Continuous *in situ* monitoring of a labelled-water pulse through a boreal Scots pine forest: vertical and horizontal fluxes

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Abstract

Water labeled with stable isotopes provides a conservative tracer, being neither produced nor consumed, for water flowpaths within soils and root systems. We added a strong, evenly distributed ^{2}HHO label to one m^2 of soil surface and continuously monitored its passage downward into the soil and upward into the stems of surrounding trees, with the objective of illuminating spatiotemporal lateral root water uptake and overlap. The study was conducted during the historic drought of 2018 in a mature Scots pine (*Pinus sylvestris*) forest growing on sandy soil in northern Sweden. Continuous *in situ* isotopic measurements of tree xylem water evidenced root system overlap of six trees within the labeled square meter. This result is consistent with previous estimates from labelled nutrient uptake

measurements at this site. However, label uptake differed sharply among trees, even within the same radius; 90% of the label was taken up by one of the two trees closest to the labelled plot. Horizontal transport rates in tree roots averaged 0.17 ± 0.05 m d^{-1}, meaning that the arrival of label pulse in tree stems was delayed by 6-33 days from first tree to last. Root water uptake by trees appeared restricted to the upper 60 cm of mineral soil, even at the peak of the drought; the volume-weighted average depth of uptake was 30.6 ± 1.2 cm throughout the study period. Label intensity of the mineral soil weakened throughout the drought, consistent with the notion that the label was being dispersed or diluted into pre-event water. Our data provide a daily and three-dimensional description of the passage of a labeled water pulse, highlighting the heterogeneity in horizontal water distribution by roots and the uneven partitioning of label among individual trees in a boreal forest.

1. Introduction

Trees take up enormous quantities of water from the soil to grow and survive. This uptake is driven by atmospheric demand for water vapor (Campbell and Norman, 2012), and is constrained by soil moisture availability and root distributions (Bachofen et al., 2024; McCulloh et al., 2003). In forests, roots of different individuals, and their mycorrhizae, extend and overlap both vertically and horizontally, thus sampling across heterogenous water availabilities (Bachofen et al., 2024; Goldsmith et al., 2019), while also competing for water and nutrients (Henriksson et al., 2021; Lutter et al., 2021). So far, vertical patterns of root water uptake have been studied more often than horizontal patterns (Beyer et al., 2016; Jackson et al., 1996; Stocker et al., 2023).

In one of the rare studies investigating horizontal uptake patterns in forests, isotopical label uptake was detected as far as 3-6 m from the stem (Sternberg et al., 2002; Sternberg et al., 2005). Whether vertical or horizontal, long-distance transport comes at a considerable carbon expense to the tree (Guswa, 2008), which must ultimately limit the benefits of long horizontal roots. The costs and benefits are complicated by the near universality of common mycorrhizal networks (Martin and van der Heijden, 2024; Simard and Durall, 2004), which could extend uptake zones beyond the range of individual root systems. At present, the physiological significance of these networks is insufficiently quantified (Henriksson et al., 2023; Martin and van der Heijden, 2024). Here, we use “root” water uptake as shorthand to refer to tree roots plus their mycorrhizal partners.

These observations raise questions about the degree of overlap in uptake zones between individual trees (Henriksson et al., 2021; Kulmatiski et al., 2020). One might argue that, at the extreme, root systems might be so territorial that we observe no overlap in water uptake and the label would be detected in only one tree around the plot. Each tree’s roots system might grow as if it owned property, perhaps avoiding other roots when they are approached (Mahall and Callaway, 1992). At the other extreme, root systems might overlap extensively, making use of the limited soil volume in their immediate vicinity, whether it is occupied by other roots or not. Most data suggest the latter (Kulmatiski et al., 2010; Lutter et al., 2021; Lwila et al., 2024), but there are also instances of some

degree of territoriality (e.g., Kuiper and Coutts, 1992). If this is a continuous variable, then quantification of this overlap might be as simple as counting the labelled trees around a 1-m^2 labelling plot. Such quantification is needed for scaling water and nutrient uptake from individual trees to forest stands (Manoli et al., 2014), as well as for estimating belowground competition in silviculture and production ecology (Lutter et al., 2021). The overlap might reveal novel underground ecological processes, for example water sharing (Kakouridis et al., 2022) or stealing among individuals, which would influence the benefits of water-use efficiency (Cohen, 1970).

Tracing water flowpaths within soils is challenging and often involves destructive sampling, which implies large disturbances to the soil column and potentially irreversible harm to the root system. *In situ* methods, based on online monitoring of soil and tree xylem water isotopic composition, allow for tracking water through the soil-plant-atmospheric continuum in real time (Beyer et al. 2020). But so far, there have been few isotopic assessments of water uptake dynamics (Gessler et al., 2022; Kühnhammer et al., 2022, 2023; Landgraf et al., 2022; Seeger and Weiler, 2021; Volkmann et al., 2016).

We studied these questions using *in situ* methods in a boreal forest in northern Sweden during the "drought of the century" in the summer of 2018 (Gutierrez Lopez et al., 2021). This episode provided a unique condition wherein long horizontal or deep vertical roots might have been important, especially if they reached into distant water sources (Bachofen et al., 2024). Such a shift in water sources might (Lindroth et al., 2020) or might not (Gessler et al., 2022) compensate for soil drying, holding canopy evapotranspiration constant (Bachofen *et al.*, 2024). Responses to this drought event have already been described elsewhere in terms of sap flow (Gutierrez Lopez et al., 2021), latent heat flux (Lindroth et al., 2020; Mensah et al., 2021), and vertical water uptake patterns (Gessler et al., 2022). Horizontal water uptake over the whole growing season was described in an earlier study at the same site (Henriksson et al., 2021), but the short-term dynamics during the peak of the drought and the recovery period have not been investigated previously.

Here, we monitored continuously and *in situ* soil and tree xylem water isotopic composition during the peak and drought recovery periods. This experiment followed the dynamics and spatial distributions of a point-labeling with ^{2}HHO in a mature pine forest. We aimed to quantify the degree of territoriality in water uptake by counting labelled tree individuals, to quantify rates of horizontal water transport through roots, and to describe the fate of a label pulse applied to the mineral soil surface of a deep sandy soil.

2. Materials and Methods

2.1 Study site

This study was conducted in a ~100-year-old naturally regenerated, homogeneous forest dominated by Scots pine (*Pinus sylvestris* L.), in northern Sweden (Rosinedalsheden, 64°10'N, 19° 45'E, 145 m a.s.l.). Understory vegetation was composed of ericaceous shrubs (*Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L.) and a layer of mosses and lichens. In 2013, the leaf area index was 2.7 m^2m^{-2}. The stand averaged 18.6 (sd = 0.1) cm in diameter and 17.5 (sd < 0.1 m) m in total height in 2013 (Lim et al., 2015). The climate is typical of the northern boreal zone: average annual temperature and precipitation are 2.4 (sd = 0.8) °C and 638 (sd = 107) mm, respectively (Klosterhalfen et al., 2023). Winters are cold and long; summers are usually cool and wet. The photosynthetically active period extends from mid-April to mid-November (Tarvainen et al., 2018; Vernay et al., 2020). A soil description was performed at a nearby forest, approximately 6 km away, under similar stand and landscape conditions. The soil was labeled a Regosol (FAO 1988) that had formed on fluvial sediment. The texture was considered loamy sand to sandy loam with lenses of silt loam at 25-45 cm depth. The clay content was less than 11% (Plamboeck et al., 1999).

The sample plot was located approximately 30 m from an eddy covariance system installed on a tower above the uniform canopy (21.5 m above the ground)) (Lindroth et al., 2020; Zhao et al., 2022). The eddy covariance system measured latent energy fluxes from the canopy over the whole study period using H_2O mixing ratios measured with a gas analyzer (LI-7200, LI-COR Environmental, Lincoln, USA) at 20 Hz frequency (Jocher et al., 2017).

The study was conducted from June to September 2018, when northern Scandinavia, like much of central Europe, underwent an exceptional drought; in Scandinavia, it was the driest period in fifty years (Gutierrez Lopez et al., 2021; Lindroth et al., 2020). From snowmelt (18 May) until 23 July, a period of more than two months, the study area received only 56 mm precipitation. The drought was then broken by a series of strong rainfall events that delivered 85 mm in eight days. We applied the soil label in the middle of the drought period and tracked its passage through the last two weeks of the drought and into the wetter period afterward.

2.2. Continuous measurements of water isotopic composition

2.2.1. Experimental design

We selected 15 Scots pines within a circular plot of 12 m radius around the labeled area (Fig. 1). To study horizontal transport of water, we applied isotopic tracer on 10th of July 2018 over a 1-m^2 area in the center of the experimental plot. We present the isotopic data in δ-notation (Gonfiantini, 1978), with units of per mil (‰) relative to the VSMOW-SLAP scale. The tracer consisted of a mixture of 25 L of deionized tap water (δ^2H: -90‰) with 200 mL of ^2H–enriched water ($^2H^2HO$, 99.5%), yielding a ^2H atom fraction of 0.0163 ($\delta^2H \cong 106{,}000‰$). The ^2H-enriched water was injected at the surface of the mineral soil (i.e., underneath the moss and humus layers) by forcing a rigid plastic tube through

the organic layer and injecting water into the created hole using a syringe. To ensure homogeneous distribution of the tracer, we applied 70 mL at each of five points within each 12.5 × 12.5 cm grid across the 1-m^2 label area, totaling 320 injections (22.4 L). After injections on the grid were complete, we injected the small volume of remaining label randomly within the labeled area.

For the sake of completeness, it should be noted that two additional labeling experiments were attempted. The first, on the 6th of June, 2018 (soon after snowmelt), consisted of applying a weaker (δ^2H = 3500‰) label to the same 1-m^2 plot. The label was briefly detected in the surface soils but did not show up in the trees at all. Therefore, we repeated the labeling with the stronger ($\delta^2H \cong$ 106,000‰) label solution described above, on July 10th. The second experiment, on the 20th of July 2018, was designed to detect deep water uptake (Burgess et al., 1998; Caldwell and Richards, 1989; Dawson and Pate, 1996; Moreira et al., 2000). We applied 1 L of deionized water containing 2.94 mL of $H_2{}^{18}O$ ($\delta^{18}O \cong$ 470‰), at a depth of 50 cm, directly under the stems of five trees (see Beyer *et al.*, 2016, 2018) for further application details). We did not detect the ^{18}O label in any of these trees (data not shown). However, to avoid potential interferences between labeling experiments, these trees were dropped from consideration. They are represented by purple symbols in Figure 1 and not addressed further.

2.3 Volumetric water content

Volumetric soil water content (VWC) was measured along a vertical profile inside the labeled area using TDR probes (5TM, Metergroup, Pullman, USA). The TDR probes were placed at 10, 30, 50, 70, and 100 cm soil depth. For our sandy soils, we used the factory-default calibration. We took particular care when installing both the TDR and the isotope probes (see 2.3.2) to avoid damaging roots during probe installation, hoping that the roots from all surrounding trees would continue to take up water normally from the labeled block of soil. We estimated total water uptake by measuring the daily decline in VWC at each depth, multiplying VWC by the soil depth represented by that sample, and accumulating the decline over all depths (the single-step, multi-layer water balance of Guderle and Hildebrandt, 2015):

$$\Sigma U = \Sigma(\Delta VWC_{10} * D_{10} + \Delta VWC_{30} * D_{30} + \Delta VWC_{50} * D_{50}) \quad \text{Eq. 1}$$

Where ΣU is the sum of uptake per day, ΔVWC_n is the daily change in VWC at depth n, D_n is the thickness of the layer represented by each VWC (always 20 cm). This approach yields a water consumption rate (ΣU) in cm day^{-1}, integrated over the upper 60 cm of mineral soil. Because the uppermost TDR probe was located 10 cm deep in the mineral soil and was covered by the forest floor, we assumed there would be little contribution of soil surface evaporation to this measured change in VWC and that it would therefore be dominated by root uptake. We return to the broader topic of surface evaporation in the discussion.

We then measured the mean depth of uptake was by weighting $\Delta VWC_n * D_n$ of each layer by its mean depth, summing, and dividing by the total uptake, thus:

$$UD=\Sigma(\Delta VWC_{10}*D_{10}*10+\Delta VWC_{30}*D_{30}*30+\Delta VWC_{50}*D_{50}*50)/\Sigma U \quad \text{Eq. 2}$$

Where UD is mean uptake depth and 10, 30, 50 cm are the depths of each VWC sensor.

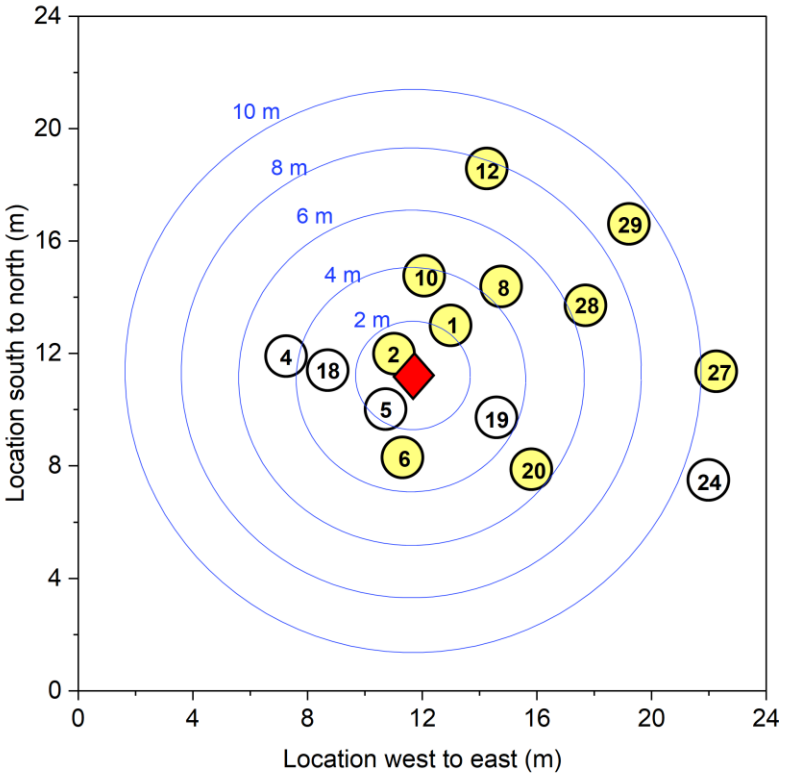


Fig. 1. Map of studied trees (numbered and yellow-filled circles). The 1-m² plot labeled with ^2H_2O is shown in the center as a red square. The trees labeled with $H_2{}^{18}O$ and subsequently dropped from the study are filled with white. Two Picarro L2130i analyzers were placed in a covered box adjacent to the plot center. Horizontal distance from the center of the labeled plot is indicated by the blue concentric rings and blue numbers. Distances of individual trees from the plot center are presented in Table S1.

2.3.2. In situ measurements of the soil and plant water isotopic composition

From June to September 2018, we measured the stable isotopic composition (δ^2H and $\delta^{18}O$) and soil VWC across depth inside the 1m² plot. The isotopic composition was measured with custom-made equilibration probes inserted at depths of 5, 10, 15, 20 ,30, 50 ,70, and 100 cm depth in the center of the labeled soil volume. The probes consisted of two PFA Teflon tubes (1/8"AD, 1/16" ID,

Teddington AB, Skogås, Sweden) that were glued (Pattex Kraft-Mix, Henkel AG & Co. KGaA, Düsseldorf, Germany) into a gas-permeable membrane (Accurel GP V8/2HF, 3 M, Germany; 0.155 cm wall thickness, 0.55 cm i.d., 0.86 cm o.d.) of 10 cm length. One of the two Teflon tubes was inserted a short distance into the probe. It provided air drawn through a desiccant tower (Drierite, Hammond, Xenia, OH, USA) reducing potential contamination with ambient water vapor. The second tube was placed at the opposite end of the probe to ensure that the air stream would equilibrate isotopically with soil water before leaving the probe. This tube was connected to a Picarro 16-port distribution manifold (A0311, Picarro Inc., Santa Clara, USA). The manifold was connected to a Picarro L2130i CRDS (Cavity Ring-Down Spectroscopy) water isotope analyzer. We verified that materials used did not affect measured isotopic composition by placing them in ambient air and comparing to measurements without soil probes. To prevent condensation, the PFA tubes were heated by electrical heating lines (TE's Raychem, TE Connectivity, Schaffhausen, Switzerland) wrapped in foam insulation tubes (Armacell, Münster, Germany).

We also measured the isotopic composition of tree xylem using the borehole equilibration method (Marshall et al., 2020). To measure xylem δ^2H and $\delta^{18}O$, a borehole was drilled all the way through the center of each tree. All trees were cored along the N-S axis at a height of 1.3 m using an electric drill with a 5-mm bit. We rinsed the boreholes with acetone to avoid the obstruction of cells by pitch (Marshall et al. 2020). The inlet of the borehole was open to the atmosphere to allow equilibration of ambient water vapor with liquid xylem water evaporated inside the borehole. We have previously shown that equilibration occurs even if ambient water vapor is added at the borehole inlet (Marshall et al., 2020). Although the continuity of the xylem tracheids is broken by the drilling, the borehole walls remain in communication with the flowing water in the adjacent xylem (Marshall et al., 2020) such that arrival time of a water label matches predictions based on sap flow (pers. comm., Kühnhammer). The outlet of the borehole was coupled to 6-mm PFA tube (Teddington AB, Skogås, Sweden) using stainless steel connectors (Swagelok) plumbed to a rotary 16-port valve (Vici, Valco Europe, Schenkon, Switzerland). The valve was connected to a second Picarro L2130i CRDS water isotope analyzer. Again, electrical heating lines wrapped in foam insulation pipes were installed to prevent condensation in the PFA tubing and the valve. This setup allowed continuous measurements of water concentration and isotopic composition of the water vapor equilibrated inside the borehole and allowed us to calculate the isotopic composition of the xylem water (Marshall et al., 2020).

All trees and the water isotope values of the atmosphere were monitored sequentially. Automatic calibration of the analyzer was performed every five hours using the Picarro Standard Delivery Module (SDM). The SDM injects a water standard of known isotopic composition into the analyzer's vaporizer. Each of the standards was measured in vapor phase for 20 minutes at two flow rates: 0.08 $\mu L\ s^{-1}$ and 0.05 $\mu L\ s^{-1}$. The two injection rates produced different water vapor concentrations, which we used to correct for the concentration-dependence of the isotope measurements. At each injection

rate, we used two standards covering the range of expected variability in δ^2H and $\delta^{18}O$: depleted deionized water (δ^2H = -94.28 ‰ and $\delta^{18}O$ = -12.87 ‰) and a homemade δ^2H-enriched standard (δ^2H = 225.8 ‰, $\delta^{18}O$ = -12.70 ‰). Any drift in instrument readings would have been detected and corrected for the multiple calibrations within each day. The isotopic composition of the two standards was determined on an isotope ratio mass spectrometer (DeltaV, Thermo Fisher) at the Stable Isotope Laboratory of the Swedish University of Agricultural Science (Umeå, Sweden). Each tree was measured for at least 30 minutes and the duration of the measurement interval for each tree was adjusted depending on the tube length. To avoid memory effects when the valve had just turned, we used only the data from the last five minutes in each valve position. To further avoid non-steady state conditions, as when condensation may have been present or stem temperatures were rapidly changing, we deleted values of water concentration with a standard deviation higher than 1,000 µmol mol^{-1} or values of water concentration that were higher than 50,000 µmol mol^{-1}.

Borehole temperature is a critical parameter for calculating xylem isotopic composition because it is needed for the conversion of water isotope values in measured vapor phase to liquid xylem water (Eq. 1-3). Copper-constantan thermocouples (Omega Engineering, Norwalk, CT, USA) were installed at approximately 2 cm depth within the borehole and in contact with the surface of the xylem tissue to measure the temperature of the xylem surface. We further filtered all data for which difference in temperature between the borehole and the atmosphere was higher than 5 degrees, which occurred most often when the temperature was rapidly changing in the morning and evening. As above, this was done to reduce the effects of condensation within the borehole or the tubing and to avoid the risk of non-steady state temperatures conditions within the borehole.

2.4 Data processing and analyses

2.4.1 Calculation of δ^2H and $\delta^{18}O$ of xylem water

After calibration and instrument drift-correction, the xylem liquid water $\delta^{18}O$ and δ^2H values were estimated using the relations proposed by Majoube (1971) for describing the equilibrium fractionation between the liquid and vapor phases of δ^2H and $\delta^{18}O$ based on the temperature (T in K) measured by the thermocouples in the borehole:

$$1000\ ln\ ln\ \alpha_{2H} = \frac{24.844x10^6}{T^2(K)} - \frac{76.248x10^3}{T(K)} + 52.612 \qquad Eq.\ 3$$

$$1000\ ln\ ln\ \alpha_{18O} = \frac{1.137x10^6}{T^2(K)} \qquad Eq.\ 4$$

The resulting α-values were multiplied by the raw isotope ratios (R, e.g., $^2H/^1H$) of equilibrated water vapor (R_{2Hvap}) to estimate liquid water isotopic ratio (R_{2Hliq}):

$$R_{2Hliq} = R_{2Hvap} * \alpha^2H \qquad Eq.\ 5$$

They were then converted back to delta notation.

The soil liquid water values were estimated using a "regression" method that uses a polynomial calibration curve to simultaneously account for isotopic equilibration (Majoube, 1971), device-specific effects of water vapor concentration, and the interaction of the sampling probe with the soil (Beyer et al., 2018, 2020; Oerter and Bowen, 2017). Regressions were fitted to measurements of dried bulk soil from the field site, placed in impermeable bags and filled with liquid water of known isotopic composition ("standards"). As with the xylem, these calibration measurements were made each time the manifold cycled through the samples throughout the field campaign. The resulting regression equations were:

$$^{2}H_{liquid} = (2.631e\text{-}04 * {}^{2}H_{vapor})^{2} + 1.216 * {}^{2}H_{vapor} - 0.001379 * H_2O + 119.9 \quad Eq.\ 6$$

with adjusted $R^2 = 0.9987$, and:

$$^{18}O_{liquid} = (-6.318e\text{-}09 * H_2O)^{2} + 0.7895 * {}^{18}O_{vapor} + 0.00005189 * H_2O + 5.754 \quad Eq.\ 7$$

with adjusted $R^2 = 0.85$.

2.4.2 Calculation of line-conditioned excess

To detect subtle increases in the δ^2H-enriched water in the trees, we used the local meteoric water line to calculate line-conditioned deuterium-excess (lc-excess) (Landwehr and Coplen, 2006). The local meteoric water line was estimated from precipitation collected at the Svartberget field station (https://www.icos-sweden.se/Svartberget), 8 km away. There were 225 samples collected at least biweekly during the whole of 2017 and 2018. The resulting LMWL was:

$$\delta^2H = 1.32 + 7.44 * \delta^{18}O \quad Eq.\ 8$$

with $R^2 = 0.97$.

The lc-excess was then calculated as:

$$lc\text{-}excess = \delta^2H_{measured} - (1.32 + 7.44 * \delta^{18}O_{measured}) \quad Eq.\ 9$$

Values of lc-excess above zero were interpreted as evidence of the uptake of δ^2H-enriched water. The advantage of this approach is that it removes background variation in δ^2H that is correlated with δ^{18}O, revealing low levels of labeling more clearly than the raw isotopic compositions. All analyses were performed using either excel or the base package of R (R Core Team (2023).

3. Results

The summer of 2018 began with only one significant rainfall after the snowpack melted. During the drought that followed, ecosystem evapotranspiration fell by about 50% but recovered immediately after the first rains (Fig. 2).

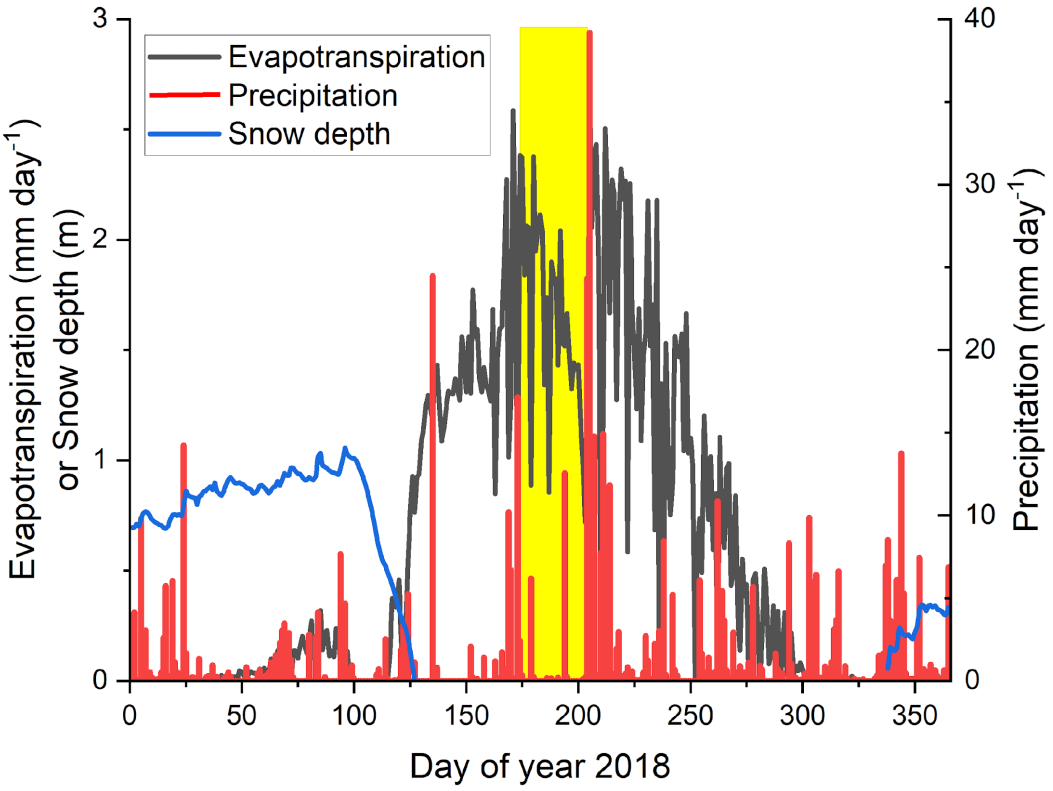
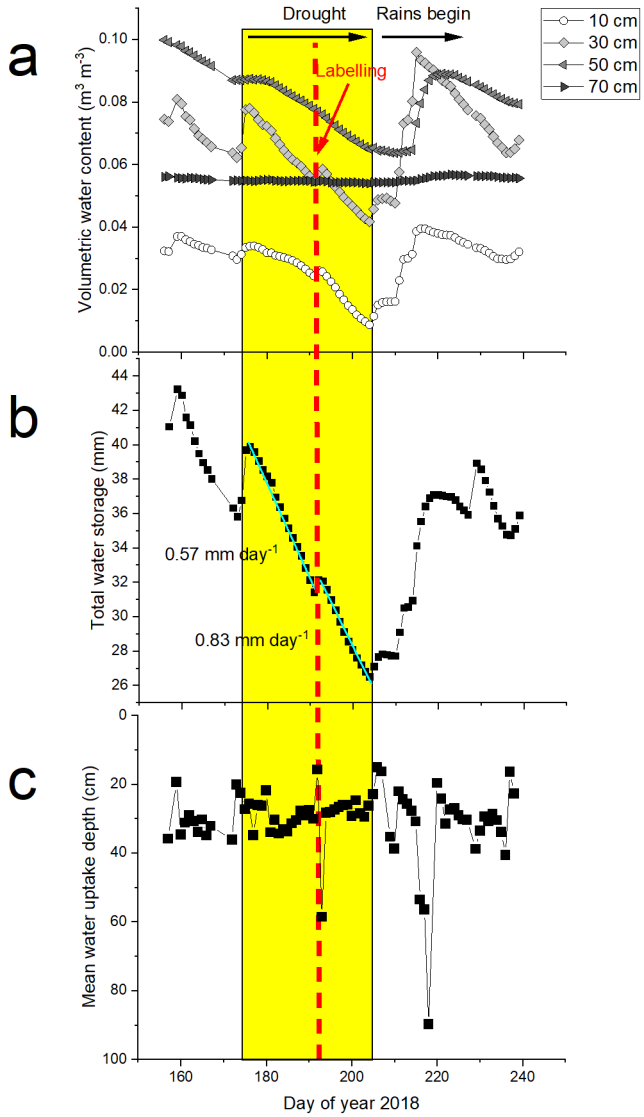


Fig. 2. Daily evapotranspiration at the Rosinedalheden boreal pine forest in calendar year 2018. The yellow box highlights the period of the drought. The drought began on day 174, more than a month after the snowpack melted (blue line), and was interrupted by only one small precipitation event (red bars). The drought ended with a series of large events beginning on day 204.

During the drought, the VWC fell at all depths within the upper 50 cm of soil (Fig. 3a). In contrast, the 70-cm depth showed almost no variation in VWC, consistent with our visual observations that there were few roots there. The isotopic labeling, which amounted to 25 mm of water on this plot,

occurred on DOY 191, and was accompanied by small, short-lived increases in VWC at 10 and 30 cm. The depth-weighted VWC in the upper 60 cm of soil (Fig. 3b) declined by 0.57 mm day^{-1} in the early part of the drought and 0.83 mm day^{-1} during the latter part (both R^2>0.99). Mean water uptake depth (Fig. 3c) reflected the steep decline at 30 cm depth, averaging approximately 30 cm throughout the drought. Brief excursions were detected after the water additions due to labeling (DOY 191) and the first rains (DOY 203). In general, mean water uptake depth moved towards the surface during the drying event. After the rains began on DOY 203, VWC sharply increased at all depths down to 50 cm, with the sharpest increase detected at 30 cm.



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315 *Fig. 3. a) Soil VWC (VWC) by depth under the labeled 1-m^2 area; b) Total water storage summed*
316 *over 60 cm of soil depth. Slopes of regression lines were fitted to the drought-induced decline,*
317 *neglecting the day after the labeling (m); c) Mean daily water uptake depth. The strong positive*
318 *excursions are associated with labeling and rainfall events. In all panels, the drought period is shown*
319 *by the yellow box and the labeling date is shown as a vertical, dashed red line at DOY 191.*

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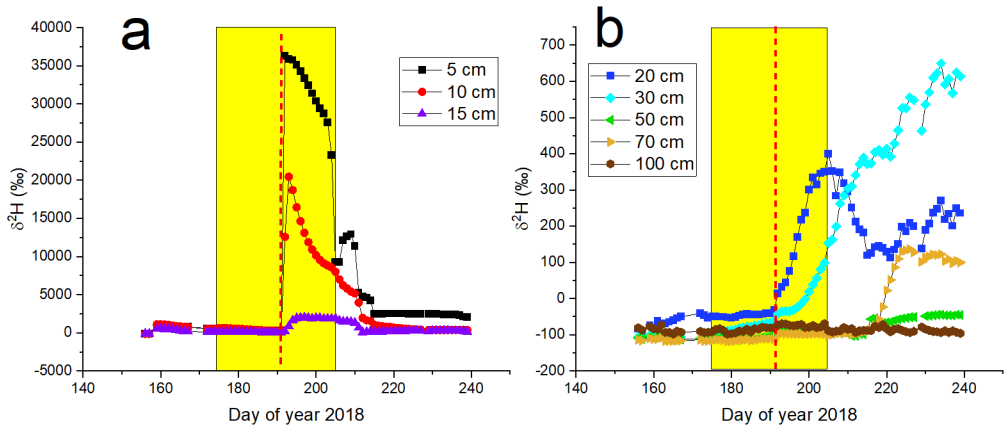
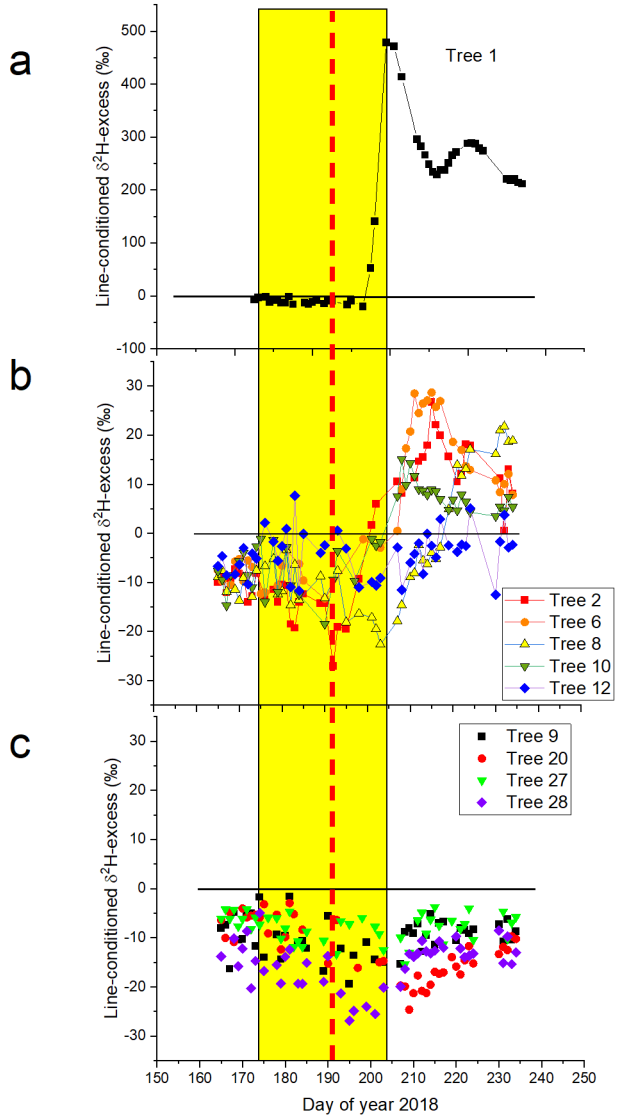


Fig. 4. *Isotopic composition (δ^2H) of soil water by depth showing a) δ^2H of depths up to 15 cm, b) δ^2H of depths 20 cm and below. Note the differences in y-axis scaling. . In both panels, the drought period is shown by the yellow box and the labelling date is shown as a vertical, dashed red line at DOY 191.*

After the ^{2}HHO labeling, the isotopic signature observed in the 5-cm probe briefly rose above 35,000 ‰, in the 10-cm probe above 20,000 ‰, and in the 15-cm probe above 2,000‰ (Fig. 4a). The probes at 20, 30, 50, and 70 cm responded more slowly and less strongly (Fig. 4b). We detected no increase in δ^2H at 100 cm depth by the end of August (DOY 240). Thus the label was concentrated in the upper 10-15 cm, especially in the period immediately after the labeling. In these upper depths, the label intensity fell by around 1,000 ‰ day^{-1} over the drought period and then dropped sharply when the rains arrived on day 204. Below, label intensity increased at 20 and 30 cm even before the rains arrived. The probe at 50 cm detected the label weakly, but the 70 cm probe detected it clearly; both the 50- and 70-cm probes responded about four weeks after the labeling. The $\delta^{18}O$ also increased at 5 and 10 cm after the ^{2}HHO labeling, but the increase was at most ≈20 ‰ (Fig. S1), three orders of magnitude smaller than for δ^2H (Fig. 4a).



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341 *Fig. 5. Line-conditioned deuterium-excess (lc-excess) of xylem water in tree stems. Values above zero,*
342 *denoted by the horizontal black line, show likely label uptake. Panel a) Tree 1, near the soil-labeling*
343 *plot; b) Labeled trees with lc-excess at some point greater than zero; c) Unlabeled trees, all more*
344 *than 4 m from the center of the labeling plot. Note that the y-axes are on different scales and the zero-*
345 *intercepts are at different locations on panel a vs panels b and c. In all panels, the drought period is*
346 *shown by the yellow box and the labelling date is shown as a vertical, dashed red line at DOY 191.*

347 Among the trees surrounding the 1-m^2 label plot, we detected the label in six (i.e. lc-excess > 0). Tree
348 1 showed by far the highest δ^2H values (Fig. 5a) and was responsible for 90% of the cumulative label
349 water uptake in the study (Fig. S2). The δ^2H of the xylem sap of this tree (at 1.3 m height) began to
350 change about four days after labeling and it reached its maximum (nearly 500 ‰) eight days after the
351 labeling (Fig. 5a). The label intensity then decreased, maintaining relatively high enrichment until the
352 end of the experiment. In contrast, δ^{18}O of this tree displayed only a slight increase (≈ 2 ‰) during

this period (Fig. S3). Four other trees on the plot increased δ^2H steadily over about two weeks after the labelling (Fig. 5b) reaching lc-excess values greater than zero. All these trees were within 4 m of the plot center. Three of the four trees within the 4-m radius began to respond before the rains started. Although it was more than 4 m from the plot center, Tree 12 also displayed lc-excess values greater than zero, but only briefly (Fig. 5b). The other trees that were more than 4 m from the plot center showed no response to the labeling (Fig. 5c).

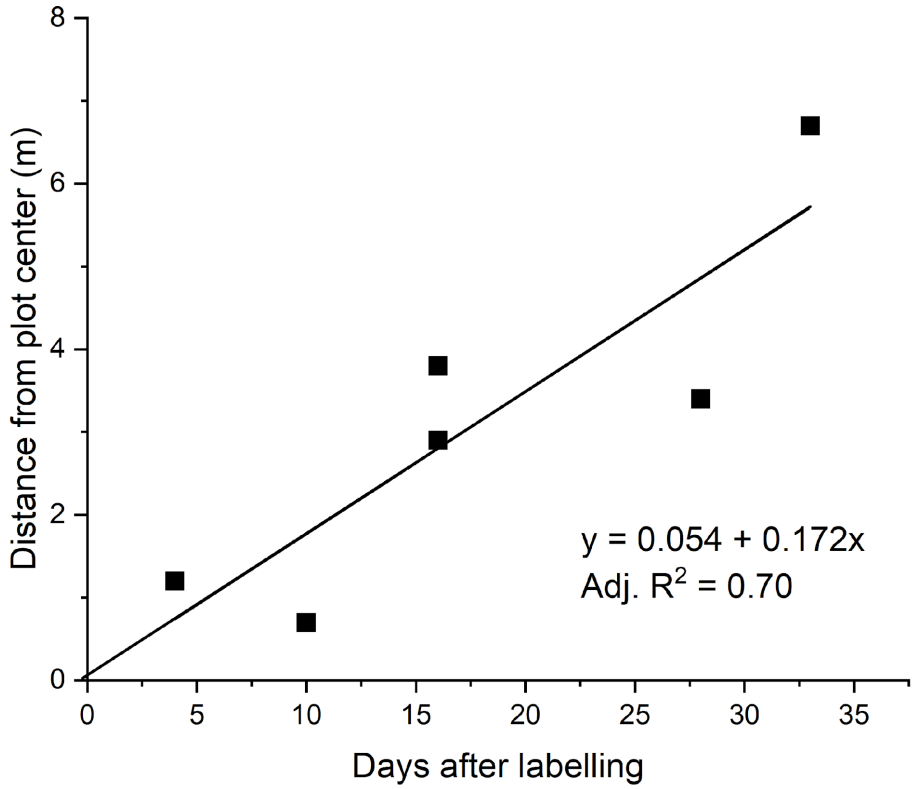

Figure 6. Distance from the plot center vs. number of days after labelling. Only trees that responded to the labeling are shown.

Among the six trees that showed label uptake, the breakthrough time varied with distance (Fig. 6). The first tree (Tree 1, 1.2 m from plot center, Table S1) required four days for label arrival and the last (Tree 12, 6.7 m from plot center) required 33 days. The x-intercept was approximately equal to

the label date (0 on this x-axis) and the slope of the regression line (0.172 (0.05) m day^{-1}) described the maximum horizontal transport rate.

4. Discussion

We added a strong δ^2H-labeled water pulse to 1 m^2 of the mineral soil surface in a mature boreal Scots pine forest and monitored water isotopic composition in soils and tree xylem continuously afterward. The label initially remained near the soil surface, but it gradually moved deeper into the soil and was eventually detected as deep as 70 cm. The label was also taken up by roots, as evidenced by the label presence in xylem water of six surrounding trees, including all trees within 4 m of the labeled plot center. The labeling occurred during a drought of rare intensity in the boreal north. We were able to observe labeling patterns both during the drought and after the rainfall events that relieved it.

Horizontal transport and xylem water isotope dynamics

The experiment was designed primarily to detect and quantify patterns of root (and mycorrhizal) overlap and dynamics of horizontal water uptake by trees. We found the label in stems of six surrounding trees, meaning that the root systems of individual trees do not express complete territoriality at this scale. We therefore conclude that all these trees had roots in the labeled 1-m^2 plot. This is far more overlap than in the crowns, which scarcely overlapped at all.

There are relatively few studies describing horizontal water uptake patterns in forest trees (Göttlicher et al., 2008; Henriksson et al., 2021; Lutter et al., 2021). Root distributions have been mapped in some temperate forests (Gao et al., 2021; Kuiper and Coutts, 1992; Lwila et al., 2024), but the presence of roots is not sufficient to predict water uptake (Göttlicher et al., 2008). Long-distance horizontal transport through overlapping root systems might increase uniformity in water availability among trees. It might also favour trees near openings by providing access to the unused soil water in the openings (Lutter et al., 2021; Moreira et al., 2000; Sternberg et al., 2002; Sternberg et al., 2005). This favouring might, as with deep vertical roots, provide a “lifeline” to trees growing under extreme drought conditions (Bachofen et al., 2024). However, it might also represent a significant carbon cost (Guswa, 2008), perhaps reducing partitioning to aboveground production (Fernandez-Tschieder et al., 2024; Marshall et al., 2023).

The rate of uptake varied among tree individuals. For example, the stem of Tree 2 was at the edge of the labeled plot (0.7 m from the center of the plot) and yet it showed far less label than Tree 1, which was slightly farther away (Figs. 5a and 5b). The label intensity detected in tree xylem became rather weak with greater distance from the plot, which we addressed by calculating the lc-excess. This parameter corrected for event-based variation using the strong correlation between natural abundance of δ^2H and $\delta^{18}O$, allowing us to detect low levels of labeling. When we focused simply on the first

appearance of the label, as defined by lc-excess greater than zero, we found that there was a correlation with distance from the plot (Fig. 6), suggesting that the label took some time to traverse the horizontal distance to the stems. The slope of this relationship, 0.17 (0.05) m day^{-1}, is an estimate of the maximum horizontal transport rate. For comparison, measurements of water flow through pine stems are typically around 1 m day^{-1} (Plamboeck et al., 1999; Tarvainen et al., 2018). Note also that the label appeared in Tree 1 in only four days. This suggests that the surface roots were alive and ready to begin water uptake, despite the severe drought (Marshall, 1986). The peak label intensity in Tree 1 appeared eight days after the labeling, perhaps due to the arrival of label via slower flow paths after its initial breakthrough (Gao et al., 2021; Seeger and Weiler, 2021; Werner et al., 2021). Alternatively, as the drought advanced, the tree could have increased the amount of water taken up from deeper soil layers (e.g., Gessler et al. 2022); however, our VWC data suggest that deep water uptake did not increase as a proportion of the total (Fig. 3c) late in the drought.

We also observed a brief, weak pulse in $\delta^{18}O$ of the surface soil horizons after the labeling. This might have occurred due to $\delta^{18}O$-enrichment of the $^{2}H_2O$ used for labeling (not measured), but we believe it is more likely interference in the spectrometer by the strong $^{2}H^{1}H^{16}O$ absorption peak with the adjacent $^{1}H^{1}H^{18}O$ peak, which has been observed in other studies using high concentrations of $\delta^{2}H$ (Beyer et al., 2016, 2018). In any case, the apparent $\delta^{18}O$ pulse was orders of magnitude weaker than the $\delta^{2}H$ pulse and can be considered negligible.

Territoriality

The water uptake benefits of long horizontal roots could be assessed not only by the detection of label in surrounding trees, but also by the label intensity. One can imagine a continuum between complete domination of uptake by a single tree, analogous to owned and defended property, vs. complete sharing of the benefit by all trees within some radial distance from the tree stem. The distinction is important, in part, because conservation of soil water for later use would be favored by territorial rooting and disfavored by extensive root overlap (Cohen, 1970). Although six trees were labeled in our plot, we detected a strong preference for uptake by one of the two trees nearest the plot center, while the other tree at a similar distance took up very little of the label. This preference remained remarkably constant even as the label pulse dissipated (Fig. S2).

In order to investigate this territoriality further, we have used the data from this earlier experiment to rank the trees from each of their plots according to label intensity (Henriksson et al., 2021) (Fig. 7). The ranking shows a strong preference for the labeling of one or two trees, with much lighter labeling in several more (Fig. 7b). Henriksson et al., (2021) found that the most heavily labeled trees were not necessarily those nearest the labeled plot. We interpret these and our results as evidence of territorial water uptake, but not to the point of exclusion.

Our results may have been influenced by choosing a microsite with particularly high tree density (1600 trees per hectare relative to 1010 (SD= 125) trees per hectare in the stand (Lim et al., 2015)). This was done to facilitate access to the central isotopic analyzer. However, in an earlier study in the same stand with 1-m^2 water-labelled plots, Henriksson et al. also reported 8 (SE=2) labelled trees per plot. We therefore conclude that our results were not atypical.

The territoriality interpretation draws attention to the orientation of the trunk boreholes in our study. Ours were oriented north-south while the boreholes in Henriksson et al. (2021) were oriented toward the plot center. We initially believed that borehole orientation would not matter because an earlier report had shown that label was evenly distributed throughout the tree circumference rather quickly in white pine (White et al., 1985). More recent evidence from our site (Tarvainen et al., 2018) suggests that the stem label distribution is not uniform near the ground. Our borehole orientation (N-S) may have contributed to the weak labeling observed for Tree 2 (on the western edge of the labeled plot) compared to Tree 1 (to the northeast) despite the proximity of Tree 2 to the labelled plot. Therefore, we recommend orienting boreholes toward the center of small labelled plots in future work.

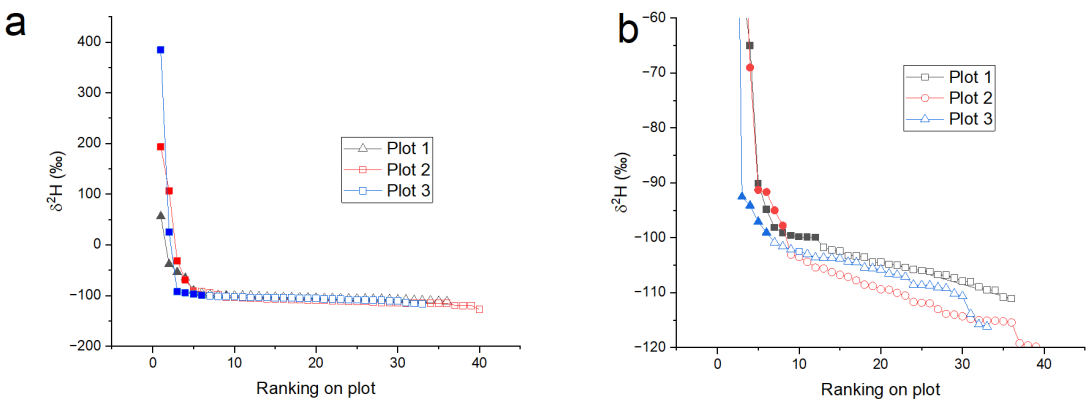

Fig. 7. Water isotopic composition (δ^2H) of xylem wood in trees surrounding three labelled 1-m^2 plots (data reprinted with permission from Henriksson et al., 2021). The trees surrounding each plot are ranked according to labeling intensity. Filled symbols represent trees that were identified as "labeled." Panel a) shows the full data range and panel b) highlights the transition from lightly labelled to unlabelled trees.

Soil water content and vertical root water uptake

We interpret daily declines in soil VWC during the drought period as evidence of tree root water uptake throughout the upper 50 cm of mineral soil (Fig. 3a). The slopes of the declines at 10 and 50cm are similar, but that at 30 cm is steeper. There was no evidence of change in water content at 70cm, consistent with our informal observation that roots were rare below 50 cm depth. The integrated water loss rate averaged 0.057 cm day^{-1} in the early part of the drought and increased to 0.083 cm day^{-1} in the last part, meaning that water uptake from these depths increased slightly as the drought proceeded. However, this conclusion is based on only three measurements across depth, the uppermost at 10 cm. Note that if there were evaporation in addition to root water uptake from the 10 cm depth, it would be included in water uptake depth estimate. Although there was almost certainly some evaporation from the injection sites at the surface of the mineral soil (0 cm depth), we speculate that evaporation was small at the measured 10 cm depth.

The maximum amount of label found in the soil was 4-5%, which is small in comparison to the amount added (see "Partial Label Recovery" in Suppl. Materials). However, we did not measure evaporation from the soil surface during the labelling and thereafter, which probably consumed a majority of the remainder. We tried to minimize surface evaporation by injecting at the mineral soil surface, but perhaps the injected water was drawn back up into the litter and moss and humus layers and above the topmost soil probes by matric forces during the injection.

After the injection, the labelled water was expected to flow downward under unsaturated conditions for as long as the water potential exceeded field capacity (Bonan, 2015). Remembering that the drought had already begun when the label was applied, the downward flow was expected to cease when field capacity was reached. One might expect a bit of short-range mixing by diffusion to continue after this point. This is consistent with the timing and intensity of the label peaks in Figs. 4a and 4b, where the label intensity decreased sharply between 15 cm (2027‰ maximum) and 20 cm depth (400‰ maximum, 8 days later). The added label could also have escaped detection by flowing down preferential flow paths (Clothier et al., 2008; Weiler, 2017), e.g. due to differences in soil density or along macropores or tree roots, leading to uneven infiltration and potentially bypassing probes and sensors. This might explain, for example, the higher maximum labelling at 70cm than at 50cm and at 30cm than at 20cm. However, the labelling patterns are largely consistent with the field-capacity scenario.

The current study follows on the classic study of Plamboeck et al. (1999), which was conducted at a nearby site with similar soils. Plamboeck et al. (1999) estimated that, without irrigation, the 2-cm humus layer provided 10% of total uptake in July. When we estimated the mean depth of water uptake (Fig. 3c), it was remarkably stable throughout this severe drought, with a slight upward shift toward the end. Our estimated mean uptake depth (30.6 (SE=1.2) cm) was similar to a depth-weighted estimate based on Plamboeck et al.'s (1999) isotopic data (30.3 (0.07) cm). Both our results and those

of Plamboeck et al. (1999) suggest a rather constant water uptake depth as a drought develops (Fig. 3c). This differs from a global meta-analysis, which found a downward shift in response to drought (Bachofen et al., 2024). If our trees cannot shift deeper under drought, then it may make the broad horizontal root distribution even more important as an emergency source of soil water.

Simplified borehole method

The method used to measure xylem water here included several simplifications of the borehole method used in previous studies. In particular, we did not use dry air (e.g., Kühnhammer et al., 2022) relying instead on the two-way exchange and equilibration of water vapor during the borehole passage (Marshall et al., 2020). In addition, we relied on the pump within the analyzer to draw air through the boreholes under negative pressure, apparently without significant air leakage into the downstream flows. This eliminates the need for the upstream pump used in some other applications (e.g., Kühnhammer et al., 2022). Finally, we did not use any screening material to inhibit microbial growth (Landgraf et al., 2022), relying instead on the natural defensive compartmentation of the xylem to serve that purpose (Shigo, 1984). Although a visible band of brown tissue formed around the borehole, our data showed that the borehole vapor continued to respond to xylem-water changes until the end of the experiment, more than three months after the boreholes were drilled. This is somewhat surprising given that the drilling must cavitate the xylem conduits that are cut, but our data, like previously published results with this method (Kühnhammer et al., 2021, 2022; Marshall et al., 2020), suggest that hydraulic contact with the xylem flow is maintained. As a reminder, we note that modeled results suggest that the equilibration is so fast that it would best reflect the isotopic composition of the water in the last few mm of the borehole (Marshall et al., 2020). Fortunately, this is where the flow is fastest and the circumference is greatest, both contributing to high proportions of the total stem flux.

One complication that remained was the placement of an analyzer in the field. Several recent papers describe methods for field sampling and storage of water vapor for stable isotope analysis (Havranek et al., 2020; Herbstritt et al., 2023; Magh et al., 2022), which would mean sacrificing the continuous data streams collected here, but would circumvent the need for an analyzer in the field and provide opportunities for more replication.

These results provide a dynamic description of soil water storage, transport, and root water uptake, including passage of the label through surrounding tree stems as they take up the label for transpiration. The timing and detection of the label are consistent with expectations insofar as it supports the notion that the root systems of several trees overlap on each square meter of ground surface. However, the concentration of the label in a single tree was inconsistent with the notion of

extensive horizontal sharing. This question deserves more attention as it influences the scaling of transpiration from trees to stands.

Data availability: The data are available on request from JM or MB.

Author contributions: JM, MD, and MB conceptualized the work. MB and JM managed data curation and analysis. Funding support was organized by MB and MD. Methodology was organized by MB, DD, PK, KK, MC, and JM. JM wrote the initial draft, but all authors contributed to writing and visualization.

Competing Interests: The authors declare that they have no conflict of interest.

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