under natural conditions, we cannot exclude unknown water sources completely. Even though we have covered~~,~~ obvious sources of precipitation, local soil water, groundwater, lake and stream water, roots may be able to access other source. For example, ~~However, the After~~ modelling work with the data used in this study showed~~s~~ that ~~we found~~ the willows ~~to probably~~might likely have a ~~much~~larger~~ higher~~ horizontal range of fine roots (>6m) and may access more distant heterogenous water sources (Smith et al., 2021) ~~than previously expected. ~~i~~the irrigation being a lot of water poured on a relatively warm soil during hot summer days during irrigation caused enriched water input into the soil, which was possibly taken up by distant fine roots of the willows.~~ Furthermore, n~~N~~umerous recent studies have shown~~n~~ that stem xylem water likely reflects the integrated effects of uptake over many weeks or months, depending on the species, age/size, seasonality and antecedent hydroclimatic variability (e.g. McCutcheon et al., 2017; Tetzlaff et al., 2021; Snelgrove et al., 2021). Moreover, recent modelling work has shown that storage, mixing and re-mobilization of ~~root water uptake~~RWU in trees may explain a lack of direct correlation between soil water and xylem water (Knighton et al., 2020). Therefore, it i~~'~~s possible that the ~~observed~~ increase of deeper soil water in the stem water pool in September was observed weeks after its root uptake. Conditioned-controlled experiments ~~maybe~~ with labelled water ~~will~~could be helpful to further investigate such possible temporal integrations of water signatures. However, the mixing model used in this study ~~likely~~ still provides a reasonable first approximation of the willows studied here. It possibly helps that willows have a rapid metabolism, supported by the high sap flow velocities and steady, sustained growth over the study period. Moreover, the two young, same-aged

trees on an immature freely-draining soil provided a relatively simple system with more limited variability in the xylem isotope signal between and within the trees than might be expected in a more heterogenous and natural riparian forest. More complex ecohydrological modelling that explicitly conceptualises the travel time of water in trees may be able to provide deeper insights (e.g. Mennekes et al., 2021). Finally, moving window analysis connecting a time-window of the soil water to the best fitting time-window of the xylem water might also be an easy, but useful approach to evaluate the temporal variability.

5.2 Using *in situ* monitoring over extended periods: potential and challenges.

In situ monitoring is usually characterised by relatively short-term periods (days to weeks) of monitoring campaigns, often under controlled experimental conditions (Volkmann et al., 2016a; Gaj et al., 2016; Kübert et al., 2020). Such studies were developed to overcome the disadvantages of low frequency destructive sampling in soil and vegetation systems (Beyer et al., 2020; Rothfuss and Javaux, 2017) where a series of methodological uncertainties may affect results (Barbeta et al., 2020; Chen et al. 2020). More recent work has demonstrated the feasibility of field application of spectroscopes in direct *in situ* monitoring to produce longer-term, reliable data of water in different compartments of the critical zone (Mennekes et al., 2021; Seeger and Weiler, 2021, Kühnhammer et al. 2022). Our study contributes to this evolution of such isotopic techniques to directly observe ecohydrological processes in soil and vegetation systems. In terms of soil isotope monitoring, our approach was very similar to that of Oerter and Bowen (2017) though we used ambient atmosphere as carrier gas rather than N_2. Consequently, we needed to dry the air using desiccantDrierite and similar to Volkmann et al. (2016a) this allowed us to achieve two hourly sampling with stable plateaus of isotope ratios from each inlet in the system (Fig. 2). Since the interval of root water uptake changes in the willow trees was uncertain, a high temporal resolution was chosen while maintaining a reasonable spatial resolution. As De Deurwaerder et al. (2020) have mentioned the xylem isotopic composition differs over the day and

~~only measuring once a day could imply higher or lower heavy isotope amounts.~~ For seasonal investigations we ~~therefore~~ suggest that fewer sub-daily measurements than we took (but at least 2 or 3 times a day) may be sufficient to capture diurnal variation~~, but this has to be further tested on other tree species~~. It might be useful to link ~~T~~the timing of these measurements ~~may be useful to link~~ to sap flow activity of ~~the~~a tree (e.g. one measurement at maximum and minimum daily sap flow, respectively). Of course, such an approach needs further investigation especially for different species.

For t~~T~~he ~~borehole method that we used to sample the~~ xylem water sampling in the trees we adapted ~~provided a test of~~ the method proposed by Marshall et al. (2020) with minor changes ~~and~~being~~its~~ the first usage in angiosperms of a temperate climate (see "3.3 Stable water isotope monitoring"). The high frequency results present an improvement on results ~~those~~ gained by destructive sampling where questions remain over exactly what is extracted by cryogenic methods (Chen et al., 2020; Barbeta et al., 2020). Our results showed moderate to no comparability between *in situ* and cryogenic extracted measurements. Similar results in cryogenic vs *in situ* extracted results ~~are the case in some studies~~were found elsewhere (Gessler et al., 2021~~; and~~ Kühnhammer et al., 2022), but in others, findings differed ~~it is not~~ (Mennekes et al., 2021). Possible reasons for differences in *in situ* vs cryogenic extracted xylem water isotopic composition ~~are~~can be released cell content due to the defrosting process, organic contamination (if measuring with an IRIS), and heterogeneity of the tree trunk. In terms of the *in situ* measurements, a much higher variability ~~of the results~~in the data or slight, undetected condensation effects, or non-equilibrium conditions might also result in off-sets to cryogenic extracted results. ~~For our data~~Here, w~~W~~e observed no off-set in δ^{18}O (cf. Volkmann et al., 2016a; Marshall et al., 2020; Gessler et al., 2021) and tests of a line width related variable (as suggested by Gralher et al., 2016) showed no effects of organic or CO_2 contamination (cf. ~~Gralher et al., 2016;~~ Beyer et al., 2020). ~~Though~~However, we did see individual differences of the willows showing the same trend of xylem water isotopic composition, while the absolute ~~results~~values differed. For a prolonged installation to

ensure equilibrium conditions it seemed advantageous to locate the boreholes further up the tree stem where increment of radial growth is lower as the stem narrows.

A particular challenge for *in situ* monitoring during the growing season is cooling and keeping the instrument <35°C in high summer temperatures. This was achieved by using a sun shade over the equipment and computer cooling fans. Despite this, the isotope time series for *in situ* water vapour measured in both soils and xylem showed diurnal variation, which, although reflecting natural processes, did include sampling artifacts that needed to be quality controlled. The soil was most enriched during the day (usually in the mornings) as atmospheric moisture demand increased evaporation. Similarly, xylem water was most enriched during periods of high sap flux during the day, and become depleted overnight. Similar variation has been observed by others (e.g. Martilla et al., 2017, De Deurwaerder et al., 2020). In general, short-term variability in the xylem isotopic composition could be greater than for soils, though longer term averages were more stable. In the current paper we have not focused on these diurnal variations, as the seasonal changes in root water uptakeRWU have been the main focus, and data was averaged on to daily time steps. Nevertheless, diurnal cycles of temperature and humidity not only contributed to diurnal variation in measurements but also caused condensation in tubing (e.g. Beyer et al., 2020; Kühnhammer et al. 2022) that could not always be prevented despite heating the tubes (see above). As a result, some data points were rejected but still sufficient data was collected to estimate daily means. This was a particular problem in cooler nights (cf. Gaj et al., 2016) and following rainfall, especially in late summer and autumn as air temperature drops faster compared to soil temperature causing a temperature gradient. The effects were most marked in the top soil layer and xylem where the sampling tubes experienced the most marked temperature variations. For much of the monitoring period flushing the system (Manifold, tubes and probes) with dry air each morning was needed. However, this daily maintenance work is of course highly labour intensive.

In this study we focused on monitoring natural abundance of isotopes in various critical zone compartments, to better understand interactions in the soil-plant-atmosphere continuum of a plot of riparian willow trees. Other studies have reported tracer experiments that focused on specific hypothesis testing under controlled conditions (e.g. Seeger and Weiler, 2021), ~~. O~~or field experiments with natural conditions, but additional labelling (e.g. Kühnhammer et al. 2022). We see these approaches as being complimentary and our results underline the usefulness of investigating natural abundances of isotopes in critical zone compartments, especially if combined with auxiliary ecohydrological measurements. Although clear tracer "breakthrough" signals are much more evident in labelling studies, there are advantages to observing conditions as they occur in nature and the realistic reaction of trees to its new rain water inputs in relation to the distribution of the water sources.

6. CONCLUSION

We conducted an *in situ* field study of stable water isotopes in soil water and the xylem of willow trees in conjunction with hydroclimatic monitoring and measurements of sap flow, stem size, soil temperature and moisture, and stable water isotopes in precipitation, lake, stream and groundwater. Our investigation delivered reliable high-frequency stable water isotope data in two soil pits at three depths and two willows in two stem boreholes with a ~2 hourly resolution over several months (4.5 months in soil and 3 months in trees).

We have shown that the stem borehole approach described by Marshall et al. (2020) successfully worked in the field and in an angiosperm species in a temperate climate. Furthermore, we adapted polypropylene membranes inside the stem boreholes ~~–~~and used dried ambient air as carrier gas reducing the risk of infection and simplifying the field setup~~which improved our results by reducing the infection risk of the tree and prevented liquid water from the tree to enter the measurement~~.

XXX

The upper soil layer was most variable in moisture content and isotopic composition. Spatial heterogeneity was shown by the two soil pits from which one was located under the canopy while the other one was in the open. The canopy covered soil pit had lower volumetric water contents, but more enriched and more variable isotopic composition than the one in open space.

The xylem isotopic composition mostly resembled top soil water and mixing models indicated that the soil~~this~~ water source at 10 cm could explain ~90% of root water uptake. The summer sap flux velocity remained high suggesting rapid xylem water travel times of a few days and sap flux velocity showed no obvious response to lower soil water conditions (i.e. reduction) or precipitation events (i.e. increase). At the end of the season, the reliance on upper soil water uptake reduced (but was still ~60%) suggesting use of additional deeper soil waters. It is likely that the willow trees preferred top soil water in summer due to nutrient supply while at the end of their growing period they shifted to layers with higher soil water content.

Although our approach delivered novel and reliable data, improvements in terms of set up and choosing locations or time periods with more variable water source availability will benefit further investigations. For long-term *in situ* xylem water investigations further research tackling challenges such as night-time tube condensation, compression of the membrane and testing on different tree species is required.

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Figures

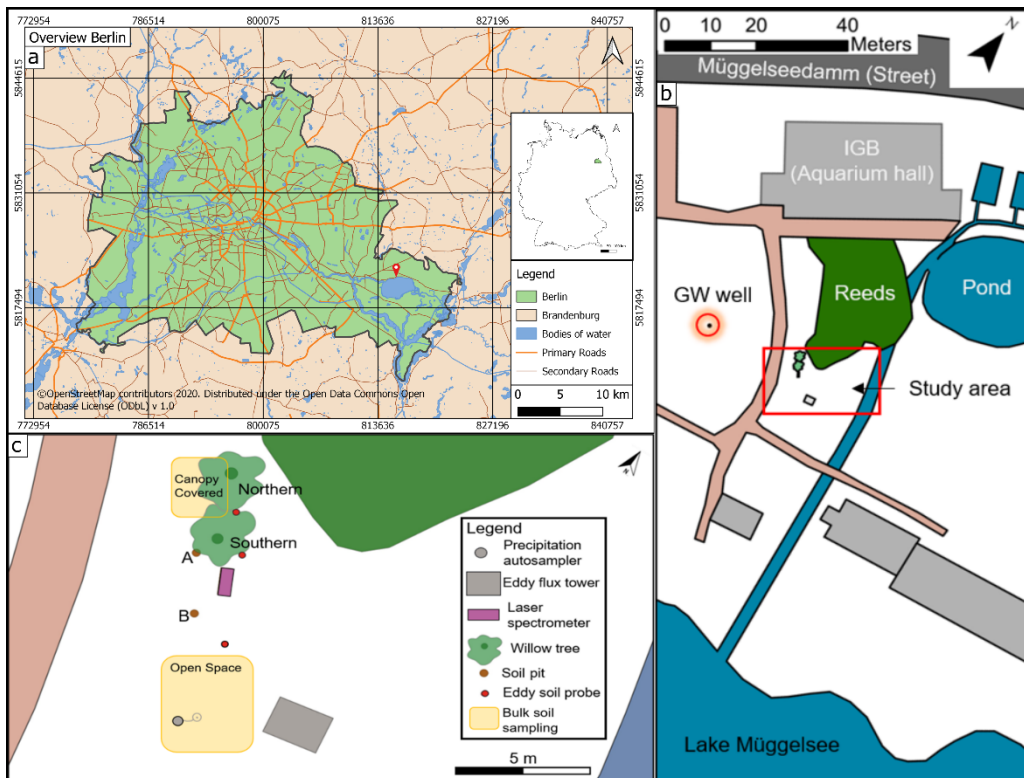



Fig. 1: Maps with a̲A̶) Inner map: Location of Berlin in Germany, Outer map: Overview Berlin with red pin showing the location of the IGB, b̲B̶) Sketch of the study site (highlighted in red rectangle) with GW well (highlighted in red circle), c̲C̶) Bird's eyes view showing all measuring points.

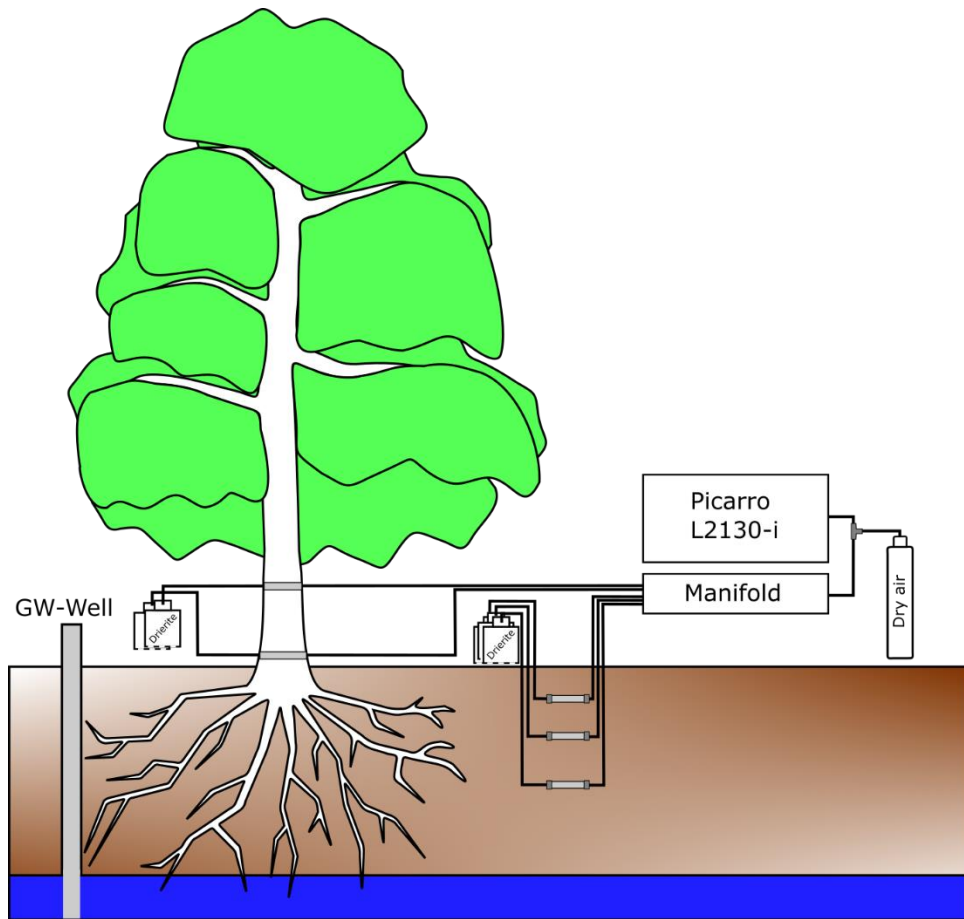



Fig. 2: Conceptual graphic of the general *in situ* isotope measuring setup.

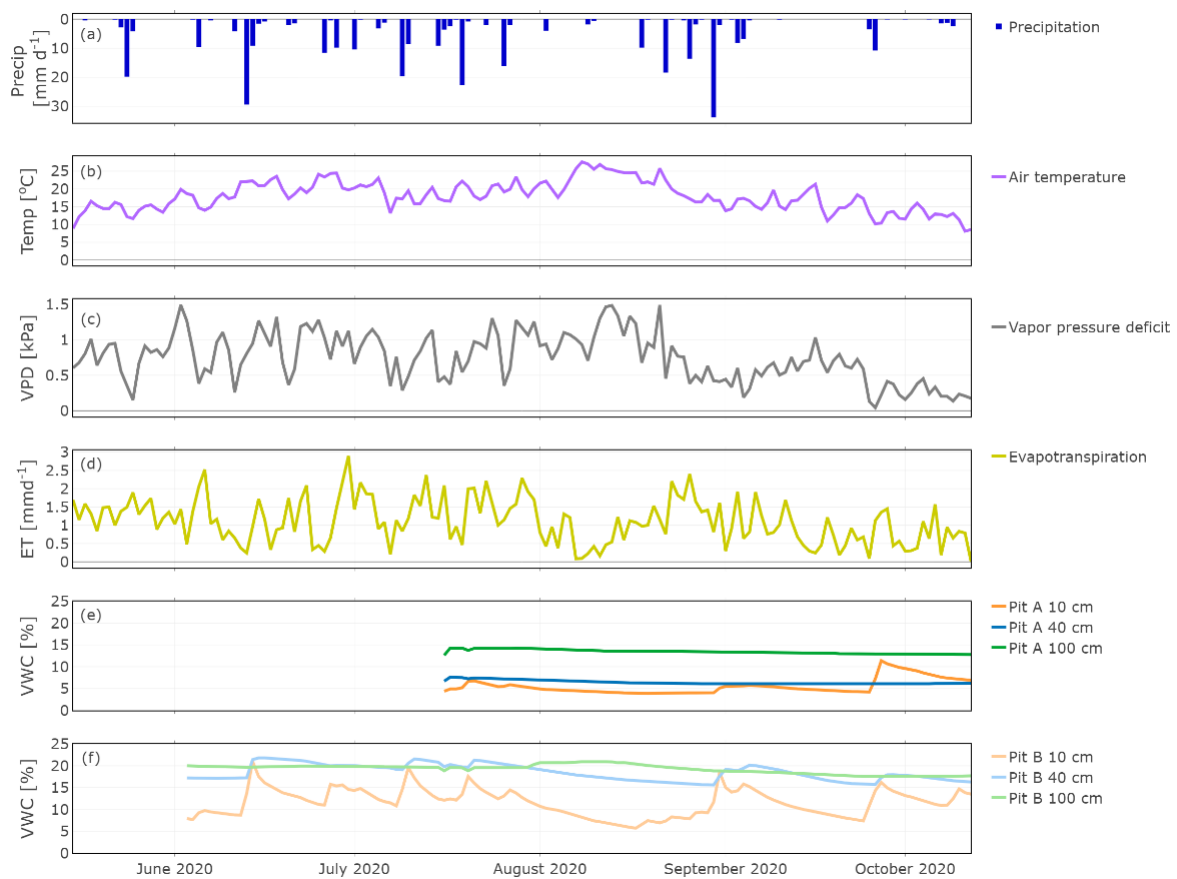



Fig. 3: Hydroclimatic conditions showing daily precipitation (a), air temperature (b), vapor pressure deficit (c), evapotranspiration (d), and soil moisture for Pit A (e) and Pit B (f).

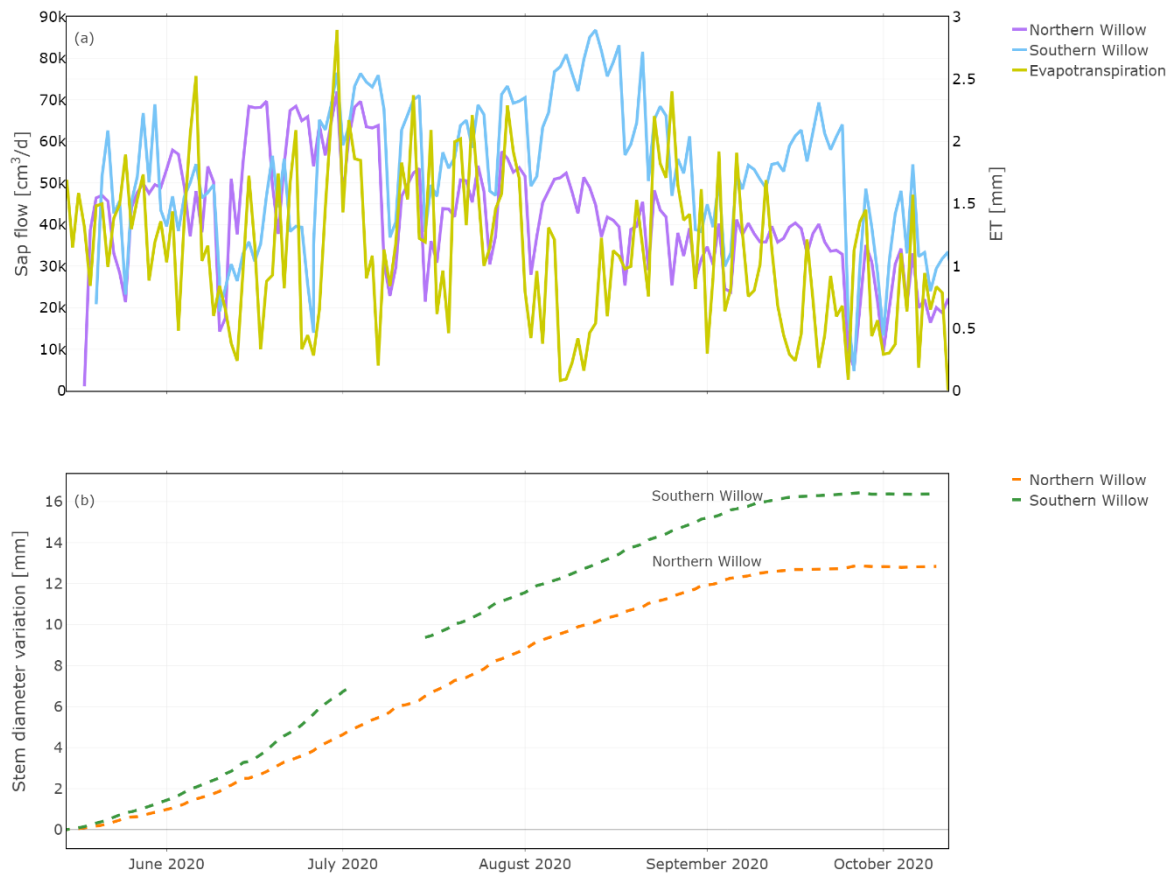



Fig. 4: Daily total sap flow ~~measured at the northern sap flow sensor~~ and stem diameter~~size~~ variation measured for the stem-radius at the Northern stem side. Plot a) shows the results of sap flow together with Evapotranspiration for comparison and b) the results of the stem diameter variation during the measuring period. ~~shows the results of Northern Willow and plot b) the ones of Southern Willow.~~

Fig. 5: *In situ* time series of daily δ²H in: Precipitation (a), Pit A and Pit B (b), and Northern Willow and Southern Willow (c).

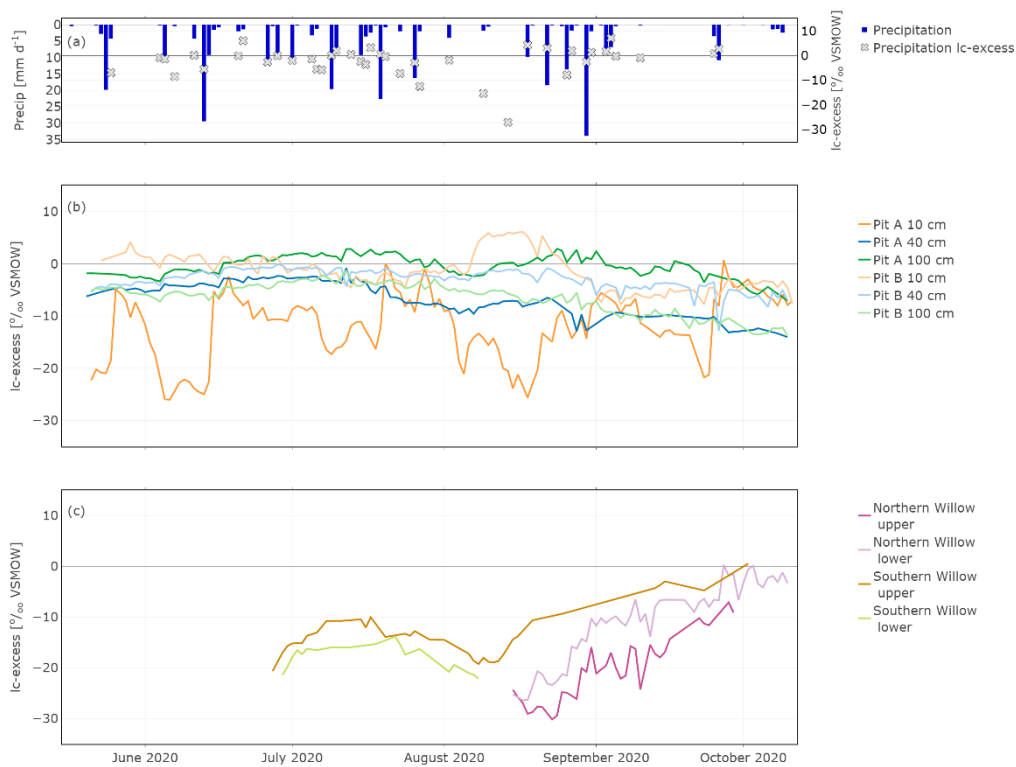



Fig. 6: *In situ* time series of daily lc-excess in: Precipitation (a), Pit A and Pit B (b), and Northern Willow and Southern Willow (c).

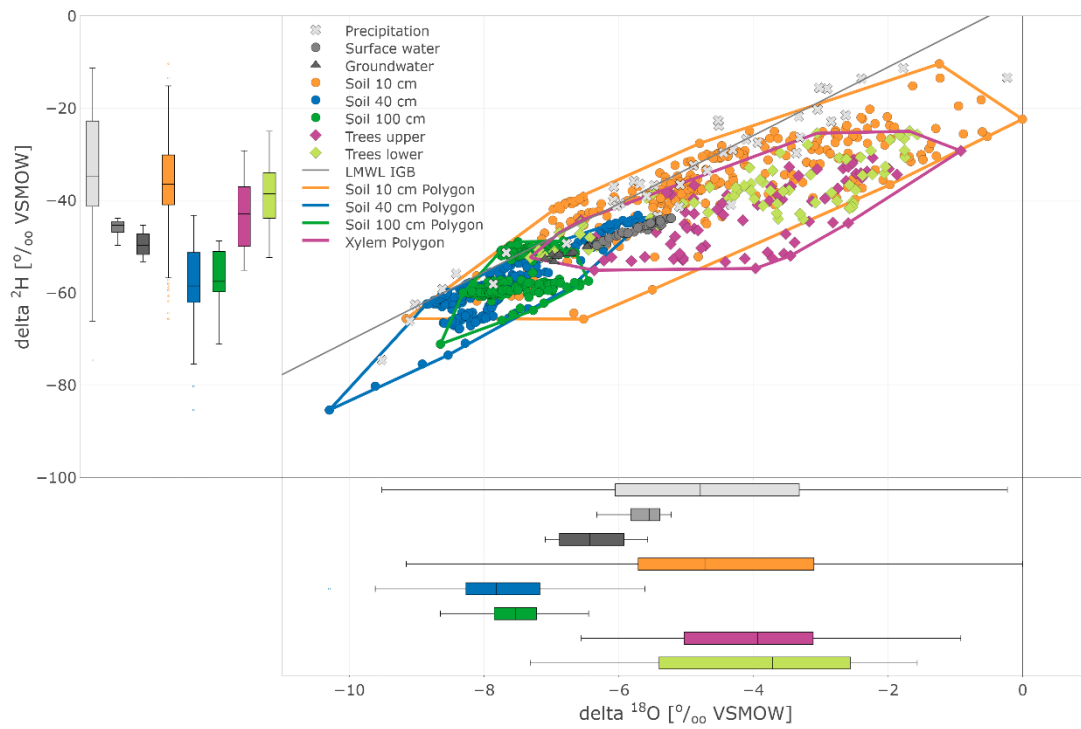



Fig. 75: Dual isotope plot of *in situ* (daily) soil and xylem as well as precipitation (daily), surface and groundwater (weekly) sampling. Soil and tree data are highlighted with boundary polygons for 10 cm, 40 cm, 100 cm and tree (upper and lower results joined) clusters. Additional boxplots show the sample distribution of the data sets.

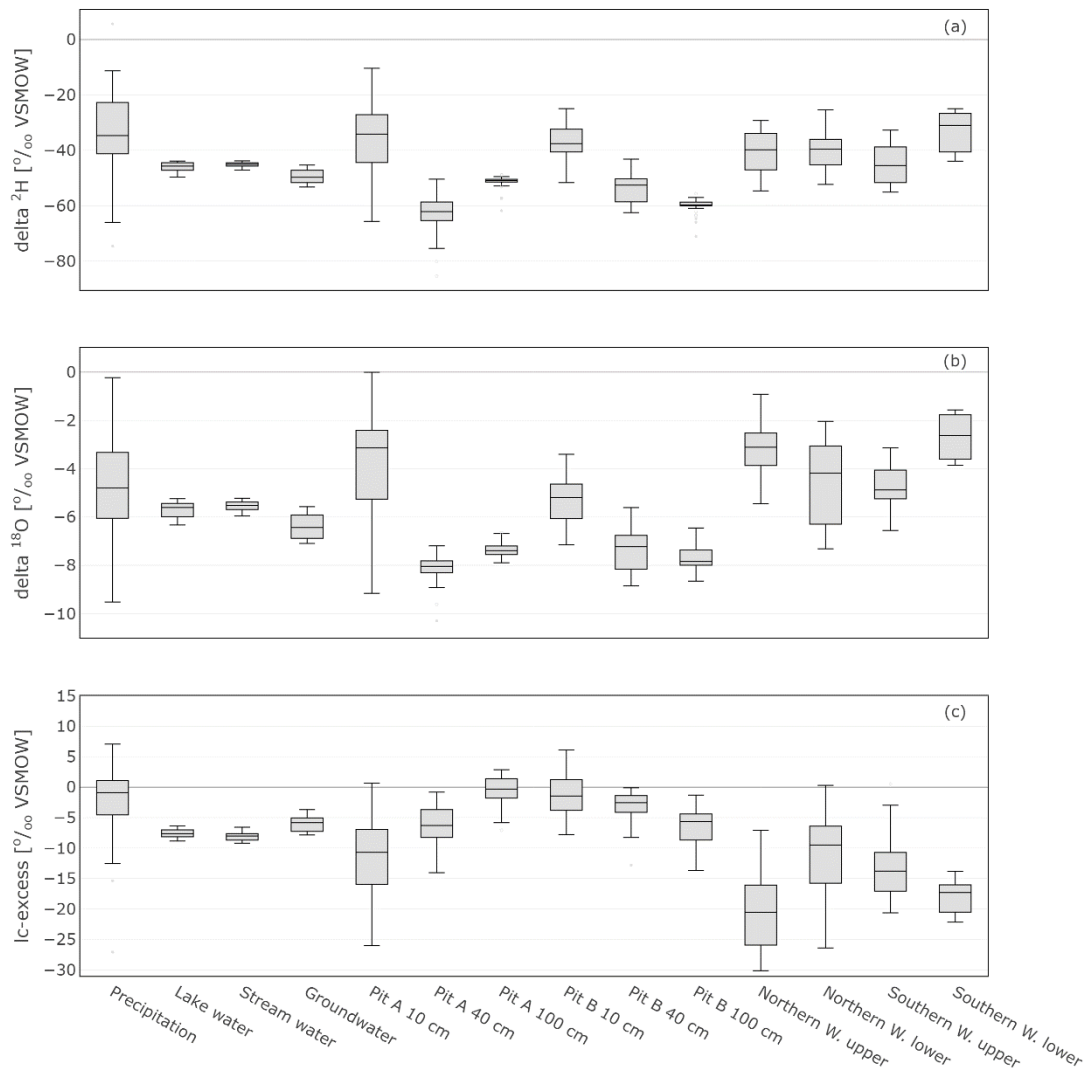



Fig. 86: Box plots showing the isotopic composition as well as lc-excess of daily precipitation sampling, weekly sampled groundwater, lake and stream water, and *in situ* sampled soil, and xylem water. The lc-excess is an indicator of evaporational effects and describes the offset of the sample from the LMWL (see Methods 3.4 Calibration and data analysis).

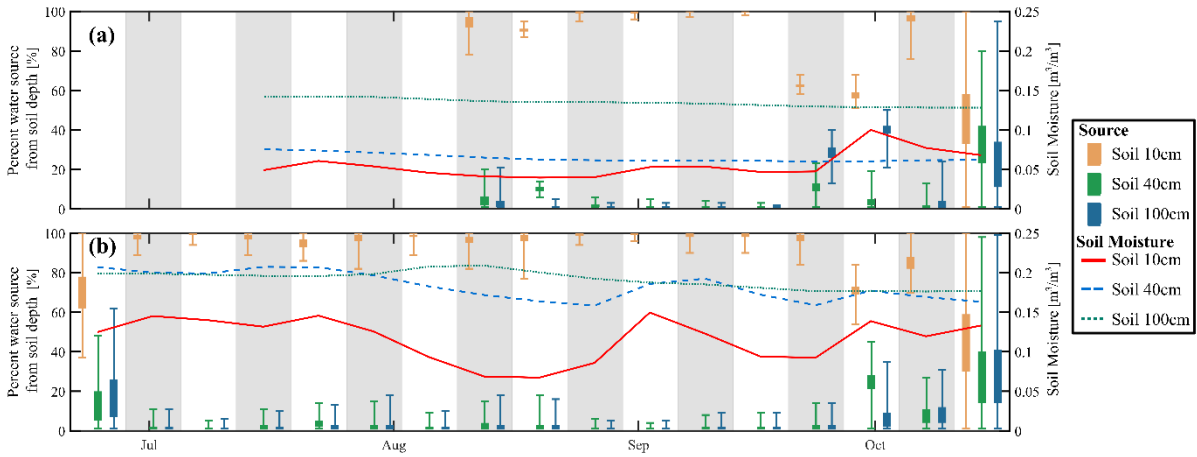



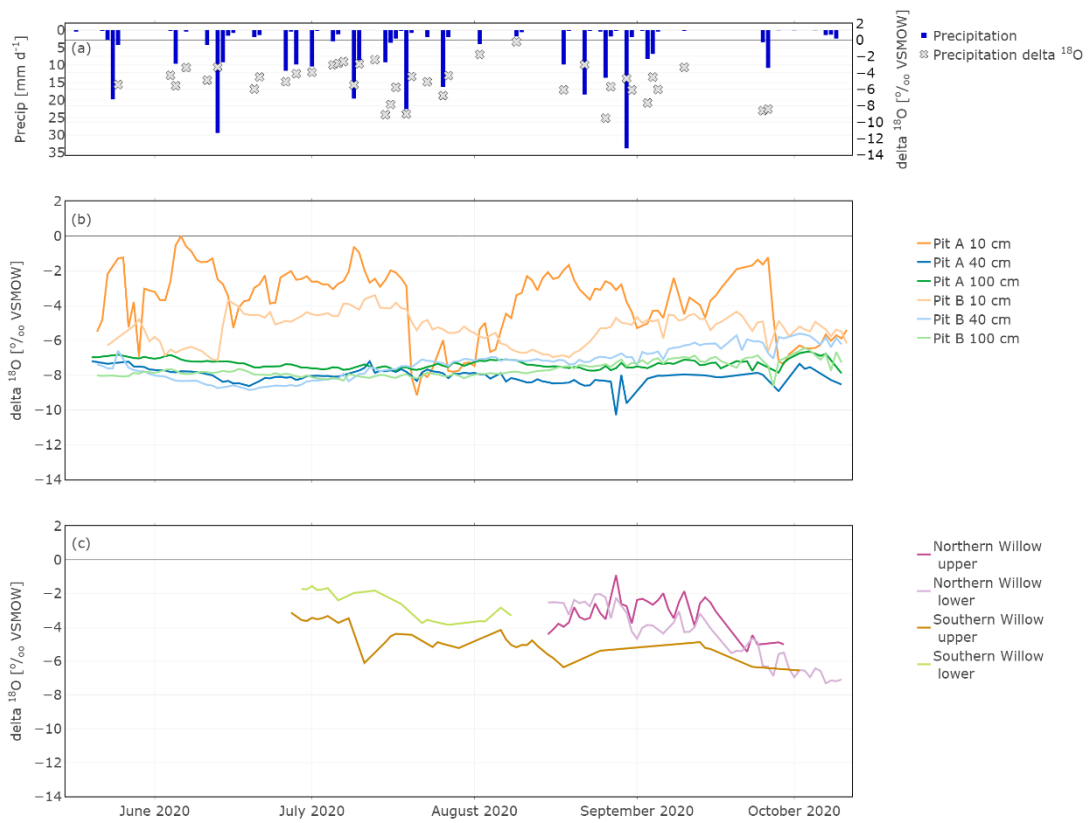
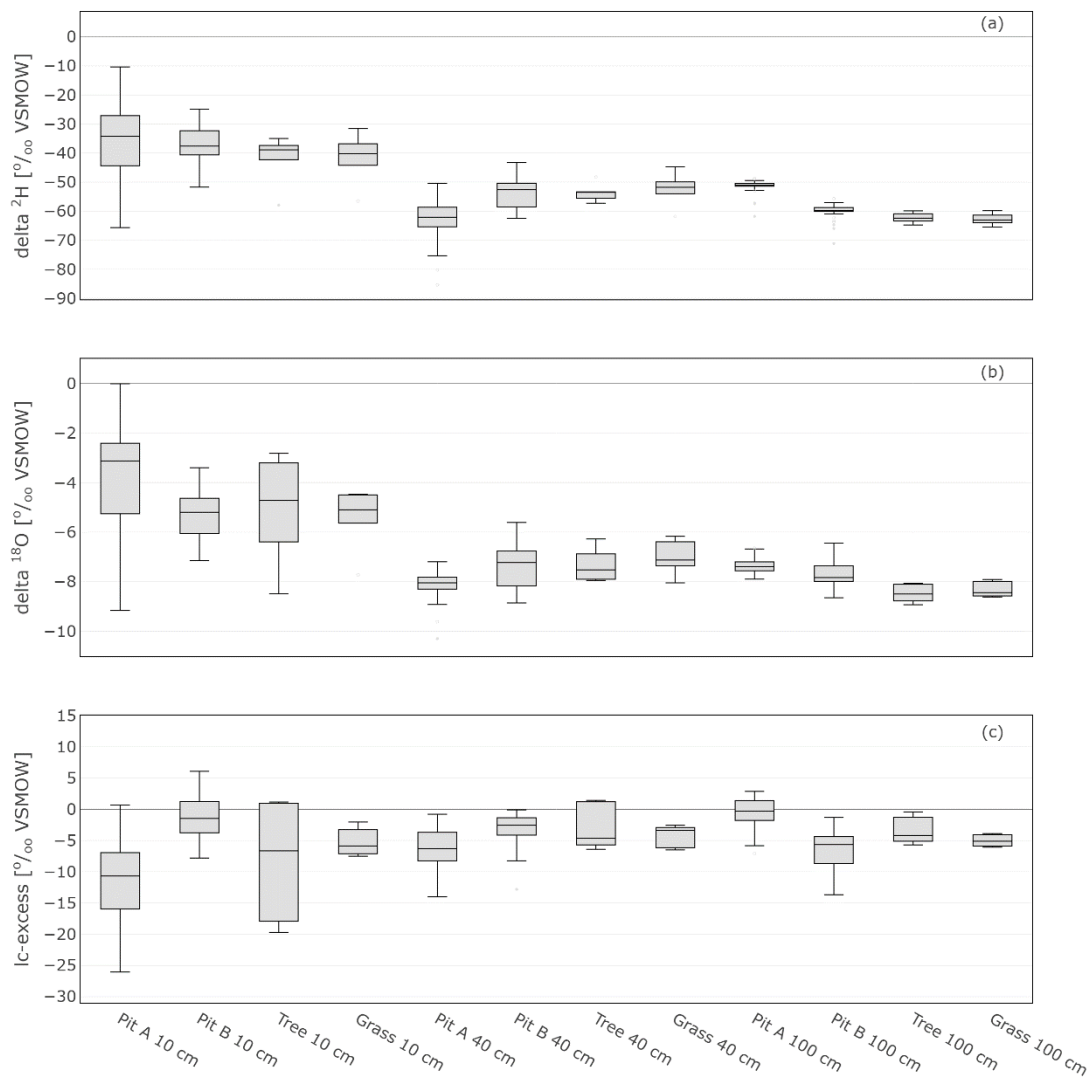
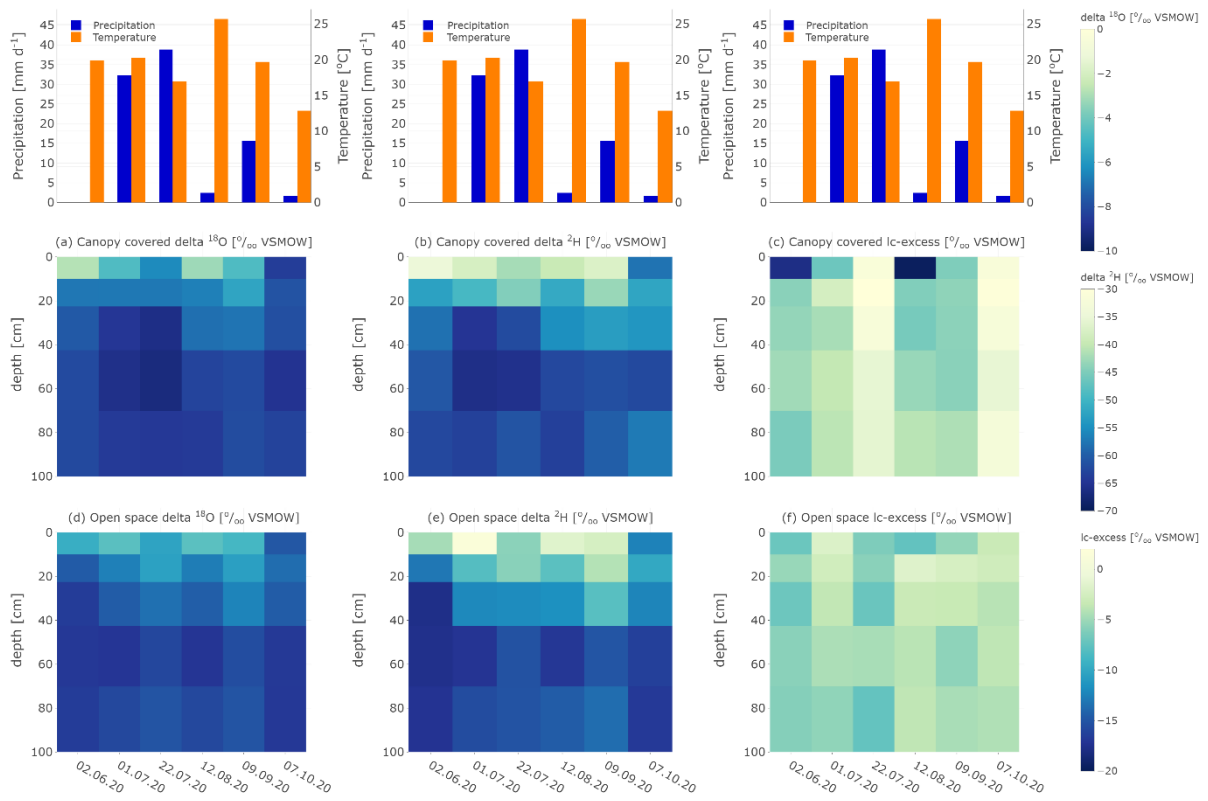
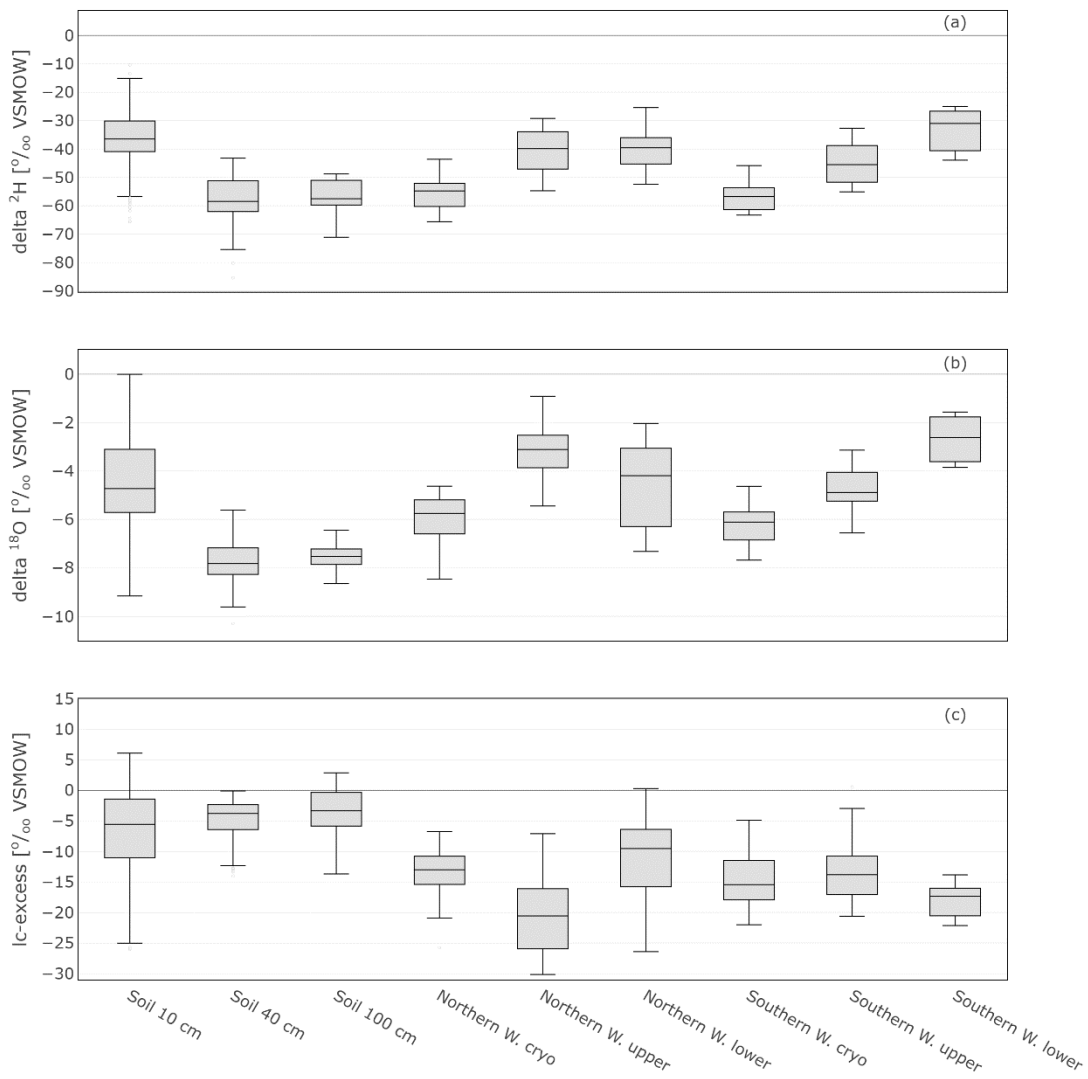
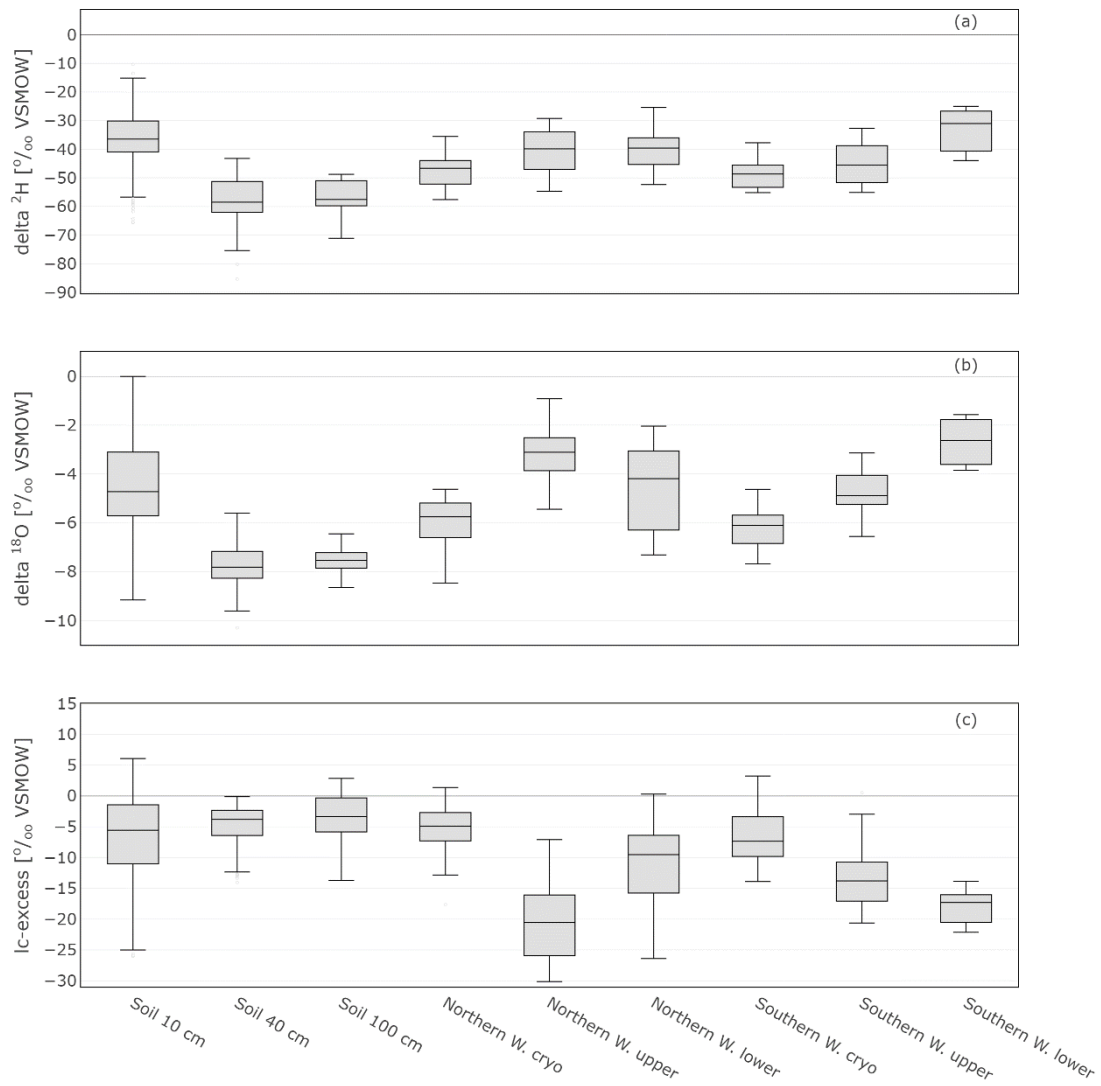
Figure 9: Estimated percentage of willow water from each soil depth in (a) Northern Willow and (b) Southern Willow. The box plots for each source show the 25th and 75th percentiles, with whiskers extending to the minimum and maximum estimated proportion. Gray and white bands show the division of each week. The right-side y-axis shows the soil moisture for each soil depth (weekly average).

Tables

Table 1: The amount (n) and 5th, 50th (median), and 95th percentiles of $\delta^{18}O$ and δ^2H (‰ VSMOW) signatures. (Surface water: Lake and Stream water).

	n	\multicolumn{3}{c}{δ^2H ‰ VSMOW}	\multicolumn{3}{c}{$\delta^{18}O$ ‰ VSMOW}
		5th percentile	Median	95th percentile	5th percentile	Median	95th percentile
Precipitation	40	-62.73	-34.67	-13.24	-9.03	-4.79	-1.70
Groundwater	23	-52.87	-49.73	-45.36	-6.99	-6.35	-5.57
Surface water	40	-48.75	-45.34	-43.90	-6.18	-5.62	-5.24
Soil 10	265	-56.08	-36.44	-24.13	-7.14	-4.72	-1.53
Soil 40	242	-66.48	-58.52	-45.36	-8.64	-7.82	-6.12
Soil 100	261	-60.49	-57.60	-50.21	-8.12	-7.53	-6.89
Canopy covered 10	6	-53.76	-35.93	-32.37	-7.97	-4.65	-2.05
Canopy covered 40	12	-62.04	-53.30	-43.56	-8.67	-7.30	-5.56
Canopy covered 100	12	-65.44	-61.82	-59.03	-9.04	-8.42	-7.95
Open space 10	6	-55.08	-40.37	-33.32	-7.40	-5.08	-4.53
Open space 40	12	-61.91	-52.95	-42.51	-8.05	-6.98	-5.63
Openspace 100	12	-65.39	-63.52	-59.86	-8.65	-8.34	-7.74
Tree upper	73	-53.01	-42.86	-32.38	-6.15	-3.94	-2.28
Tree lower	73	-51.08	-38.51	-25.96	-7.01	-3.72	-1.76
Tree cryo	29	-63.07	-55.63	-46.34	-7.61	-5.85	-4.68

Table 2: Variance of Euclidean distance between *in situ* and direct equilibrium ("bulk") soil stable water isotopes and between cryogenic extracted twig and *in situ* xylem stable water isotopes. Euclidean distances were calculated between the δ^2H (or $\delta^{18}O$) bulk (or cryogenic extracted) and δ^2H (or $\delta^{18}O$) *in situ* point and the 1:1 line. *In situ* data were taken from the day of sampling bulk (or twig) samples. The results from "cryo cor" refer to corrected~~altered~~ δ^2H values where 8.1 ‰ was added (cf. Chen et al. 2020).

	\multicolumn{3}{c}{$\delta^{18}O$ [‰ VSMOW]}	\multicolumn{3}{c}{δ^2H [‰ VSMOW]}	
	Min	Mean	Max	Min	Mean	Max	n
bulk vs *in situ*	0.00	0.68	2.00	0.01	4.62	12.12	34
cryo vs *in situ*	0.07	1.62	3.24	5.13	12.08	18.01	7
cryo cor vs *in situ*	0.07	1.62	3.24	1.20	6.81	12.89	7

S Fig. 1̲3̶: *In situ* time series of daily ¹⁸O in: Precipitation (a), Pit A a̲n̲d̲ (̶b̶)̶,̶ Pit B (b̲c̶), a̲n̲d̲ Northern W̶i̶l̶l̶o̶w̶ ̶(̶d̶)̶,̶ and Southern Willow (c̲e̶).

S Fig. 2̶1̶: Box plots showing *in situ* and bulk soil sampling results at certain depths. For comparison bulk soil sampling results were estimated via mean of the depths above (for example 10 – 20 cm and 20 – 40 cm samples to calculate the result for 40 cm)

S Fig. 32: Monthly bulk soil sampling isotopic results from two locations of the site. "Canopy covered" (a – c) is closer to Northern Willow and Pit A and "Open space" (d – f) is closer to Pit B and Southern Willow. On top of the plots is antecedent precipitation as 7d sum before sampling and the daily mean temperature.

S Fig. 4 Box plots showing daily means of *in situ* soil and xylem water isotopic composition as well as the monthly cryogenic vacuum extracted twig water isotopic composition of the two willow trees.

S Fig. 5 Box plots showing daily means of *in situ* soil and xylem water isotopic composition as well as the monthly cryogenic vacuum extracted twig water isotopic composition of the two willow trees. Cryogenic vacuum extracted water results of δ^2H is corrected by 8.1 ‰ after Chen et al. (2020).

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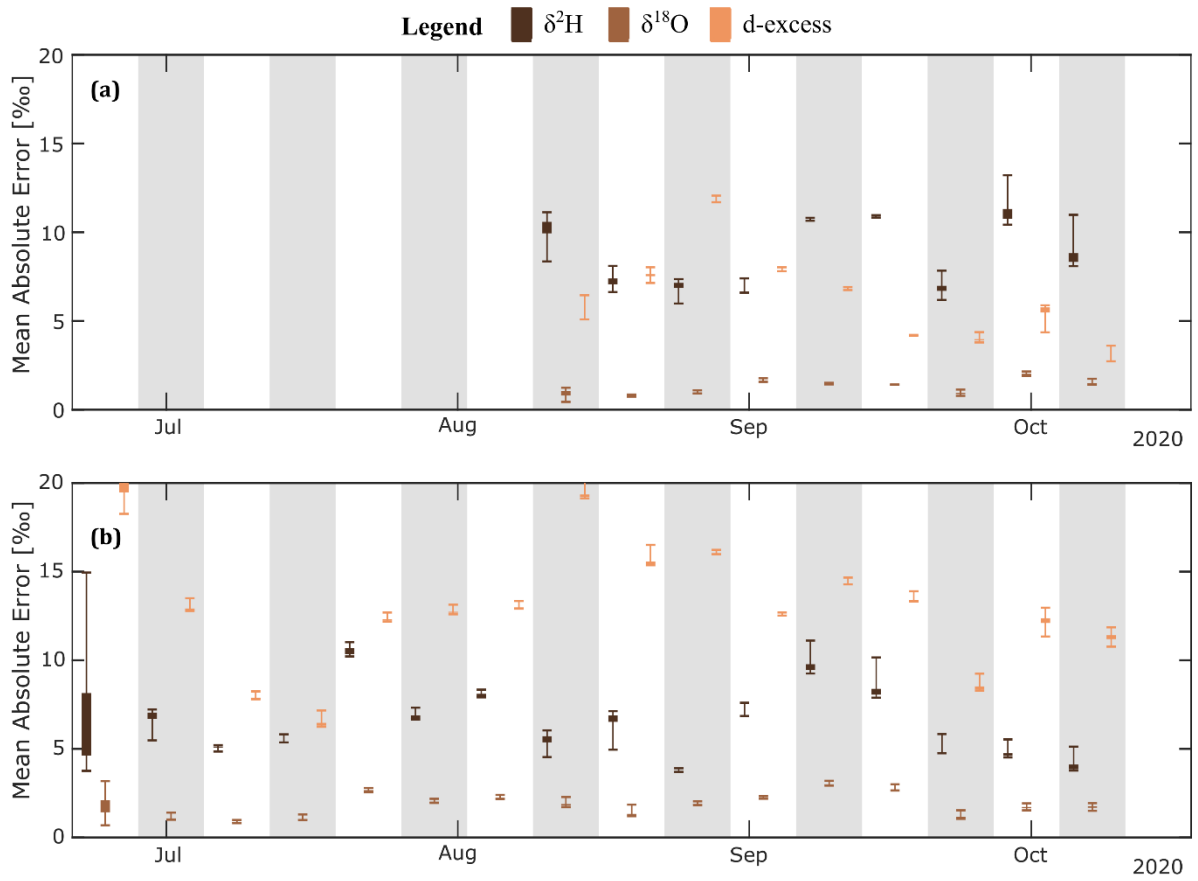
S Figure 6: Box plot of mean absolute error for each isotope and week for (a) the Northern Willow, and (b) the Southern Willow