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Tree water deficit and dynamic source water partitioning

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Abstract

The stable isotopes of hydrogen and oxygen (δ^2H and $\delta^{18}O$, respectively) have been widely used to investigate tree water source partitioning. These tracers have shed new light on patterns of tree water use in time and space. However, there are several limiting factors to this methodology (e.g. the difficult assessment of isotope fractionation in trees, and the labor-intensity associated with the collection of significant sample sizes) and the use of isotopes alone has not been enough to provide a mechanistic understanding of source water partitioning. Here, we combine isotope data in xylem and soil water with measurements of tree's physiological information including tree water deficit (TWD), fine root distribution, and soil matric potential, to investigate the mechanism driving tree water source partitioning. We used a 2 m^3 lysimeter with willow trees (*Salix viminalis*) planted within, to conduct a high spatial-temporal resolution experiment. TWD provided an integrated response of plant water status to water supply and demand. The combined isotopic and TWD measurement showed that short-term variation (within days) in source water partitioning is determined mainly by plant hydraulic response to changes in soil matric potential. We observed changes in the relationship between soil matric potential and TWD that are matched by shifts in source water partitioning. Our results show that tree water use is a dynamic process on the time scale of days. These findings demonstrate tree's plasticity to water supply over days can be identified with high-resolution measurements of plant water status. Our results further support that root distribution alone is not an indicator of water uptake dynamics. Overall, we show that combining physiological measurements with traditional isotope tracing can reveal mechanistic insights into plant responses to changing environmental conditions.

1. Introduction

Plant water use studies using the stable isotopes of hydrogen and oxygen (δ^2H and $\delta^{18}O$) as tracers have shed considerable light on ecohydrological processes (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002; Penna et al., 2018). Following early work by Dawson and Ehleringer (1991), many studies subsequently investigated patterns of tree water use within a given soil profile and other available water sources (e.g. streamflow, groundwater) (Dawson & Pate, 1996; Flanagan, Ehleringer, & Marshall, 1992; Jackson et al., 1999; Meinzer et al., 1999; Snyder & Williams, 2000). Brooks et al., (2010) showed the importance of considering different flow domains within the soil pore space and the seasonal lags from precipitation to the origin of water used by trees. Subsequently, Allen et al., (2019) showed that species growing in the same environment used water with distinct seasonal origin. Xu et al., (2019) showed that trees transpire water from first rainfall event that recharged soil water storages during the wet season, and supported transpiration during the dry season. While some synthesis (*e.g.* Evaristo, Jasechko, & McDonnell, 2015) and field-based investigations have found that the use of tightly bound water by trees is widespread across different biomes (Goldsmith et al., 2012; Hervé-Fernández et al., 2016), others have not (Geris et al., 2015; Qiu et al., 2019). Nevertheless, the mechanisms governing tree water source apportionment are still unclear (Berry et al., 2017; Penna et al., 2018). At the catchment scale, where diverse assemblages of vegetation differentially affect the dynamics of water ages in storage, describing these mechanisms is vital for our understanding of the water balance and the fundamental processes controlling the apportionment of streamflow and evapotranspiration (Rinaldo et al., 2015).

Recent soil and xylem water investigations have shown that enhancing the temporal and spatial sampling resolution of water isotopologues is key to improving our understanding of tree water use patterns that are linked to soil water dynamics and heterogeneity of flow paths (Goldsmith et al., 2019; Sprenger & Allen, 2020; Sprenger, Llorens, Cayuela, Gallart, & Latron, 2019). However, even higher sampling resolution reveals patterns of tree water use that cannot be explained by using stable isotopes alone. Volkmann et al., (2016), using hourly-resolution isotope measurements, showed different species's response to newly available water. In particular, one of the two species (which grew under the same conditions and had similar root distribution as the others) readily took- up newly available water after a rewetting event, whereas the others relied on water in deeper layers and delayed uptake of the new water. These species-based heterogeneous responses to soil water availability can be attributed to distinct water-use strategies and physiological traits (Choat et al., 2012; Frederick C. Meinzer et al., 2016; Rodriguez-Iturbe, Porporato, Laio, & Ridolfi, 2001). Thus, combining variables that add physiological understandings about plant's response to water availability along with tracers could allow plant water source investigations to go beyond the limitations imposed by the use of δ^2H and $\delta^{18}O$ alone (e.g. the uncertainty regarding fractionation process (Barbeta et al., 2019; Martin-Gomez et al., 2016; Poca et al., 2019), and co-extracted organic interference with regards to isotope analysis (Martín-Gómez et al., 2015; Nehemy et al., 2019)). Physiological monitoring at the time of uptake could enable a more mechanistic process-based interpretation of tree water sources and provide a more precise record of how trees use water.

Tree's response to water availability can be assessed by tree water status. While soil water status ('dry' vs. 'wet') has been used as the guiding criteria for sampling campaigns and interpretations about patterns of plant water source apportionment (Evaristo, McDonnell, Scholl, Bruijnzeel, & Chun, 2016; Hervé-Fernández et al., 2016), no studies, that we are aware of, have yet considered tree water status in this same regard. Plant water status is the plant's internal water balance that results from the difference between transpiration and water uptake (Klepper, Browning, & Taylor, 1971; Slatyer, 1967). This reflects plant's response to soil-water supply and atmospheric demand driven by water potential gradients, and it is highly regulated by plant traits (Fu & Meinzer, 2019; Hsiao & Acevedo, 1974). Plant water status can be characterized using different methods based on leaf water potential, hydraulic conductance, and plant's relative water content (net changes in water in plant tissue) (Jones, 2007). While measurements of predawn and midday leaf water potentials using pressure bombs (Scholander, Hammel, Bradstreet, & Hemmingsen, 1965) is often used to measure plant water status, this approach is labour intensive, destructive, and provides low temporal data resolution. Alternatively, the use of relative water content offers a more integrative physiological metric to plant water status because it accounts for the key roles of water storage and capacitance (Martinez-Vilalta et al., 2019; Schulte, 1992).

One way of measuring net water changes in plant tissue at high-temporal resolution is through tree water deficit (TWD in μm). TWD also known as 'tree water deficit-induced stem shrinkage', provides information about plant's relative water content in relation to its hydration state (Zweifel et al., 2015). The importance of TWD has been gaining popularity in ecophysiological investigations (Dietrich, Zweifel, & Kahmen, 2018; Kathy Steppe, 2018; Roman Zweifel, Haeni, Buchmann, & Eugster, 2016) and can be determined by measuring changes of stem radius with automated dendrometers (Hinckley & Bruckerhoff, 1975; R Zweifel, Zimmermann, & Newbery, 2005). This allows for continuous and non-destructive measurement of plant water status and natural incorporation of temporal plant responses to soil water availability (Brinkmann, Eugster, Zweifel, Buchmann, & Kahmen, 2016; Roman Zweifel et al., 2020). Previous studies have shown that TWD measurements provide key information about drought stress during the growing season (Drew, Richards, Downes, Cook, & Baker, 2011; Köcher, Horna, & Leuschner, 2013; Oberhuber, Kofler, Schuster, & Wieser, 2015). However, there is a lack of understanding about how plant water status measurements (as inferred through TWD), and responses to soil water potential gradients, affect source water partitioning. Predicting whether a tree is water- limited or has access to sufficient water supply would be difficult to by ascertain using soil matric potential alone. This is difficult because tree's access to water is dependent on species specific hydraulic traits that drive its capacity to maintain xylem hydraulic conductivity under decreasing soil water potentials and increasing atmospheric demands (McDowell et al., 2008; Sperry, Adler, Campbell, & Comstock, 1998; Sperry & Love, 2015). Tree water status can integrate this information and it inherently considers different water use-strategies across species in the same environment.

Here we present results from a controlled experiment that shows how plant water status affects source water apportionment and tree water uptake. We test whether coupling high-temporal resolution plant water status with isotope measurements can provide a mechanistic understanding to observed tree water source partitioning. To do this, we leverage the 'SPIKE II' experiment. This experiment was carried out in a large (2.5 m^3) weighed lysimeter

planted with two willow trees (*Salix viminalis*) with measurements of plant and soil water isotopic (δ^2H and $\delta^{18}O$) ratios, coupled with measurements of plant hydraulics, root distribution, climatic variables, soil water content and soil matric potential. We use this high-resolution data to answer the question: How does plant water status affect water use partitioning? More specifically, we ask: 1) What are the main environmental drivers of tree water deficit? 2) Do these drivers explain patterns in plant water source partitioning? Our work combines high spatial and temporal resolution measurements in a controlled environment (as advocated by Freyberg et al., (2020) and Penna et al., (2018)).

2. Material and Methods

2.1. Experimental layout

Our experiment was conducted on a vegetated continuously weighed soil lysimeter, situated at the École Polytechnique Fédérale de Lausanne (EPFL), in Switzerland (Figure 1). The lysimeter is located outside and is exposed to atmospheric conditions and precipitation inputs. The experiment consisted of monitoring the lysimeter for a period of 43 days after the application of an isotopically labeled irrigation event on May 16th, 2018, ending on June 29th,2018.

The lysimeter column was the same as described in Queloz et al., (2015) and consisted of a fiberglass-polyester cylindrical tank with 2.5 m depth and 1.12 m^2 base area. The column was filled in 2012 with soil (a mixture of equal proportions of local loamy sand and lacustrine sand from Lake Geneva) on top of a 0.5 m layer of gravel (to facilitate drainage and avoid clogging the outlet), and two small *S. viminalis* clones (originated from branch cutting of the same tree) were planted in the lysimeter. The *S. viminalis* species was chosen because of its ability to withstand drought and flood conditions (Frédette et al., 2019; Queloz et al., 2015). While the soil remained undisturbed since 2012, the trees were cut at the base in 2014 and allowed to regrow. At the beginning of our experiment, the tree's main stems had regrown to over 3 m. The soil surface of the lysimeter was at ground level, located in an open grass field. The base of the lysimeter was accessible by an underground chamber, where free drainage at the bottom was quantified using a tipping bucket (Casella Measurement, UK). The lysimeter sat on three load cells (HBM, Germany) connected to a digital transducer (AD103C, HBM) and weight was electronically logged (AD Panel32 software, HBM) at 20 s intervals.

2.2 Atmospheric and soil conditions

An automatic weather station (MeteoMADD, MADD Technologies Sàrl, Switzerland) located five m away from the lysimeter provided air temperature (Ta, in °C), solar radiation (Rn, in Wm^{-2}) and relative humidity (RH in %) at 15 min intervals. We calculated vapour pressure deficit (VPD) based on relative humidity and air temperature records. We used frequency domain reflectometry probes (FDR; 5TM Devices Inc., USA) to monitor and record volumetric water content at four different depths (10, 25, 125, 175 cm, with 2 probes at each depth). A soil-specific calibration curve was measured in the lab during the experimental set up (Queloz et al., 2015). Soil matric potential (Ψ_S) was monitored at four depths (25, 75, 125, and 175 cm) within the lysimeter using TensioMark® probes (ecoTech UmweltMeßsysteme, GmbH, Germany). Soil matric potential measurements were recorded as pF. All measurements were recorded at 15 min intervals using a CR1000 data logger (Campbell Scientific).

We monitored the soil water storage and occasionally irrigated the lysimeter with tap water (-87.84 ‰ ± 0.86 δ^2H and -12.01 ‰ ± 0.18 $\delta^{18}O$) to avoid tree water deficit conditions in the early phase of the experiment. We then suppressed irrigation during the final period to generate drier soil conditions and to induce changes in plant water status. Irrigation was always carried out at night to minimize evaporation (and thus minimize isotope fractionation), using a drip irrigation system. The isotope tracer application took place on May 17th and consisted of 25 mm of tap water mixed with deuterium oxide (D_2O; 99.5% Cambridge Isotopes, Cambridge, MA, USA) and oxygen-18 water ($H_2{}^{18}O$; 97.7% Medical Isotopes, Pelham, NH, USA). The mixture had an isotopic composition of 256.55 ‰ ± 1.0 δ^2H and 29.63 ‰ ± 0.2 $\delta^{18}O$, which had been prepared to lie along the local meteoric water line (LMWL). We applied this tracer to create a more heterogeneous isotopic soil water profile and to enable detection of source water uptake patterns by plants.

2.3 Plant water status measurements

Our plant hydraulic measurements were conducted on the 3 main stems (two belonged to a single tree and root system, while the third belonged to the other clone). We monitored stem radius change using a non-invasive automatic small diameter dendrometer (DD-S; Ecomatik, Germany; type small diameter dendrometers, accuracy ±1.5 µm). Dendrometers were installed around the base of each main stem and in a location free of branches. The initial diameter of the willow stems were 29.73, 28.15, and 29.02 mm. Stem radius change was recorded every 15 min with a datalogger (DL15, Ecomatik, Germany). These measurements allowed observations of tree diel dynamics including growth-induced irreversible stem expansion caused by the development of new stems cells, and reversible shrinking and swelling of the living stem cells due to changes in water storage following method outlined in Zweifel et al., (2016) and Zweifel (2016), and extensively used by others (e.g. Brinkmann et al., 2016; Drew et al., 2011; Obojes et al., 2018). We refer the reader to section 2.7.1 for detailed information.

We measured sap flow rates (J_s, in mm day^{-1}) just above the dendrometer sensor on the three main stems with a heat balance gauge (EXO-Skin SGA19; SGA 25, SGA 25 Dynamax, Houston, TX, USA). We installed these sensors immediately above the dendrometers where there were no branches. We were not able to install the sensors closer to the base of main stems, or below the dendrometers, because it requires a smooth surface, which were only found at ~ 50 cm height. Sap flow sensors consisted of a heater band that was constantly supplied with power. A pair of thermocouples located at the top and bottom of the band measured temperature gradients associated with conductive heat loss during transpiration. This system used a heat balance method to estimate sap flow density following methods described in detail in Lascano et al., (2016). Sap flow measurements were taken every minute and the average over 15 min was recorded with a datalogger (CR1000 and AM16/32 multiplexer; Campbell Scientific). We selected the heat balance method because it was non-invasive and suited to small diameter stems. We also equipped the tree with a leaf psychrometer (PSY 1, ICT International, Australia) to provide high-resolution measurements of leaf water potential (Ψ_l in MPa). Leaf water potential was recorded every 15 min. As per equipment requirement, we rotated the psychrometer every 4-5 days to a new leaf on the same branch.

2.4 Sampling of tree water and soil water sources

We sampled the tree and soil water sources in the lysimeter between May 16th and June 29th, 2018. We collected samples for isotope analysis of: precipitation, bulk soil water, mobile water (porous cups) following (Brooks, Barnard, Coulombe, & McDonnell, 2010), tree xylem and phloem water (extracted) . We obtained bulk soil water by sampling five different depths (10, 25, 50, 80 and 150 cm, with two replicas per depth) every four days throughout the experiment. Samples from the 10 and 25 cm depth were collected through vertical cores using a 3 cm diameter auger. The deeper soil samples were collected through lateral cores using the same auger through small ports on the side of the lysimeter tank. We collected samples progressively inward from the same two access points. This was done to avoid creating a large number of ports at the same depth. We collected approximately 8 cm of soil per sampling event and discarded the first 3 cm to ensure that we were not sampling any enriched soil water due to any possible evaporation at the soil-lysimeter column interface. We closed the access point with a sealed PVC pipe of the same diameter to minimize possible preferential leakage, evaporation, or condensation of water in the empty space. For the surface soil sampling locations at 10 and 25 cm, we filled the open space with a dry soil (same soil in the lysimeter column). We also flagged the vertical sample locations to avoid future resampling of those areas. Soil samples were immediately stored in 12 mL Exetainer® vials (Labco Ltd, Lampeter, UK).

For mobile water sampling, we used a system of radially-inserted ceramic porous cups combined with an automated pump to collect soil water (Queloz et al., 2015). We collected this mobile water every other day from five different depths (10, 25, 50, 100, and 150 cm) at three different locations distributed radially within the lysimeter. We vacuumed down simultaneously each porous cup in the lysimeters to 600 hPa for six hours prior to sample collection. We collected natural precipitation from a collector installed 2 m away from the lysimeter. Precipitation was collected immediately after the event, or in the morning following the event on occasions when precipitation occurred overnight. We collected bottom drainage at the base of the soil column every time there was outflow. The bottom drainage was first routed to a tipping bucket to measure flow and then a volume corresponding to three tilts of the tipping bucket (18 mL), which was directed to an automatic sampler through a solenoidal valve. We immediately filtered the samples with 0.45 μm disk filters into a 2 mL vials (2 mL Clear Vial, Canadian Life Science) and tightly sealed them to prevent evaporation.

We sampled xylem from branches for isotope analysis. Frequent sampling was possible because of the crown architecture of the trees with numerous long branches. We collected xylem samples each day for the first eleven days (until May 23th). We then changed to every other day until June 10th. After this period, we followed bulk soil sampling frequency and changed xylem sampling to every four days. We returned to every day until the end of the experiment on June 22nd. The variability in sampling frequency during the experiment was to allow high-temporal frequency observations of transpiration dynamics, while preserving the architecture of the crown by decreasing frequency during other periods. We selected suberized branches with mature bark to avoid evaporative fractionation that might occur through unsuberized stems (Dawson & Ehleringer, 1993), and sampled during midday when transpiration was at peak (Hervé-Fernández et al., 2016; Lai, Ehleringer, Bond, & U, 2006) . We recorded the length, diameter and branch sampling locations in reference to the three main tree stems. Immediately after removal of a branch section, we covered the wound with silicone to minimize any possible evaporation from the exposed

surface. The sampled xylem was quickly separated from bark and inner bark, chopped and stored in 12 mL Exetainer® vials. Phloem samples (inner bark 'strips') removed from xylem, and sampled from the same branch, were also stored in separated vials for posterior water extraction. All samples from this experiment were stored in a refrigerator at 4°C.

2.5 Water extraction and isotope analysis

Isotope analysis of collected waters were conducted at the Watershed Hydrology Lab at the University of Saskatchewan. Mobile water (water held above 600 hPa), precipitation, and bottom drainage were analyzed via laser spectrometry - in liquid mode (OA-ICOS ; Los Gatos Research Inc., USA). Laboratory precision was ± 1.0 ‰ and ± 0.2 ‰ δ^2H and $\delta^{18}O$, respectively. We extracted water from bulk soil samples, and plant material using cryogenic vacuum distillation method, following Koeniger et al., (2011). Isotopic composition of extracted bulk soil water was later analyzed on OA-ICOS . However, because of the risk of spectral interference from co-extracted organic compounds from plants (Millar, Pratt, Schneider, & McDonnell, 2018), isotope analyses of plant water samples were performed at the National Hydrology Research Centre Stable Isotope Laboratory using isotope ratio mass spectrometry (Elementar Isoprime and Delta V plus IRMS). Laboratory precision for this specific project was ± 0.81 ‰ and ± 0.12 ‰ δ^2H and $\delta^{18}O$ (n =8; water standards), respectively.

2.6 Spatial fine root distribution

At the end of the experiment, we sampled fine roots to assess root functional traits. We determined the fine root length density (RLD, cm of root per cm^3 of soil) (Gregory, 2006), root tissue density (RTD, g of dry root per root volume cm^3), and specific root length (SRL, m of root per g of dry root mass) (Ostonen et al., 2007) throughout the 2 m deep soil profile. We collected three soil cores using a cylindrical soil auger (internal auger diameter of 54 mm) distributed radially around the main willow stems. We sampled every 25 cm until reaching the bottom of the lysimeter. We sieved and carefully washed soil samples individually using a 250 µm sieve to obtain fresh root samples. The fresh roots from each depth and cores were stored in plastic bags at -6 ˚C until analysis. Individual samples from each depth and cores were scanned on a flatbed scanner (Epson model V700) at 400 dpi. Images were analyzed using WinRHIZO software (Regent Instruments Inc.) to acquire the root length (cm) and volume (cm^3) among root diameters classes. Several root functional traits were calculated for fine roots (< 2 mm) only. We converted root length per depth to root length density (RLD) based on the associated soil volume (572.55 cm^3). We obtained dry root mass (< 2 mm) by oven drying the roots at 65 ˚C for 48 h. We then calculated specific fine root length (SRL) as the ratio of fine root length and fine root dry mass (m g^{-1}). Root tissue density (RTD) was obtained by the ratio of fine roots (< 2 mm) mass (g) per associated root volume (cm^3).

2.7 Data analysis

Data analyses and visualization were done using R, version 3.5.1 (R Development Core Team 2015).

2.7.1 Computation of tree water deficit and definition of 'dry' and 'wet' periods

We calculated TWD to obtain the overall plant water status based on the assumption that plant physiological responses change according to atmospheric and soil conditions (Dietrich et al., 2018; Drew et al., 2011; Zweifel et al., 2005). We first verified that the stem radius variation on the three stems showed similar diel

cycle patterns. Then, we used the averaged value of stem radius from the three stems to determine TWD following the approach developed by Zweifel et al. (2016) (Figure 2). TWD is the difference between the past maximum stem radial record (SR_{max}) and current stem radial record (SR_t). The continuous SR_{max} record indicates the irreversible growth (GRO) (Figure 2; green line for individual stems). TWD is the reversible, tree water deficit-induced stem shrinkage. Zero TWD indicates that tree water storage compartments and cambial zone were fully hydrated, close to saturation, and stem water potentials are close to zero. TWD values above zero represent water loss from stem and indicates that stem water storage is not near saturation and stem water potentials are below zero.

We defined periods of tree water status based on continuous observations of TWD, also following Zweifel et al., (2016). Our definition of 'dry' or 'wet' is not based on soil moisture conditions but rather on the physiological response of trees to environmental conditions, which is achieved by continuously monitoring plant water status. A 'dry period', termed here *water deficit period*, implies that the stem is below a full hydration state over 24 h, that is, the TWD does not return to zero at night. During *water deficit period*, daily variability in TWD still shows diurnal patterns of stem shrinkage in response to transpiration demands and decrease in stem water potential, but stem radius remains in a partially shrunk state and water storages are not refilled to saturated conditions overnight. This corresponds to commonly used pre-dawn water potential, which decreases when plants are not able to refill storages at night (Larcher, 2003). Tree *water deficit period* ends when TWD returns to 0. In contrast, a 'wet period', termed here *no deficit period*, implies that the stem returns to full hydration state within a 24 h cycle, and TWD returns to zero at night-time. This condition can last for many days when trees are well hydrated and able to refill storages.

We further investigated the relationship between TWD and midday leaf water potential (Ψ_l) to explore the hydraulic coupling between these two variables for our investigated tree species. This relationship describes the change in water potential in relation to changes in relative water content. We did this by fitting a sigmoidal function as done by Dietrich et al., (2018).

2.7.2 Statistical analysis: Environmental drivers of plant water status and water source

Measurements of meteorological, soil matric potential, TWD and sap flux were aggregated to daily values for comparison of temporal dynamics. We investigated the relationship between TWD and transpiration rates with environmental variables using general linear models (stats, glm function in R). We used one-way analysis of variance (ANOVA) with Tukey's HSD (honest significance difference) post-hoc test to compare distinct periods of tree water status. We compared the isotopic composition of soil from different depths and sampling methodology using Kruskal-Wallis (Kruskal & Wallis, 1952) and post-hoc test (Dunn, 1964). We adjusted *p* values according to Benjamini and Hochberg (1994) method, as the data set were not normally distributed. We categorized soil water above and below field capacity and sampling methodology following Brooks et al., (2010) and Berry et al., (2017) . However, the mobile water collected with the use of porous cups and bulk soil water that was obtained through cryogenic extraction of soil from different depths were not significantly distinct ($p > 0.05$). Thus, we only used bulk soil water as source for water uptake since this sampling method already incorporates tightly bound water and mobile water isotopic signatures.

3. Results

3.1 Tree water deficit (TWD)

Figure 3 shows TWD throughout the seven-week experiment. Stems showed daily fluctuations in TWD, with maximum values reaching 110.16 µm in a day (June 15th). We identified three main periods of water status based on the continuous measurement of TWD. During the first two weeks of the experiment (May 16th to May 31st), the plant returned to a fully hydrated state overnight and thus was classified as *no deficit period.* A clear *water deficit period* was instead observed from June 14th to June 23rd, when TWD consistently remained below zero. We also observed an intermediate condition (from June 01st to June 10th), which we termed *intermittent water deficit period,* as it was characterized by equal days of TWD = 0 and TWD < 0 in the daily cycle. Hereafter, we refer to these periods in our analysis. We note that these three water status periods were statistically distinct ($F = 22.18$; $p = 7.83e\text{-}07$). The daily averages of TWD during each period were 18.8, 34.2, and 51.8 µm, for *no deficit period*, *intermittent water deficit period*, and *water deficit period*, respectively.

The total transpiration rates measured through sap flow sensors were compared to the transpiration rates estimated from the high-resolution lysimeter weight variations. While the two estimates had almost identical patterns throughout the entire experiment, values from the sap flow sensors were systematically lower. This is likely due to experimental limitations, such as tree architectural constraints where sap flow sensors were installed above a few long branches, thereby affecting measurements. To address this, we rescaled sap flow measurements to match the total transpiration obtained from the lysimeter weight variations. Transpiration rates were statistically higher during water deficit period ($F = 12.89$; $p < 0.001$) (Figure 4 F). We found a daily average transpiration of 11.5 mm d^{-1} (± 2.97) (± SD) throughout the experiment. Specifically, a daily average of 9.78 (± 2.8), 11.4 (± 1.8), and 14.3 mm d^{-1} (± 1.9) during, *no deficit period, intermittent water deficit period* and *water deficit period,* respectively. Transpiration rates were statistically related to TWD (Figure 5).

Leaf water potential measurements were interrupted on June 1st, due to a sensor failure. Thus, recorded data only included *no deficit period,* where the tree was well hydrated. During this shorter period, midday leaf water potential (Ψ_{midday}) ranged from 0 to -6.16 MPa and showed a direct relationship with TWD (Figure S1; Supplementary material).

3.2 Environmental conditions and plant hydraulic response

Figure 4 shows atmospheric conditions during the experiment. The *water deficit period* occurred during a 10 day dry spell, when no precipitation occurred, and average temperatures (21.6 ˚C ± 2.38) (Mean ± SD), solar radiation (257 kPa ± 18.8), and especially vapour pressure deficits (VPD) (1.18 kPa ± 0.19) were higher than in other periods. Precipitation during the experiment totalled 136 mm, 49% of which was recorded during *no deficit.* Total irrigation was 466 mm of which 54% occurred during *intermittent water deficit* period. This resulted in variable total soil water storage (mm) in the lysimeter and changes in Ψ_S throughout the experiment (Figure 4B).

Figure 6 shows variability in soil matric potential observed throughout the experiment. Soil matric potential was also variable with depth. Values of soil matric potential were 1.53 (± 0.09) at 25 cm, 1.57 (± 0.16) at 75 cm, 1.86 (± 0.05) at 125 cm, and 1.67 pF (± 0.04) at 175 cm during the *no deficit period.* During the *intermittent water deficit*

period, soil matric potential at 25 cm was relatively lower than other depths (higher moisture content). Values of soil matric potential ranged from, 1.58 (± 0.09) at 25 cm, 2.08 (± 0.22) at 75 cm, 2.19 (± 0.19) at 125 cm, and 1.86 pF (± 0.10) at 175 cm. During *water deficit period*, we observed the highest soil matric potential of 3.37 pF at 25 cm during the entire experiment (closest to the permanent wilting point of 4.2 pF). Soil matric potential at 175 cm showed the highest measured soil matric potentials during the *water deficit period*. Values of soil matric potential varied from, 2.28 (± 0.71) at 25 cm, 1.97 (± 0.49) at 75 cm, 2.06 (± 0.25) at 125 cm, and 1.80 pF (± 0.12) at 175 cm. Total soil water storage also dropped to about half during this period, from 391 mm to 228 mm (Figure 3B). We also observed visually that leaves started to wilt towards the end of this period.

Tree water status showed change in relationship with atmospheric and soil conditions throughout the experiment (Table 1; Figure 7). TWD showed a strong relationship to atmospheric variables during *no deficit period*, whereas no relationship was observed with soil matric potential. During *intermittent water deficit period*, TWD had a strong relationship with soil matric potential at 25 cm, but not with soil matric potential at lower depths. During *water deficit period*, soil matric potential in deep layers had the largest explanatory power over TWD. Soil water storage (mm) also showed a strong relation to TWD during *water deficit period*. In contrast to TWD, we found no relationship between transpiration rates and soil matric potential. Transpiration rates were driven mainly by VPD during *no deficit* and *intermittent water deficit period* and only responded to changes in atmospheric conditions. During the *water deficit period* transpiration rates showed stronger relationship with air temperature.

3.3 Root distribution

We found roots at all depths of the lysimeter, including fine roots (< 2 mm) at 200 cm. Overall, the fine root length density (RLD) showed a decline from 14.24 cm cm^{-3} ± 5.81 (mean ± SD) at 0-25 cm to 6.47 cm cm^{-3} ± 2.99 (mean ± SD) at 200 cm (Figure 8). Fine roots represented 99% of the total root length density (RLD) sampled in the lysimeter, of which the large majority (> 90% for all depths) were < 0.5 mm in diameter. Root tissue density (RTD) was higher at shallow soil layers, with greatest value of 0.29 g cm^{-3} ± 0.03 at 50 cm deep (Figure 8). Overall, the willow showed relatively high specific root length (SRL). Deep soil layers had longer SRL than shallow soil, with highest value of 132 g cm^{-3} ± 54.99 (mean ± SD) at 125 cm deep (Figure 8).

3.4 Water source apportionment

Bulk soil water (n = 130) isotopic compositions ($\delta^{2}H$ and $\delta^{18}O$) were significantly distinct along the soil profile ($p < 0.01$) for both isotopes. Soil layers at 10 and 25 cm deep were significantly distinct ($p < 0.01$) from soil layers at 50, 80 and 150 cm for both isotope signatures throughout the experiment. We thus define shallow soil layers as 10 and 25 cm and deep soil layers from 50 to 150 cm. The depth-average isotopic composition of shallow soil layers was significantly enriched in both ^{2}H and ^{18}O compared to deep layers (Figure 8). The shallow soil layers became more enriched during *no deficit period* due to mixing with the highly-enriched labeled tracer irrigation, and during the *intermittent water deficit period* as a result of more enriched precipitation in ^{2}H and ^{18}O ($\delta^{2}H$ = -41.41 ‰ ± 4.62 and $\delta^{18}O$ = -6.62 ‰ ± 0.29). We also observed large standard deviations in the isotopic composition of the soil profile as a result of spatial isotopic variability in the lysimeter.

Figure 9 shows the isotopic composition of xylem water throughout the experiment (n = 59). Xylem water plotted in between water sources during *no deficit period*. We observed an enrichment of 12.19 ‰ and 1.94 ‰ for δ^2H and $\delta^{18}O$, respectively on May 17th after the irrigation of the tracer. The xylem became even more enriched in both ^2H and ^{18}O on May 21st (δ^2H = -51.48 ‰ and $\delta^{18}O$ = -5.76 ‰) in comparison to pre-irrigation values on May 16th (δ^2H = -73.89 ‰ and $\delta^{18}O$ = -9.55 ‰). The isotopic composition of xylem water was significantly distinct from deep soil layers for both δ^2H and $\delta^{18}O$ during *no deficit period* ($p < 0.05$). During the *intermittent water deficit period*, xylem isotopic compositions overlapped with shallow soil layers isotopic values. The mean isotopic composition of xylem during this period (δ^2H = -62.0 ‰ ± 5.85 and $\delta^{18}O$ = -7.29 ‰ ± 1.24) was more similar to mean shallow isotopic signatures (δ^2H = -61.3 ‰ ± 3.93 and $\delta^{18}O$ = -7.79 ‰ ± 0.488), than deep soil layers isotopic signature (δ^2H = -67.3 ‰ ± 1.68 and $\delta^{18}O$ = -8.88 ‰ ± 0.126). However, we did not find any statistical difference among xylem and shallow or deep soil layer isotopic composition during this period. During this same period, but after June 4th, the average isotopic composition of xylem became more enriched (δ^2H = -58.2 ‰ ± 2.92 and $\delta^{18}O$ = -6.46 ‰ ± 0.63) in both ^2H and ^{18}O as a probable result of enriched precipitation values that fell throughout this period (δ^2H = -41.41 ‰ ± 4.61 and $\delta^{18}O$ = -6.62 ‰ ± 0.29) and resulted in decreased soil matric potential at the soil surface (Figure 6). During *water deficit period*, the isotopic composition of xylem shifted to deep soil layers. The isotopic composition then became more depleted in both ^2H and ^{18}O (δ^2H = -69.0 ‰ ± 2.23 and $\delta^{18}O$ = -8.04 ‰ ± 0.266) in comparison to the previous period. The xylem isotopic composition was significantly distinct from shallow soil layers for both δ^2H and $\delta^{18}O$ ($p < 0.05$). Overall, xylem water isotopic composition plotted in between the isotopic composition of mean shallow and deep soil layers during *no water deficit period* (Figure 10). This composition shifted closer to shallow soil layers during *intermittent water deficit period* becoming enriched in both isotopes when compared to previous period, and then shifting to deeper layers during *water deficit period* (Figure 10).

3.5 Spatial variability of xylem isotope composition across branches

At the end of the experiment (June 29) we collected multiple xylem samples from the three main stems (labelled ‘N’, ‘SE’, and ‘SW’) and their secondary branches. Branch and stem diameter of the willows were homogenous, and we did not observe large variability in this regard. The mean and standard deviation of $\delta^{18}O$ and δ^2H grouped by main stem and by height of main stem (base vs crown) are reported in Table 2. Results show that there was moderate variability across samples (± 0.40 ‰ for $\delta^{18}O$ and ±1.65 ‰ for δ^2H). However, this was not related to the particular stem or height as no statistical difference was found across the three main stems ($p > 0.05$; n = 12) nor across samples taken from the base and the crown of the tree ($p > 0.05$; n = 12). The maximum difference in the mean isotope composition across the main stems was 0.2 ‰ for $\delta^{18}O$ and 2.1 ‰ for δ^2H.

4. Discussion

4.1 Soil drying triggers temporal response in plant water status

Our data showed strong response of TWD to atmospheric demand and soil moisture decline. During the *no deficit* (i.e., ‘wet’) *period*, plant water status was driven mainly by solar radiation and vapour pressure deficit. The daily variability in TWD is associated with stomatal response to short-term changes in environmental conditions and dependent on stem capacitance to offset the drop in water potential driven by atmospheric water demands (Steppe,

De Pauw, Lemeur, & Vanrolleghem, 2006; Zweifel, Item, & Häsler, 2000). We observed that TWD was reversed at night and driven by atmospheric conditions when the tree was not water limited by soil moisture in the root zone. The increase in transpiration rates and the decline in soil moisture during periods of *intermittent water deficit* and *water deficit* triggered an increase in TWD. As soil moisture declined, limited water availability ultimately lead to long-term increases in TWD as daily transpiration demands were not completely reversed at night by the replenishment of water in the elastic tissues. This resulted in the plants remaining in a partially shrunken state (Zweifel, Item, & Häsler, 2001). Soil depths with the highest matric potential (more water available for uptake) during the two periods of deficit ('water stress') explained most of the daily variability in TWD. This was the shallow soil layer (25 cm) during *intermittent water deficit period*, and deep soil layer (175 cm) during *water deficit period.* This may indicate that tree water supply was limited to the zones of moisture availability. We thus conclude that changes associated with tree water status were dynamic and one consequence of our findings are that such measurements may provide a better understanding of long-term species response to environmental conditions, even in open field settings.

Previous studies have shown agreement among TWD with pre-dawn and midday potential values. The latter suggests that TWD is a good proxy for tree water status (Brinkmann et al., 2016; Dietrich et al., 2018). In this study, the relation between midday leaf water potential and TWD also supports these findings (see Supplementary material). Although, limited by midday water potential data continuity. Water potential is a key physiological parameter, but often limited to point and labor-intensive destructive manual measurements (Kathy Steppe, 2018), or by sensitivity and high-maintenance psychrometer equipment that is prone to fail in field conditions. This highlights that measurements of TWD via sturdy dendrometers can offer more robust and continuous information about plant water status (Zweifel et al., 2015).

Contrary to TWD, transpiration rates were driven mainly by vapour pressure deficit and solar radiation. Soil water content was weakly related to transpiration rates, when matric potential declined, and soil moisture was limited. We observed an increase in transpiration due to atmospheric demand and air temperature during the *water deficit period*. We interpret this observed higher transpiration rates as a result of the tree's stomatal behaviour. Transpiration rates are maintained irrespective of soil water availability to maximize gas exchange. This lack of water regulation is usually found in *S. viminalis* and other willow species along with high transpiration rates (Frédette et al., 2019a). Species-specific hydraulic traits confer different stomatal controls and distinct risk of hydraulic failure at a given water supply in the soil (Choat et al., 2012). Because of the strong relationship between transpiration rates and TWD, the observed high transpiration rates during periods of limited soil moisture resulted in increased water deficit. However, the transpiration rates of species investigated here did not indicate direct physiological response to changes in soil moisture conditions; rather, this was mediated by the net change in water storage within the plant.

We are unaware of any work using TWD or any other measure of relative tree water content to improve the understanding of plant water source partitioning in higher-temporal resolution. The changing relationship among TWD and environmental variables have been shown elsewhere at seasonal scales, and previous studies have shown

the relative importance of soil moisture to TWD increases with increasing soil dryness. For example, field measurements of TWD in *Callitris intratropica* have been shown to be strongly coupled with atmospheric variables during the wet season, but soil water availability was shown to be of primary importance in determining patterns of TWD during the dry season (Drew et al., 2011). Brinkmann et al., (2016) documented that in one year, five investigated species (*Fagus sylvatica*, *Picea abies*, *Fraxinous excelsior*, *Abies alba* and *A. pseudoplatanus*) showed increased TWD in response to declining soil moisture. However, during the previous growing season with wetter soil conditions, only two of the investigated species (*P. abies* and *F. excelsior*) indicated significant TWD response to changes in soil moisture. Furthermore, Oberhuber et al., (2015a) showed that trees from the same species under different developmental stages can show distinct TWD in response to changes in environmental conditions. Tree water status of saplings of *Picea abies* were strongly affected by changes in soil moisture, while the water status of mature trees was not affected by soil moisture. Collectively, this past research supports the idea that adopting soil moisture as guiding criteria to understand 'dry' or 'wet' conditions is only suitable towards a broad-brush understanding of water availability to trees and a tree's water status. This is because different species, even individuals of the same species at different development stages, have distinct responses to changes in soil water availability. Indeed, it would be difficult to predict when the tree is under stress based on soil moisture alone. Tree water status is greatly dependent on available water to individual trees, which can influence water uptake patterns (Carrière, Ruffault, et al., 2020). This was clear even in very early work on soil water status where Holmes and Robertson, (1959, pp. 103) noted that "it is clear that any accurate description of the moisture status of a soil will not be simple. The plant is the only true indicator" of soil water status. TWD is a single descriptor that integrates the controls on plant water stress (net changes among storages and transpiration). Our work shows that studies looking at plant water apportionment may greatly benefit from deploying dendrometers and computing TWD.

4.2 Plant water uptake and plant water status

Our study supports the notion that water uptake is a dynamic process, as observed by others with high frequency measurements of δ^2H in field conditions (Volkmann et al., 2016). While the steady-state assumption is common in many ecohydrological investigations (Penna et al., 2018), the patterns we observed in the xylem along with soil water δ^2H and $\delta^{18}O$ indicate changes in source water partitioning. Plasticity in root water uptake at seasonal scales have also been observed previously (Antunes, Díaz-Barradas, Zunzunegui, Vieira, & Máguas, 2018; Carrière, Martin-StPaul, et al., 2020; del Castillo, Comas, Voltas, & Ferrio, 2016; Ellsworth & Sternberg, 2015; Martín-Gómez, Aguilera, Pemán, Gil-Pelegrín, & Ferrio, 2017). However, the mechanisms that explain changes in depth of uptake (and hence presumably water age in the transport volume) relative to water availability are difficult to quantify at finer temporal scales. Our high-temporal resolution measurements of soil matric potential and plant water status, along with stable isotope ratios of δ^2H and $\delta^{18}O$ for xylem and soil layers, suggest that identifying changes in plant water status may help to explain shifts in uptake depths. Changes in the relationship between TWD and soil matric potential appeared to indicate a shift in plant water uptake. The depth with the strongest relationship to TWD appeared to represent the depth where most of the water is supplying transpiration. During the *intermittent water deficit period*, xylem isotopic composition was similar to shallow layers (10 and 25 cm) and soil matric potential at 25 cm explained most of the variability of TWD. This is also supported by rainfall events that occurred throughout

this period. These increased matric potential at shallow layers and resulted in enriched isotopic composition, which was later measured in the xylem. Similarly, during the *water deficit period*, variability in TWD was driven by soil water potential at deeper and wetter layers, and xylem isotopic composition was similar to soil water at this depth. Zweifel et al., (2005) already raised the question of whether changes in the relationship among TWD and soil matric potential can represent changes in depths under which roots are withdrawing water. Our high-resolution measurements of $\delta^{2}H$ and $\delta^{18}O$ in xylem and soil support this early hypothesis made by Zweifel et al., (2005). Importantly, here we show that changes in the relationship between soil matric potential and TWD are matched by shifts in source water partitioning. During the *no deficit period*, trees appeared to be using a mixture of (pre-tracer) shallow and deep layers in the lysimeter, but we detected some uptake of the tracer after irrigation with an observed small enrichment in xylem water.

Fine root assessments confirm that tree water uptake was possible across the entire profile. Our measured willow root system was 2 m deep and was characterized by high fine root length density (RLD) and high specific root length (SRL). These values are comparable with the literature that also show higher root length density (RLD) of willows growing in well drained soils (Cunniff et al., 2015; Jackson & Attwood, 1996). Maximum rooting depth and specific root length are important to water supply acquisition and plant survival during dry periods (Fort et al., 2017; Palta et al., 2011). However, the proportional distribution of fine roots did not explain the patterns of source water partitioning as is often assumed in hydrological climate models (Feddes et al., 2001). For instance, Volkman et al., (2016) showed that two tree species growing in field conditions with similar fine root density distribution had different water uptake patterns. Kühnhammer et al., (2020) used a controlled set- up to measure transpiration in herbaceous species and showed that root water uptake patterns were better described by water availability in the soil profile than by root length density distribution. Our results further support that root distribution alone is not an indicator of water uptake dynamics.

The discussion that follows explores mechanistically the findings presented here and alternative hypothesis to isotopic observations in this experiment.

4.3 How plant water status drives changes in water uptake depth: three alternative hypotheses

The principal finding of this study is that a relationship exists between plant water status and patterns of source water partitioning. The main hypothesis and two alternative hypotheses are proposed to explain these findings: (1) soil drying triggers change in tree water status and results in a shift in source water uptake; (2) fractionation processes may bias source water interpretations during periods of water deficit; or (3) use of internal storage explains changes in xylem water isotopic composition.

4.3.1 Hypothesis 1: Soil drying triggers changes in water status and results in shift in water uptake

Water flow at the soil-plant-atmosphere continuum is driven by water potential gradients and is regulated by plant hydraulic conductance (Nobel, 2009). The driving force of water uptake by roots is the negative gradient of hydrostatic pressure between plants and soil matric potential. The ability of plants to withdraw water from soil depends on plant water potential being lower than soil water potential – i.e. the "'tug-of-war' on a hydraulic rope" as described by Sperry et al. (1998, pp. 347). Using Darcy's Law, the flow of water from soil to roots (Q) is the product of the hydraulic conductivity of the tree (K) and the transport driving force ($\Delta\Psi$) [$Q = -K\,\Delta\Psi$] (Sperry et al., 1998).

Thus, the relative proportion of water withdrawn per depth observed in a specific time period is directly dependent on the water potential difference (Ψ_s) between the tree and the soil at a specific depth .

Our first hypothesis is that soil drying results in a shift in water uptake. The observed larger relative contributions of depths with higher soil matric potential to transpiration is a result of less hydraulic resistance and greater plant-soil driving force at that depth. It has been observed that reduced soil moisture at depths reduces root hydraulic continuity with the soil (Carminati et al., 2013; Huck, 1970; North & Nobel, 1997), and root hydraulic conductance (North & Nobel, 1992). Thus, the soil depth that offered the least resistance to root water uptake—a weaker "tug of water"— more water to move into the transpiration flux. Alternatively, the soil depth with lowest matric potential would have offered more resistance, thus less water was energetically available to transpiration from that depth.

The larger contribution of water from soil layers with higher matric potential (more water) to xylem isotopic composition is in accordance with root water uptake theory and models that incorporate water uptake compensation mechanisms (Cowan, 1965; Javaux, Schröder, Vanderborght, & Vereecken, 2008; de Jong van Lier, Dam, Metselaar, Jong, & Duijnisveld, 2008). These suggest that plants can increase water uptake in wetter soil layers to compensate for uptake reduction in dryer layers— for maintaining water potentials and transpiration rates. However, soil moisture availability cannot explain patterns of plant water uptake alone. Volkmann et al., (2016) showed with high-temporal resolution isotope measurements that not all species respond similarly to increases in moisture availability. At their study site, *F. sylvatica* acquired water in approximately equal proportions from shallow and deep soil layers before, during, and following a rewetting event, while *Quercus petraea* withdraw water from deeper soil layers during drought, and delayed uptake of new event water at surface. This highlights that not all species show similar short-term responses to changes in water availability. Volkmann et al., (2016) proposed observed variability in species response may then be a result of trees specific hydraulic traits.

Our data shows that by combining stable isotope measurements with measurements that provides information about trees specific responses to water availability in high-temporal resolution can help to solve short-term (within days) water uptake dynamics. We can observe changes in plant water status in response to water availability at high temporal resolution to understand changes in uptake. When soil moisture is limited, the depth that controls TWD can be identified as the depth of uptake. Our results support Dubbert et al., (2019) who provided evidence that assessment of plant water use cannot be done with stable isotopes alone. Incorporating measurements that provide physiological understanding of plant response to moisture availability is key because species-specific hydraulic properties determine differences in the risk of hydraulic failure at a given water supply (Choat et al., 2012), which results in heterogeneous responses to drought across forest ecosystems (Anderegg et al., 2016).

Further studies are necessary to understand the use of TWD as an indicator of source water uptake shifts across a range of species with distinct drought response strategies. Plant species exist along a spectrum of stomatal regulation and leaf water potential that defines species hydraulic strategies (Fu & Meinzer, 2019; McDowell et al., 2008; Skelton, West, & Dawson, 2015; Tardieu & Simonneau, 1998). At one end of the spectrum are isohydric species that respond to drought by decreasing stomatal conductance and limiting water loss from transpiration. At the other end

of the spectrum anysohydric species which maintain high stomatal conductance and transpiration during drought, at the cost of embolism. Because of less restricted stomatal control on leaf water potentials, anysohydric species allow decline in leaf water potential as a result of declining soil water potential (Klein, 2014; Frederick C. Meinzer et al., 2016). Although additional physiological measurements would have been necessary to identify the hydraulic behaviour of *S. viminalis*, the continuous increase in transpiration rates during deficit periods (at declining soil water potentials) may indicate a more anysohydric behaviour. This may explain the close relationship between TWD and soil storage during this period. We are not able to find any literature for this species specifically, however, other riparian species from the Salix genera have been found to behave as either isohydric or anysohydric (Pivovaroff, Cook, & Santiago, 2018). A better understanding of TWD and shifts in patterns of water use across species with distinct hydraulic strategies, as well the length of drought period and timing of species response (Martin-StPaul, Delzon, & Cochard, 2017) will be, necessary to provide a more complete understanding of the applicability of this approach.

4.3.2 Hypothesis 2: Fractionation processes during periods of water deficit

We observed an enrichment in xylem isotopic composition in δ^2H and $\delta^{18}O$ during the *intermittent water deficit period* as a result of the larger contribution of enriched water in shallow soil layers to transpiration, as opposed to drier soil in deep layers. The shallower soil layers received additional isotopically enrich water in-puts (in both isotopes) from precipitation events, increasing soil moisture and likely plant water uptake at this depth. We also observed that during the deficit period, there was a shift to depleted isotopic composition in deeper layers as the soil in the shallow layers dried. However, the possibility that fractionation processes occurred because of physiological processes and biased source water interpretations during these periods should be further explored.

Our second alternative hypothesis is that fractionation occurs during periods of water deficit. Previous studies have shown isotopic enrichment of xylem isotopic composition as a consequence of reduced transpiration rates (Ellsworth & Sternberg, 2015; Martin-Gomez et al., 2016). The enrichment in xylem water isotopic composition in these studies was observed after defoliation and leaf cover loss minimizing transpiration and resulting in enriched isotopic ratios in both δ^2H and $\delta^{18}O$ (around + 4 ‰ in $\delta^{18}O$; + 20 ‰ in δ^2H for both studies). It was proposed that observed enrichment under reduced transpiration rates is a result of increased xylem residence times, reducing the input of unenriched soil water and allowing for cumulative evaporative enrichment via bark (Martin-Gomez et al., 2016). One could argue that during periods of water stress in the lysimeter, there was a decrease in transpiration rates which could have resulted in enriched xylem isotopic composition. However, we did not observe reduction in transpiration rates during *intermittent water deficit* or *water deficit period*; in fact, it increased. It could be hypothesized that observed enrichment is a result of the mixing of xylem water with enriched water from the leaves (Brandes et al., 2007; Ellsworth & Williams, 2007). This may occur directly through the back-diffusion of enriched water from the leaf veins to the xylem (Dawson & Ehleringer, 1993; Farquhar & Lloyd, 1993). Although, we observed that leaf water was more enriched in both ^2H and ^{18}O (data not shown) than xylem water, our data did not show a difference in xylem water isotopic composition between base and crown of the tree. One would expect that xylem isotopic composition would be more enriched closer to the leaves if back-diffusion was an important process, but no evidence of this was found. For *Salix*, Busch et al., (1992) showed that the isotopic composition of xylem

water in proximity to the crown was not different from xylem isotopic composition at breast height. The isotopic variability was minimal within the tree. Minor variability within the tree crown and with larger variations at the landscape-level have been previously reported (Cernusak, Farquhar, & Pate, 2005).

We did not observe depleted xylem isotopic ratios during *intermittent water deficit period*. Instead, during this period, xylem water was enriched in both ^{2}H and ^{18}O. However, xylem δ-values were depleted during the *water deficit period*. This could be a result of increased aquaporin expression, and preferential uptake of lighter isotopes during water stress. Previous studies have reported increased aquaporin expression during periods of water stress (Lovisolo & Schubert, 2006; Parent et al., 2009; Rodríguez-Gamir et al., 2019). This occurs because the anatomy of the root tissue may change to avoid loss of water to drying soil by developing apoplastic barriers that decrease water permeability of the root membrane cell (Steudle, 2000). This in turn results in increased importance of alternative transmembrane water transport (i.e., cell-to-cell pathway) in roots. The transmembrane pathway is mediated by aquaporins, which are water channels proteins that facilitate water transport across cell membranes (Brunner, Herzog, Dawes, Arend, & Sperisen, 2015). The prevalence of transmembrane water transport instead of aploplastic is especially important for stable isotope investigations. It has been hypothesized that water transport via aquaporins can result in fractionation due to lower permeability of heavier isotopes, specially for ^{2}H (Chacko, Cole, & Horita, 2001). This was the proposed mechanism to explain depletions in both ^{2}H and ^{18}O isotopic composition in xylem water relative to source water in the presence of mycorrhiza (Poca et al., 2019), and in early findings that reported xylem water depleted in ^{2}H in xerophytic and halophytic species (Ellsworth & Williams, 2007). Nonetheless, the focus has been on static scenarios where apoplastic transport is permanently constrained because of the high degree of suberization and lignification of the root membrane cells (i.e. forming the Casparian strip) in xerophytic and halophytic species, or where mycrohryza structures are associated with root systems (Ellsworth & Williams, 2007; Poca et al., 2019). However, there is a lack of understanding on the effects of periodic water deficits and how that may enhance aquaporin expression. In a controlled experiment, Barbeta, Gimeno, et al., (2020) showed that $\delta^{2}H$ offset between xylem and soil water disappeared during drier conditions when compared to well watered conditions. We observed similar trends in the xylem water timeseries. We found no offset between xylem and soil $\delta^{2}H$ values during *water deficit*. However, our data do not support the explanation provided by Barbeta et al., (2020) because we did not observe reduced trends in transpiration during *water deficit*. Although, the cause offset is unknown the possibility of increased aquaporin expression cannot be excluded. The deep soil layers in our lysimeter study showed more negative δ-values and higher matric potential, which may also explain larger uptake of water from this depth. Although, there is a small difference in the offset between soil water and xylem water $\delta^{2}H$ and $\delta^{18}O$, both indicate deep water use. This highlights the need to monitor the impact of tree water status not only on source of uptake depth but possible changes in root morphology that may enhance pathways that increase isotopic fractionation, and impact source water interpretations.

4.3.3 Hypothesis 3: Storage water isotopic composition influences xylem water

Water contained as internal storage in bark is hydraulically connected to water in the xylem and contributes daily to transpiration (Hölttä, Mencuccini, & Nikinmaa, 2009; Scholz et al., 2008; Steppe et al., 2006; Steppe, Cochard, Lacointe, & Améglio, 2012). Water is transferred from elastic storages (i.e. phloem, parenchyma,

cambium) to buffer changes in water potentials and to maintain xylem hydraulic integrity (Goldstein et al., 1998; Mcculloh, Johnson, Meinzer, & Woodruff, 2014; Meinzer, James, Goldstein, & Woodruff, 2003; Scholz et al., 2007; Steppe & Lemeur, 2004). The radial flow of water from elastic storages to xylem can potentially affect our water source interpretations.

Our third hypothesis to explain how plant water status drives changes in water uptake depth is that internally stored tree water isotopic composition influences xylem water. The largest water storage within bark is phloem water. Phloem water $\delta^{18}O$ and $\delta^{2}H$ signatures are rarely reported in the literature. A previous study showed that for *Eucalyptus globulus*, phloem water was more enriched in $\delta^{18}O$ by 0.5 to 0.8‰ compared to xylem water (Cernusak et al., 2005). The isotopic enrichment of phloem water in trees is much smaller than others reported for herbaceous species (Cernusak, Pate, & Farquhar, 2002; Cernusak, Wong, & Farquhar, 2003). Interestingly, the phloem water isotopic composition of *S. viminalis* in our lysimeter experiment were systematically more depleted in ^{16}O and ^{1}H compared to xylem water across the 14 days measured (data not shown). This was unexpected based on previous reported work (Cernusak et al., 2005). There are, however, extremely limited reports of direct phloem $\delta^{18}O$ and or any $\delta^{2}H$ measurements in tree species in the literature. Cernusak et al., (2005) found phloem enrichment in relation to xylem during two sampling campaigns, but no enrichment was found on a third campaign, showing similar composition between phloem and xylem. The mean observed difference between phloem and xylem for *S. viminalis* in our experiment was -0.74 ‰ ± 0.53 in $\delta^{18}O$ and -2.46 ‰ ± 2.60 in $\delta^{2}H$. These results support recent studies showing that bulk stem water (including phloem, parenchyma, intracellular and xylem water) are more depleted in ^{2}H than solely sap water (xylem water) (Barbeta, Burlett, et al., 2020). Thus, the radial transfer of water from phloem to xylem in *S. viminalis* could potentially deplete xylem water in ^{18}O and ^{2}H. This might be especially true during water stress, when radial transfer can be highly important to help with embolism repair and to buffer larger offsets in xylem water potentials (Pfautsch, Hölttä, & Mencuccini, 2015). But because xylem-phloem a bidirectional flow mediated by aquaporins (Rodríguez-Gamir et al., 2019; Sevanto, Hölttä, & Holbrook, 2011; Steppe et al., 2012) we hypothesize that another possible explanation to more negative phloem in $\delta^{2}H$ and $\delta^{18}O$ values could be a result of preferential recharge of lighter isotopes in phloem by aquaporins coming from xylem during the evening when trees are refilling its storages as observed on stem radius measurements.

The influence of phloem to xylem water isotopic composition will be dependent not only on the difference between both δ-values but also on radial water flow ratios. The contribution of elastic storages to daily transpiration rates can be as little as 1% (Betsch et al., 2011) or 2-5% (Salomón, Limousin, Ourcival, Rodríguez-Calcerrada, & Steppe, 2017), or as much as 19% (Cermák, Kucera, Bauerle, Phillips, & Hinckley, 2007). We can estimate maximum daily elastic water storage contribution for *S. viminalis* in the lysimeter by using maximum observed stem radial shrinkage of 110.16 μm along with tree volume. Based on estimated tree volume obtained from biomass measurements and allometric relations, and assuming that radial shrinkage is entirely due to the loss of liquid water (Zweifel et al., 2016), we estimate a potential maximum contribution of 0.1 mm of water from inner bark to total transpiration during the observed period. This represents approximately 0.9% of measured average daily transpiration in the lysimeter. Our approach is limited, yet this supports the idea that phloem water storage will not result in significant changes in xylem water isotopic composition for the investigated individuals even if large radial

contributions (110.16 μm) were observed in periods of water stress. Beyond bark storage (phloem and parenchyma), water stored in the xylem (sapwood) can affect xylem water isotopic composition and resulting source water interpretations. A recent study has shown that accounting for internal tree water storage and mixing could improve estimations of root water uptake when using δ^2H and $\delta^{18}O$ (Knighton et al., 2020). Based on our estimated sapwood volume from destructive measurements and allometric equations, and considering reported 50% fresh biomass water content for *S. viminalis* (Wróbel & Wróbel, 2017) we estimated a total sapwood storage of approximately 3.5 mm of water. This volume is approximately one third to a quarter of the total daily water transpired by the willow during the experiment (11 – 14 mm). Such high transpiration rates are also found for other willows which gives them a "water wasting" reputation (Frédette, Labrecque, Comeau, & Brisson, 2019b). This may suggest that the willow has high turnover rates, which may not result in long residence times for sufficient mixing of sapwood storage with source water. Knighton et al., (2020) also showed that influence of storage on isotopic composition of transpiration for small trees with high transpiration rates is negligible. This is also supported by the species high wood density (ρ = 0.55 g cm^{-3} (Berthod, Brereton, Pitre, & Labrecque, 2015)), which characterize species with small stem capacitance (Oliva Carrasco et al., 2015). Thus, relatively small volume of water is released from storage tissues per change in water potential during transpiration. Nonetheless, variability in hydraulic capacitance (Salomón et al., 2017), atmospheric conditions (Zweifel et al., 2001) and flow rates and lags (Köcher et al., 2013) needs to be considered for a truthful assessment of storages contribution to transpiration.

4.4 Future tree water deficit research needs for source water understanding

From a methodological point of view, we show that combining δ^2H and $\delta^{18}O$ with TWD is relevant to plant water source research. Measures of water pools in plant tissue can provide a more comprehensive measure of tree hydraulic functioning than other metrics that emphasize flow (Martinez-Vilalta et al., 2019). Thus, tree water status may be a useful tool to understand not only tree responses to environmental changes such as drought (Anderegg et al., 2018), but also to improve our understanding of plant response to water availability and patterns of source water partitioning. TWD can complement stable isotope information and potentially help to identify source water partitioning by providing mechanistic understanding of tree water uptake patterns. Shifts in depth of water uptake could be identified with changes in TWD along with measurements of soil matric potential, *e.g.* observed more negative soil water potentials at shallower layers along with an increase in TWD, may indicate that contributions from shallow layers to transpiration are limited; whereas, recovery of TWD along with a decrease in soil matric potential in specific layers (more available water) may indicate larger contributions of this layer to the transpiration stream. This method could improve tree water use investigations. If the use of the TWD approach proves useful to indicate shift in patterns of tree water uptake in a wide range of species, then this methodology could be introduced to larger scale forest water use investigations. Such adoption of TWD and soil matric potential measurements to monitor tree water use across different species could lessen the reliance on continuous and intensive isotopic monitoring. Such measurements could also provide mechanistic parameterization of ecohydrological process for larger scale models. This approach could be also useful in cases where sources are not isotopically distinct (e.g., homogeneous soil water isotopic composition in the tropics), or in cases where fractionation processes may limit the use of isotopes (e.g., mycorrhiza fungi, xerophytic and halophytic species).

Future investigations of plant water use should go beyond soil water status as the only guiding criteria for sampling and interpretations of tree water sources. A comprehensive understanding of tree water deficit along with isotopic analysis could be an important step forward in understanding how trees drain the critical zone. The coupling of tree hydraulic (hydrometric) measurements to improve isotope interpretations of source water uptake is perhaps analogous to how catchment science has begun to couple precipitation-runoff hydrometrics with isotope tracing to improve understanding of the flow *and transport* mechanisms generating runoff. And like the results of that coupling, we see much new potential for developments in understanding ecohydrological processes. As an initial step, we advocate for continuous monitoring of TWD and soil matric potential across different species along with high-temporal resolution of xylem and soil water isotope sampling.

5. Conclusions

We combined fine root distribution mapping, stable isotope tracing and plant water status monitoring to systematically investigate plant water use. We used TWD as an integrative measure of plant hydraulics to understand tree response to atmospheric and soil matric potential. The combined isotopic signature and TWD measurement showed that short-term variation in source water partitioning is determined mainly by plant hydraulic response to changes in soil matric potential. Changes in relationship between TWD and soil matric potential indicate shifts in depth of uptake, while patterns of fine root distribution do not provide information about source water partitioning. These findings challenge the common assumption of a more static and uniform tree water use in space, and provide empirical support for root water uptake models that incorporate compensation mechanisms. Further field-based investigations are needed to understand whether these findings are observed in the field where roots are in an unconstrained environment and for species with distinct hydraulic strategies.

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Table 1. Results of the relationship between TWD and transpiration rates and single environmental variables. Results marked with *** helps to visually identify $p < 0.001$, and ** $p < 0.01$. * $p < 0.05$. Values in bold indicate variables with Adjusted R^2 (R2-adj) ≥ 0.60. Rn = solar radiation; T = Air temperature; VPD = vapour pressure deficit; Storage = soil water storage in the lysimeter; Soil ψ25 = soil matric potential at 25 cm depth; Soil ψ75 = soil matric potential at 75 cm depth; Soil ψ125 = soil matric potential at 125 cm depth; Soil ψ175 = soil matric potential at 175 cm depth.

Variable	Period	Statistics	Rn	T mean	VPD	Storage	Soil Ψ25	Soil Ψ75	Soil Ψ125	Soil Ψ175
TWD	No water deficit	R^2-adj	**0.54**	0.18	0.30	0.00	0.11	-0.07	-0.06	-0.06
		p-value	**0.001** **	0.059	0.016 *	0.321	0.108	0.952	0.705	0.678
	Intermittent deficit	R^2-adj	0.17	0.35	0.34	0.27	**0.62**	-0.12	0.01	0.02
		p-value	0.134	0.042 *	0.045 *	0.071	**0.004** **	0.885	0.320	0.303
	Water deficit	R^2-adj	0.42	-0.12	0.17	0.60	0.11	**0.90**	**0.91**	**0.94**
		p-value	0.024 *	0.964	0.134	0.005 **	0.180	**0.00001** ***	**0.00001** ***	**0.000001** ***
Transpiration rates	No water deficit	R^2-adj	**0.71**	0.33	**0.73**	-0.07	-0.07	-0.07	-0.07	0.18
		p-value	**0.000** ***	0.012 *	**0.000** ***	0.870	0.944	0.917	0.987	0.058
	Intermittent deficit	R^2-adj	**0.60**	**0.67**	**0.80**	0.42	0.02	0.16	-0.12	-0.12
		p-value	**0.005** **	**0.002** **	**0.000** ***	0.025 *	0.301	0.139	0.935	0.821
	Water deficit	R^2-adj	-0.08	0.47	-0.09	-0.12	-0.04	0.07	0.31	0.23
		p-value	0.580	0.017 *	0.649	0.800	0.442	0.227	0.055	0.093

Table 2. Spatial variability of xylem isotope composition across branches resulting from destructive sampling at the end of the experiment (June 29th). The three main stems were sampled across different heights and are indicated as N, SE and SW, while "Base" and "Top" indicate samples closer to the soil surface and to the crown, respectively.

	n	$\delta^{18}O$	sd	$\delta^{2}H$	sd
N	4	-9.17 ‰	± 0.32	-78.2 ‰	± 0.76
SE	4	-8.89 ‰	± 0.42	-76.1 ‰	± 1.23
SW	4	-9.37 ‰	± 0.43	-78.0 ‰	± 2.79
Base	6	-9.16 ‰	± 0.53	-78.0 ‰	± 2.46
Top	6	-9.13 ‰	± 0.29	-76.8 ‰	± 0.99

Figure 1: Lysimeter experiment set up and design.

Figure 2: Stem radius fluctuations of willow tree's main stems in gray tones. The difference between individual stem radius fluctuation and GRO defines TWD. The main three stems are represented: Stem 'N' is "north" stem, 'SW' is "south west" stem, and 'SE' is "south east" stem. The insert shows a dendrometer used in this experiment.

Figure 3: Average tree water deficit TWD. Colors indicate the different TWD periods. Blue indicates *no water deficit period*; yellow indicates *intermittent water deficit period*; red TWD indicates *water deficit period*.

Figure 4: Environmental conditions and transpiration rates during experiment. Period shaded in blue corresponds to *no deficit period*, in yellow corresponds to *intermittent water deficit period*, and red corresponds to *tree water deficit period*. Dashed line in respective colours represent mean value per period. A. Input = Precipitation (grey) and irrigation (black); B. Storage; C. Temp = Temperature; D. Rn = Solar radiation; E. VPD = Vapor pressure deficit; J_s = Transpiration rate.

Figure 5: Relationship between daily transpiration rates (J_s) and tree water deficit (TWD). This relationship is described by J_s = 1.955e-01 + 1.256e-02 TWD + -9.369e-05 TWD^2.

Figure 6: Soil water matric potential throughout the experiment. Top panel shows TWD and bottom panel shows changes in soil matric potential at different depths.

Figure 7: TWD and environmental variables relationship. Values for each linear relationship and period is reported in Table 1.

Figure 8: Fine root (< 2 mm) functional traits. RLD = root length density; SRL = specific root length; RTD = root tissue density.

Figure 9: Isotopic composition of shallow, deep, xylem, tracer, irrigation (tap) and precipitation, throughout the three periods of plant water status. Panel A shows TWD. Panel B and C show $\delta^{18}O$ and $\delta^{2}H$ timeseries, respectively. Inserts show period with higher sample frequency.

Figure 10: $\delta^{18}O$ and $\delta^{2}H$ plot for the different periods of plant water status, *no deficit period*, *intermittent water deficit period*, *water deficit period* (panel A, B, and C respectively). Xylem (Xy), shallow soil water (Sh), deep soil water (Dp), precipitation (Pp) and tap water (Tp) and respective evaporation line (slope is based on Benettin et al., (2019) for the same lysimeter and site) are shown in the same colour. Each plot shows the Local Meteoric Water Line (black) as references. Boxplots show average (line) per data type.