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Contrasting stem water uptake and storage dynamics of water-saver and water-spender species during drought and recovery

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Running head:

Stem water uptake and storage in pine and oak

ABSTRACT

Drought is projected to occur more frequently and intensely in the coming decades, and the extent to which it will affect forest functioning will depend on species-specific responses to water stress. Aiming to understand the hydraulic traits and water dynamics behind water-saver and water-spender strategies in response to drought and recovery, we conducted a pot experiment with two species with contrasting physiological strategies, Scots pine (*Pinus sylvestris*) and portuguese oak (*Quercus faginea).* We applied two cycles of soil drying and recovery and irrigated with isotopically different water to track fast changes in soil and stem water pools, while continuously measuring physiological status and xylem water content from twigs. Our results provide evidence for a tight link between the leaf-level response and the water uptake and storage patterns in the stem. The water-saver strategy of pines prevented stem dehydration by rapidly closing stomata that limited their water uptake during the early stages of drought and recovery. Conversely, oaks showed a less conservative strategy, maintaining transpiration and physiological activity under dry soil conditions, and consequently becoming more dehydrated at the stem level. We interpreted this dehydration as the release of water from elastic storage tissues as no major loss of hydraulic conductance occurred for this species. After soil rewetting, pines recovered pre-drought leaf water potential rapidly, but it took longer to replace the water from conductive tissues (slower labelling speed). In contrast, water-spender oaks were able to quickly replace xylem water during recovery (fast labelling speed), but it took longer to refill stem storage tissues, and hence to recover pre-drought leaf water potential. These different patterns in sap flow rates, speed, and duration of the labelling, reflected a combination of water use and storage traits, linked to the leaf-level strategies in response to drought and recovery.

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INTRODUCTION

Drought is projected to occur more frequently and intensely in the coming decades due to climate change (Bréda et al. 2006, Choat et al. 2012, IPCC 2021), and the extent to which drought will affect forest functioning mostly depends on species-specific responses to water stress and their associated physiological traits (Moreno-Gutiérrez et al. 2012, Valladares et al. 2015). Traditionally, plant hydraulic strategies have been classified along the isohydric-anisohydric spectrum, based on their stomatal conductance regulation level (Tardieu and Simonneau 1998, Klein 2014, Martínez-Vilalta et al. 2017). Water-saver species, generally associated to leaf-level isohydric strategy, avoid water losses during drought by closing their stomata as soon as a slight increase in soil tension is perceived (Tardieu and Simonneau 1998, McDowell et al. 2008, Klein 2014). Conversely, water-spender species that are generally linked to leaf-level anisohydric strategy, can maintain carbon fixation keeping high stomatal conductance and transpiration during drought (Tardieu and Simonneau 1998, McDowell et al. 2008, Klein 2014). However, these water-use strategies are not always consistent for a given species, since they are modulated by ambient conditions and can substantially fluctuate in response to soil or atmospheric dryness (Barlett et al. 2014, Hochberg et al. 2017, Novick et al. 2019). In this regard, quantifying response-based metrics across a range of water availability or during a dry-down period can benefit the interpretation of plant trait interactions (e.g., Klein 2014, Martínez-Vilalta et al. 2014, Meinzer et al. 2016). On the other hand, water use strategies can be linked to species-specific differences in water uptake patterns (West et al. 2012). For example, plants with a water-spender strategy generally develop a deeper rooting system to have access to more reliable water moisture. On the contrary, plants with a water-saver strategy generally develop a more superficial rooting pattern and then, usually rely on short and dynamic precipitation events for their growth and survival (see e.g., Filella and Peñuelas 2003, West et al. 2012, Moreno-Gutiérrez et al. 2012b, Klein et al. 2013, del Castillo et al. 2016).

In this regard, the analysis of the isotopic composition of xylem water, and of possible water sources for the plant (soil at different depths, groundwater, stream, fog, or dew water) has been extensively applied to assess the spatial and temporal dynamics of plant water uptake (Ehleringer and Dawson 1992). The basis of this approach is that the potential water sources available to plants show contrasting isotopic signatures, which can be traced back from the values in xylem water (e.g., Filella and Peñuelas 2004, Máguas et al. 2011, del Castillo et al. 2016; Martín-Gómez et al. 2017). In addition to the study of the use of different water sources in natural conditions, isotope labelling experiments with tracers such as deuterated water (D_2O) offer additional information about the short-term dynamics of water uptake and internal transport in plants (Piayda et al. 2017; Brinkmann et al. 2018; Kahmen et al. 2021). Once we add the tracer into the soil or directly into the trunk, we can calculate the tracer speed and residence time by destructively sampling different plant organs, or measuring transpired water vapor (e.g., Calder et al. 1992; Meinzer et al. 2006; Schwendenman et al. 2010; Ferrio et al. 2018; Rodriguez-Robles et al. 2020). These parameters are indicators of how water moves inside the trees and can be transformed into variables such as sap flow velocity and stem hydraulic capacitance. There is a direct dependence of stem internal water transport and storage properties to wood anatomy and wood density (James et al. 2003, Meinzer et al. 2003). Generally, angiosperms exhibit higher tracer velocity and shorter residence times than conifers (i.e., total sap flux and water turnover rate per sapwood volume are greater), related to highly efficient vessel-formed xylem and higher wood density (Meinzer et al. 2006). The magnitude of hydraulic capacitance – understood here as tracer residence time – is also strongly related to wood anatomy (Köcher et al. 2013, Oliva Carrasco et al. 2015), as parenchyma, fibres, vessels, and tracheids represent important sites for plant water storage (Holbrook 1995). In this regard, there is recent evidence of variations in the isotopic composition of the different water pools inside the stem (xylem water *vs*. water from storage tissues and adsorbed water to fibres), which might

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be associated to contrasting species-specific wood anatomy characteristics and hydraulic properties (Barbeta et al. 2022). Therefore, the combination of isotope tracing techniques together with traditional physiological measurements such as gas exchange, water potential, sap flow or xylem water content during a wide range of soil or atmospheric drought, can reveal more about transpiration and internal water transport in trees (James et al. 2003, Meinzer et al. 2003, Marc and Robinson 2004, Meinzer et al. 2006, Rodríguez-Robles et al. 2020). Additionally, this technique can be largely appropriate to understand the connection between leaf and stem physiological traits involved in drought and recovery, and improve their integration in whole-tree water transport models. Hereof, while the link between water-use strategies and the dynamics of leaf gas exchange has been intensively studied (see e.g., Klein 2014, Martínez-Vilalta & Garcia-Forner 2017, Meinzer et al. 2017), the relationship between leaf hydraulic strategies and stem internal water fluxes alongside their ability to rely on stored water in stems – also addressed as hydraulic capacitance – is relatively unknown (Matheny et al. 2015, Yi et al. 2017).

Furthermore, it is common for coexisting species, such as the ones studied here that coexist in the medium-high mountains in the Mediterranean basin, to employ contrasting whole-plant hydraulic strategies which result in distinct, species-specific patterns of transpiration and growth (e.g. Poyatos et al. 2008, Martinez-Vilalta et al., 2014, Anderegg, 2015, Martín-Gómez et al. 2017). Within this context, the aim of the present study was to develop a more comprehensive understanding of the links between whole-tree water transport properties and associated physiological traits. Particularly, we were interested in analysing the relationship between leaf hydraulic properties and the ability to rely on stored water in different plant species with contrasting xylem anatomy and water-use strategies. For this, a drought-recovery pot experiment was conducted using two species: Scots pine (*Pinus sylvestris*) and portuguese oak (*Quercus faginea*). Scots pine is an evergreen conifer, that has a xylem formed only by tracheids (with a narrow lumen and low theoretical hydraulic conductivity) and typically maintains a tight stomatal control (i.e., isohydric leaf response) and a water-saver strategy (e.g., Irvine et al. 1998, Poyatos et al. 2008, Martínez-Vilalta et al. 2009). Conversely, *Quercus faginea* is a marcescent oak with ring-porous xylem, characterised by large earlywood vessels that can provide high hydraulic conductivity, high transpiration rates and a typically water spender strategy, with less stomatal control (i.e., anisohydric leaf response) (Corcuera et al. 2004, Peguero-Pina et al. 2016, Alonso-Forn et al. 2021). Bearing this in mind we hypothesised that (H1) pines would be more sensitive to drought and they would show an earlier response to a decrease in soil water content than oaks; (H2) under moderate drought conditions, oaks would recover faster than pines due to their larger vessel diameter and higher transport capacity; and (H3) the speed of xylem labelling would be faster in oaks than in pines due to their higher transport capacity.

MATERIALS AND METHODS

Description of the experiment

Commercial saplings of Scots pine (*Pinus sylvestris* L.) and portuguese oak (*Quercus faginea* L.) located at the Experimental Fields of the Universitat de Lleida (Lleida, 31T 0.596293 41629816, 170m high) were used in a pot experiment in summer 2015 (8th-28th July). During this period, average minimum and maximum temperature were 19.6 ºC and 39.4 ºC, respectively, with relative humidity ranging between 29.4% and 81.5 %. There were two precipitation events occurring overnight, on the 18th-19th July (68 mm) and 20th-21st (27.6 mm), during which the trees were covered with plastic bags to prevent foliar rehydration (See Fig. S1 for the time evolution of main meteorological variables during the experiment). Eight saplings per species (4 and 6 years old, for oaks and pines, respectively) were cultivated in pots filled with a forest clay loam soil (40 cm depth, 20 L of substrate). Trees were

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approximately 0.7 to 1 meter high and 1 to 2 cm of stem diameter at 25 cm high. Despite the similar height, aboveground biomass of pines was about 3-fold larger than in oaks (235.1 ±32.1g and 75.2 ±6.8g, respectively), whereas belowground biomass was slightly larger in oaks (49.1 ±8.1g in pines; 59.2 ±8.3g in oaks; for further details on biomass distribution see Table S1). To minimise isotopic fractionation caused by soil evaporative enrichment, the surface of the soil pot was directly covered with aluminium foil and alluvial rocks on top. A cone-shaped roof was fixed around the tree stem to prevent water inputs from eventual precipitation events (see Fig. S2 for an overview of the experimental setup). Plants were divided into two groups, in which two different water labelling treatments were applied during two consecutive soil irrigation-drought cycles (Fig. S2D, E). Before the experiment, plants were maintained at well-watered conditions being irrigated every 1-2 days with tap water; however, in order to facilitate the labelling of soil water, just before the first labelling event we kept the plants without irrigation for 3 days. The day the experiment started, plants were generously irrigated (until approximately field capacity, letting the pots largely drain), then left to transpire without watering for 8 days until reaching soil wilting point (around -1.5 MPa, 13% of soil water content); then watered again with a different isotopic composition as in the previous irrigation and left to dry for another 8 days. The first group (Fig. S2D) was initially irrigated with tap water (-9.79 ±0.24‰ for δ^{18}O and -68.25 ±3.42‰ for δ^{2}H VSMOW) and afterwards with depleted water (-23.25‰ for δ^{18}O and -166.102‰ for δ^{2}H) (later called "Depleted labelling"). The second group (Fig S2E) was first irrigated with deuterated water (-9.16 ±0.04‰ for δ^{18}O and +343.02 ±0.96 for δ^{2}H), followed by tap water (later called "Deuterated labelling"). To assess the effectiveness of our set up to prevent soil evaporation, we also completed a blank test for one tree per species. We irrigated the pots until field capacity, defoliated the trees to avoid plant water consumption and left them without watering throughout the experiment, while we were weighing the pots. The results showed negligible water losses after 12 consecutive days (less than 0.8% weight loss); indicating that our experimental set-up was largely preventing soil evaporation.

Sampling and measurements

Distal twigs from the tree branches were sampled at the time of maximum vegetative activity (around 10-12h solar time) on the day before watering, the day after and on the 2rd, 4th, and 6th day of drought. Soil cores (15 mm diameter × *ca.* 150-200 mm height) were sampled on the same days as twigs and were divided into 3 samples: 0-5cm, 5-10 and 10-15cm depth. Twig xylem and soil sampling were complemented with measurements on the same sampled twigs, for xylem water potential (ψ_{xylem}) with a pressure chamber (Scholander and Hammel 1965) and gas exchange with an infra-red gas analyser (IRGA, Walz GFS-3000, Heinz Walz GmbH, Effeltrich, Germany). The IRGA was equipped with a LED-Array/PAM-Fluorometer 3055-FL and a cuvette for conifers 3010-V80. Chamber conditions were set to mimic ambient conditions (CO_2 concentration: 400 ppm; photosynthetic photon flux density: 1200 µmol m^{-2} s^{-1}; cuvette temperature and relative humidity: 25-33ºC, 40-70%). All parameters were recalculated to actual leaf area following Von Caemmerer and Farquhar (1981). In oaks, leaf area was calculated as the projected area in the cuvette (usually one leaf covered all the surface) and for pine the projected area was determined by approximating needle surface as a thin rectangle (length × width) and multiplying by the total number of needles inside the chamber. Throughout the experiment, pot weight was measured with balances, and sap flow was monitored with "baby gauges" SF62, coupled to the Sap flow meter T4.2 (EMS Brno, Brno, Czech Republic). Sap flow sensors were installed between 20 to 40 cm height depending on each tree branch distribution. We inserted the probes (7mm length) into the external part of the stems in order to avoid non-conducting heartwood but sufficiently deep to have correct measurements (Peters et al. 2018), and we adjusted depth of the probes for every tree by checking sap flow measurements before the experiment. Sap flow data were downloaded and analysed with Mini32 software ver.403.34 (EMS Brno, Brno, Czech Republic). Sap flow was standardised by the leaf area above the sensor, considering progressive losses due to sampling (at the end of the experiment, 22.2 ±2.3% for pines, and 19.3 ±1.4% for oaks). To correct for vertical temperature gradients, we removed the sap flow estimates of the two control (leafless) trees (see Fig. S2 for an example of the steps in sap flow data standardisation). To quantify artefactual variations in sap flow caused by vertical temperature gradients within the stem (Do & Rocheteau, 2002), in the same leafless, non-transpiring trees, we installed sap flow sensors which were subsequently used to correct measured values in transpiring trees. Leaf-specific hydraulic conductance (K_h) of the whole plant was calculated from sap flow rate per unit area between 12 and 15h solar time, and the difference between xylem water potential and maximum soil water potential, using average values for each species and day. Standard error was calculated following the rules of error propagation, assuming independent measures. We considered sap flow rates during a period immediately after the determination of xylem water potential to (1) account for time-lags between leaf transpiration and sap flow, (2) minimise the interference due to gas exchange and water potential measurements (e.g., shadowing, water loss after cutting) and (3) to attain a more integrated value for potential water flow including 3 hours of measurements.

Sample collection and water extraction for isotopic analyses

For xylem sampling, bark and phloem from 1-2 twigs were removed and the peeled xylem was immediately placed in air-tight glass tubes (Duran GL-18). The tubes were frozen on liquid nitrogen directly after sampling and kept frozen until processing. Xylem and soil water were extracted by cryogenic vacuum distillation (Ehleringer & Dawson 1992) at the Dept. of Crop and Forest Sciences of the Universitat de Lleida. Sample tubes were placed in a heated silicone oil bath (120°C), and connected with Ultra-Torr^{TM} unions (Swagelok Company, Solon, Ohio, USA) to a vacuum system (*ca.* 10^{-2} mbar), in series with U-shaped collector tubes, cooled with liquid N_2. After an extraction time of 2 h (soil) and 1.5 h (xylem), trapped water was transferred into 2 ml vials, and stored at 4°C until analysis. Preliminary recovery tests showed that these were the most suitable conditions to ensure complete distillation (Palacio et al. 2014, Martín-Gómez et al. 2015). In order to calculate twig-xylem and soil water content (XWC, SWC respectively, in %), all twigs and soil samples were weighed before and after distillation and the following formula was applied:

$$XWC, SWC\ (\%) = (\text{Sample weight before (g)} - \text{sample weight after (g)}) \,/\, \text{Sample weight before (g)}$$

Isotopic analyses

We analysed the isotope composition of water samples by Cavity Ring-Down Spectroscopy (CRDS) in a Picarro L2120-*i* isotopic water analyser (Picarro Inc., Sunnyvale, CA, USA) at the Serveis Científico-Tècnics of the Universitat de Lleida (Lleida, Spain) and a Picarro L2130-*i* in the Stable Isotopes Facility of the Institute for Landscape Biogeochemistry at ZALF (Müncheberg, Germany). Generally, 6 replicates of 1 µl were injected into the vaporizer, keeping the last three injections for calculation. When analysing deuterium-enriched samples, to minimise memory effect, 9 replicates were injected and only the last three injections were used for calculation. With this method, we observed negligible memory effects, and rather homogeneous values. Average within-sample standard deviation was <0.2‰ for $\delta^{18}O$ and <0.7‰ for $\delta^{2}H$). After calibration with three internal standards (the same in both laboratories), isotope composition was expressed in per mil notation ($\delta^{18}O$ and $\delta^{2}H$, for oxygen and hydrogen, respectively), relative to VSMOW (Vienna Standard Mean Ocean Water). As described in Martín-Gómez et al. (2015), residual organic compounds in the distilled water can interfere with the analysis of plant and soil samples using CRDS, but it is possible to overcome this with a post-processing correction. However, we found generally a minimal level of contamination in most of our samples (out of 170 non-labelled samples, only 8 soil samples were flagged, and none of them showed unusual delta values). Because of this reason, and together with the fact that we were using labelled water, we decided to use raw values in all cases.

Estimation of soil water potential: water retention curves and Rosetta Model

In order to estimate soil water potential (ψ_s in MPa) from soil water content (SWC, in %) we used the software Rosette V1.2 (Marcel G. Schaap, 2002, University of Arizona) to calculate unsaturated hydraulic properties (e.g., water retention parameters or hydraulic conductivity) from surrogate soil data such as soil texture and bulk density. From previous soil analysis, we characterised texture data (29.14% sand, 38.49% silt, 32.37% clay) and SWC at field capacity (0.33MPa, 21% SWC) and wilting point (1.5MPa, 13% SWC). We calculated dry bulk density using wet and dry distillation weight, pot weight at field capacity and pot volume, obtaining an average value of 0.64±0.06 g/cm^3. Using these data as model inputs and resolving the Van Genuchten (1980) equations with the calculated parameters (saturated and residual water content, and curve shape parameters), we obtained ψ_s values for a defined SWC.

Statistical analyses

All statistical analyses were performed in R v4.2.1 (R core team 2022). We tested significant differences in time and between species for all physiological variables using generalised linear mixed models, based on Restricted Maximum Likelihood - REML ($\alpha = 0.05$), preferentially using the function *lmer* from package lme4 v1.1-31 (Bates et al. 2015). Species x phase and Species x day were considered as fixed effects, including repetition (i.e. individual) as a random effect. For hourly sap flow data, we included Species x day as fixed effects, and repetition and hour as random effects. Over- and under-dispersion of model residuals was checked, including outlier tests, using the *simulateResiduals* function from package *DHARMa* v.0.3.1 (Hartig 2022). Only minor issues were found, which were solved with a log-transformation. Differences among sampling times for each species were tested with the Tukey test ($\alpha = 0.05$) using the package *emmeans* v1.8.4-1 (Lenth 2022). Alternatively, we tested the inclusion of first-order temporal autocorrelations in the model using the function *lme* from package *nlme* v3.1-152 (Pinheiro et al. 2022), obtaining nearly identical results (see Supplementary material, Appendix A1 support statistics). Unless otherwise stated, results presented refer to the *lmer* model without autocorrelation. Inter-specific differences in the association between variables were tested with general linear models with the built-in function *lm* from package *stats* v.3.6.2 (R core team 2022), using log-transformed variables when required. Graphs were created using Sigma Plot version 12.5 (Systat Software, Inc., San Jose California USA). Means are shown together with their associated standard error of the mean.

RESULTS

Evolution of sap flow rates under drought limitation

The two irrigation events increased hourly and total daily sap flow rates within one day after the water was added (Figs. 1A, 1B; support statistics for all figures can be found in supplementary material, Appendix A1). Despite the large uncertainty associated with sap flow measurements, we found a significant increase in mean hourly sap flow rates from 12th to 14th July for pine (p<0.001) and from 19th to 21st (p<0.001). In oaks, we found a significant increase from 11th to 13th July (p=0.008), and from 19th to 21st (p<0.001). After the irrigation peak, pines showed a faster decline (p<0.001 from 14th to 16th July and from 22nd to 24th). than oaks (p<0.001 from 14th to 17th July and from 22nd to 27th). Nevertheless, after the second irrigation, sap flow did not fully recover to the initial values (p<0.001). Overall, the decrease in sap flow rates induced by drought was larger and faster in pines than in oaks (respectively, around 81% and 52% of maximum decrease in daily water use). Daytime mean hourly sap flow was significantly higher for oaks than for pines during the first drought (p<0.001 on the 16th), but only marginally higher during the second drought (p=0.056 on the 25th), reaching even lower values at the end of the experiment (p=0.005 on the 26th). Daily water use, calculated from weight loss, showed similar trends (Figs. 1A, 1B). For pines, peak values were found on the 14th and the 22nd July, and minima on the 18th and 26th (p<0.001). In oaks, we found a significant decline from 14th to 18th July (p<0.001), but not between 22nd and 26th. However, as for sap flow, oaks did not recover to the initial values (p=0.018 from 14th to 22nd July), but this was not the case for pines (p=0.998 from 14th to 22nd July). Similar results were found considering mean values of daily water use calculated from sap flow and weight loss for each of the experimental phases (watering 1, drought 1, watering 2 and drought 2), showing more significant effects of drought in pines than in oaks (Table 1). Daily evolution of sap flow patterns during well-watered conditions (Fig. S4) showed that pines reached maximum transpiration in the morning (9-10h solar time), reduced their transpiration during midday (12-15h) and recovered again during the late afternoon (16-18h); whereas oaks tracked the evaporative demand more closely, showing maximum transpiration rates in the afternoon (14-16h) when maximum vapour pressure deficit (VPD) occurred.

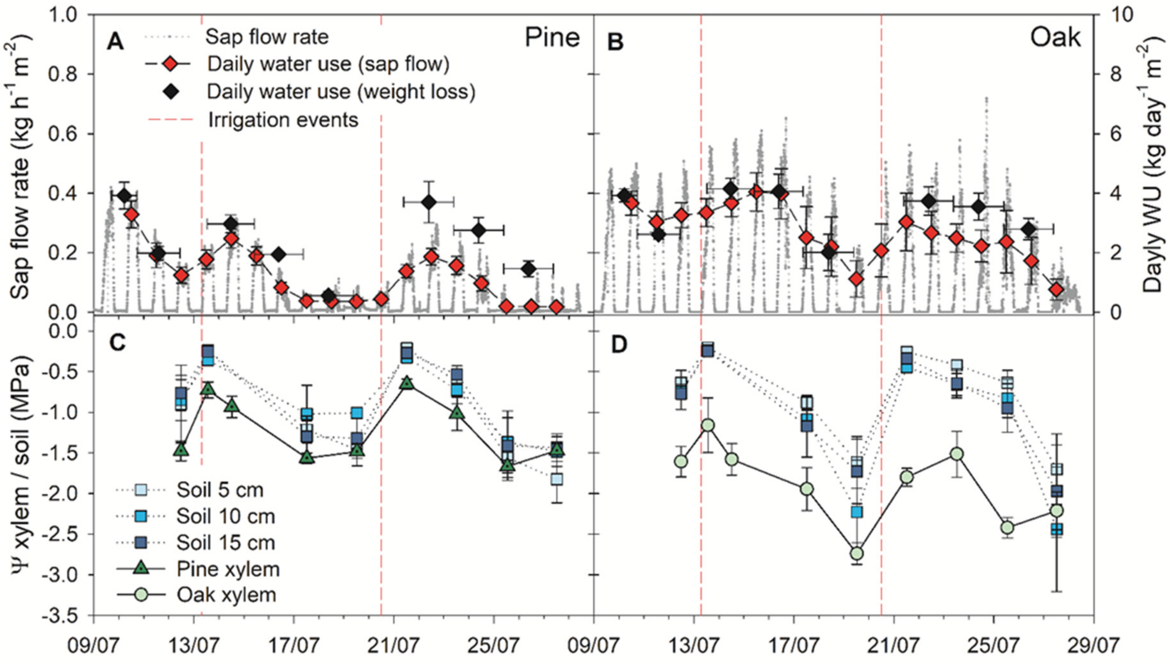

Figure 1. Time evolution of sap flow, daily water use (A, B) and soil and xylem water potential (C, D) for pine (left) and oak (right). Red dashed lines indicate the days we irrigated (13/07/15 and 21/07/15). Sap flow rate (A, pine; B, oak) was corrected for thermal gradients by removing the apparent sap flow measured in two defoliated trees (used as blank) and standardised by the leaf area above the sap flow sensor (see Figure S3 for details). Daily weight loss (A, pine; B, oak) was standardised by total leaf area, calculated as the area determined at the final harvest and accounting for the area that was removed progressively during sampling. Error bars represent standard errors. Horizontal error bars in daily water use (A, B) show the timespan between pot weights.

Table 1 Mean and standard error for the main soil and physiological variables during the four phases of the experiment, including between parentheses the number of replicates. Letters denote significant differences between groups, according to the Tukey posthoc test ($p<0.05$). For each variable, p-values of the fixed terms of the linear mixed model are shown (Species, Period and their interaction). "*lmer(log)*" and "*lmer*" indicate whether the variable was log-transformed or not. Maximum and minimum Soil Ψ, water potential of the wettest and driest soil layer, respectively; Xylem Ψ, late-morning (10-12h solar time) xylem water potential; g_s, A_n, E, late-morning stomatal conductance, net assimilation, and leaf-level transpiration rates under ambient conditions (recalculated from measured g_s and ambient vapour pressure deficit); Daily WU-sf and Daily WU-w, integrated daily water use per leaf area, calculated from sap flow and weight loss, respectively.

Sp/ Phase	**Phase**	**Maximum Soil Ψ (MPa)**	**Minimum Soil Ψ (MPa)**	**Xylem Ψ (MPa)**	**g_s (mmol m^{-2} s^{-1})**	**A_n (µmol m^{-2} s^{-1})**	***E* (mmol m^{-2} s^{-1})**	***Daily WU-sf* (kg day^{-1} m^{-2})**	***Daily WU-w* (kg day^{-1} m^{-2})**
Pine									
W 1	12/7-14/7	-0.4 ±0.10^{b}(12)	-0.6 ±0.16^{b}(12)	-1.1 ±0.11^{b}(13)	211 ±107^{bc}(4)	13.7 ±1.84^{c}(4)	3.2 ±1.61^{bc}(4)	1.8 ±0.6^{bc}(5)	3.3 ±0.1^{cde}(5)
D 1	17/7-19/7	-0.9 ±0.11^{a}(8)	-1.5 ±0.15^{a}(8)	-1.5 ±0.04^{a}(8)	45 ±13^{ab}(8)	3.1 ±1.02^{b}(8)	0.9 ±0.25^{ab}(8)	0.4 ±0.2^{a}(5)	0.5 ±0.1^{a}(5)
W 2	21/7-23/7	-0.3 ±0.05^{b}(12)	-0.5 ±0.09^{b}(12)	-0.8 ±0.12^{b}(8)	254 ±51^{c}(8)	9.1 ±1.13^{bc}(8)	4.3 ±1.03^{c}(8)	1.6 ±0.4^{bc}(5)	3.7 ±0.3^{cde}(5)
D 2	25/7-27/7	-1.3 ±0.15^{a}(12)	-1.8 ±0.20^{a}(12)	-1.5 ±0.07^{a}(11)	26 ±11^{a}(4)	-0.3 ±0.17^{b}(4)	0.7 ±0.31^{a}(4)	0.2 ±0.2^{a}(5)	1.4 ±0.1^{ab}(5)
Oak									
W 1	12/7-14/7	-0.3 ±0.06^{b}(12)	-0.5 ±0.11^{b}(12)	-1.5 ±0.14^{b}(14)	355 ±88^{bc}(4)	12.9 ±1.53^{c}(4)	5.3 ±1.40^{bc}(4)	3.4 ±0.5^{c}(5)	4.2 ±0.2^{e}(5)
D 1	17/7-19/7	-1.1 ±0.21^{a}(8)	-1.8 ±0.36^{a}(8)	-2.3 ±0.20^{a}(8)	224 ±41^{ab}(7)	11.2 ±1.69^{c}(7)	3.9 ±0.79^{ab}(7)	1.9 ±0.8^{ab}(3)	1.7 ±0.2^{ac}(3)
W 2	21/7-23/7	-0.3 ±0.03^{b}(11)	-0.6 ±0.07^{b}(11)	-1.6 ±0.17^{b}(7)	360 ±78^{c}(7)	14.1 ±1.30^{c}(7)	4.9 ±0.66^{c}(7)	2.7 ±0.7^{bc}(3)	2.8 ±0.3^{bd}(3)
D 2	25/7-27/7	-1.2 ±0.29^{a}(11)	-2.0 ±0.50^{a}(11)	-2.3 ±0.14^{a}(9)	49 ±15^{a}(4)	3.2 ±1.85^{ab}(4)	1.3 ±0.41^{a}(4)	1.6 ±0.8^{abc}(3)	2.3 ±0.2^{abcd}(3)
Model		lmer(log)	lmer(log)	lmer	lmer	lmer	lmer	lmer(log)	lmer
Sp		0.974	0.945	**<0.001**	**0.034**	**<0.001**	**0.020**	**0.017**	0.335
Phase		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**
Sp:Phase		0.394	0.469	0.253	0.677	**0.035**	0.383	**0.005**	**<0.001**

Response of xylem and soil water potential to drought and recovery

In line with sap flow time evolution, soil and xylem water potential increased with irrigation and started to decline concurrently as long as the trees were consuming the water in the pots, with strongly significant ($p<0.001$) differences between watering and drought phases, and non-significance differences ($p=0.650-1.000$) between the two watering phases and the two drought phases (Table 1; Figs. 1C, 1D). We did not find significant differences between species in ψ_{soil}, both showing a significant decline from 13th to 17th July and a significant recovery ($p<0.001$) from 17th to 21st July. During the second drought cycle, however, oaks showed a slower soil drying than pines (pines showed a significant decline in maximum ψ_{soil} already from 21st to 23rd July -$p=0.004$-, whereas oaks showed differences only with the 27th July; $p<0.001$). Regarding ψ_{xylem}, pines showed significantly higher values than oaks during the first experimental drought (19th July; $p=0.002$) and the subsequent recovery (21st July; $p=0.009$). Differences during the second drought phase were only marginally significant (27th July; $p=0.057$). For pines, changes during the first drought were not significant ($p=0.159-0.326$), but the subsequent recovery was significant, and the second drought showed significant changes from 21st to 25th July ($p=0.036$). Conversely, oaks showed a strongly significant ($p<0.001$) decline from 13th to 19th July, and a delayed recovery (only significant from 19th to 23rd July, $p<0.001$). During the second drought, we only found a marginally significant decline from 23rd to 25th July ($p=0.054$).

When irrigated after the first drought period, the recovery of xylem water potential was faster in pines than in oaks, reaching their maximum ψ_{xylem} on the next day after watering ($p=0.045$ from 17th to 21st July). Conversely, despite the ψ_{soil} indicating that the soil was completely wet the next day of irrigation ($p<0.001$ from 17th to 21st July), the oaks did not reach maximum ψ_{xylem} until two days after the watering (only significant from 19th to 23rd July, $p<0.001$). Irrigation was used to achieve field capacity, around 21% of soil water content for this clay loam soil. However, the conditions under which the plants stopped their water uptake under drought were different for both species. Pines dried the soil to 13.7±0.60% of volume (with a midday xylem water potential around -1.5MPa). In contrast, oaks dried the soil to 12.3±0.70% of volume (with a midday ψ_{xylem} of ca. -2.7 MPa), and even under these conditions they maintained a significant leaf-level transpiration rate (3.9 ±0.79 mmol m^{-2} s^{-1}).

Variations in gas exchange and hydraulic conductance with drought and recovery

In agreement with sap flow patterns and daily water use, pines showed lower values than oaks in late-morning (10-12h solar time) stomatal conductance (g_s; p=0.034), net photosynthesis (A_n; p<0.001), and leaf-level transpiration rates (E; p=0.020), and the three variables showed significant differences among experimental phases (p=0.0004-0.0014) (Table 1). Only A_n showed a significant Species x Phase interaction (p=0.035). Pines showed significant declines in both drought phases (p=0.001-0.002), declining to 23% of the initial values during Drought 1, and remaining with values close to zero during Drought 2. Conversely, oaks did not show significant changes in A_n among Watering 1, 2 and Drought 1 (p=0.733-0.999), maintaining significantly higher values than pine during Drought 1 (p=0.001), but suffered a significant decline during Drought 2 (p<0.001). Pairwise comparisons showed only marginally significant differences in g_s and E between Drought 1 and Watering 2 for pines (p=0.061-0.062), and between Watering 2 and Drought 2 for g_s oaks (p=0.032). We did not find significant differences in E among experimental phases for oaks (p=0.173-0.990). Considering the ability to recover after drought, pines showed a full recovery in g_s (121% of the values during the first watering phase), but not in A_n, which reached only 66% of the initial values during the second watering, although still significantly higher than during Drought 1 (p=0.027) (Table 1). In oaks, a clear recovery was observed in both variables during the second watering cycle (101% and 110% of the initial values, in g_s and A_n, respectively).

Coupled to these results, we found a significant exponential relation between ψ_{soil} and gas exchange parameters, particularly strong in pines (A_n, p=0.005; g_s, p=0.029), showing a consistent decline in g_s and A_n with decreasing Ψ_{soil} during drought (Figs. 2A, 2B). Models showed only significant differences between species in the intercept (A_n, p<0.001; g_s, p=0.017), although this could be partly due to the poorer fit of these relationships in oaks (p=0.046-0.058), suggesting that gas exchange parameters were less sensitive to soil water potential. This could be linked to the fact that oaks were able to track with xylem Ψ the changes in soil Ψ, hence maintaining a relatively constant gradient in water potential (Fig. 2C). Although VPD was high throughout the experiment (3-5 kPa), we still found a significant negative effect (p<0.001) on A_n and g_s (Fig. S5). Again, differences between species were only significant for the intercept (A_n, p=0.001; g_s, p=0.006). However, contrary to the response to Ψ_{soil}, oaks showed a stronger response than pines (p<0.001 and p=0.058-0.078, respectively). This trend should be taken with caution, as it was largely driven by the last measurement round, coinciding with the lowest xylem Ψ in oaks. Furthermore, despite K_h exhibiting a tendency to decline with decreasing soil and xylem Ψ in both species (Fig. 3), correlations were strongly significant (p<0.001) for pines, both against soil and xylem water potential, whereas for oaks it was only significant (p=0.048) against soil Ψ (Fig. 3A). The ANOVA (see Appendix A1 in supplementary file) revealed a change in the response against soil Ψ from the first drought cycle to the second cycle, showing significant trends only for the second cycle (Fig. S6). However, these differences must be taken with caution, as they are based on regressions with only four points. Conversely, no significant effects of the drought cycle were found for the association between xylem Ψ and K_h (Appendix A1).

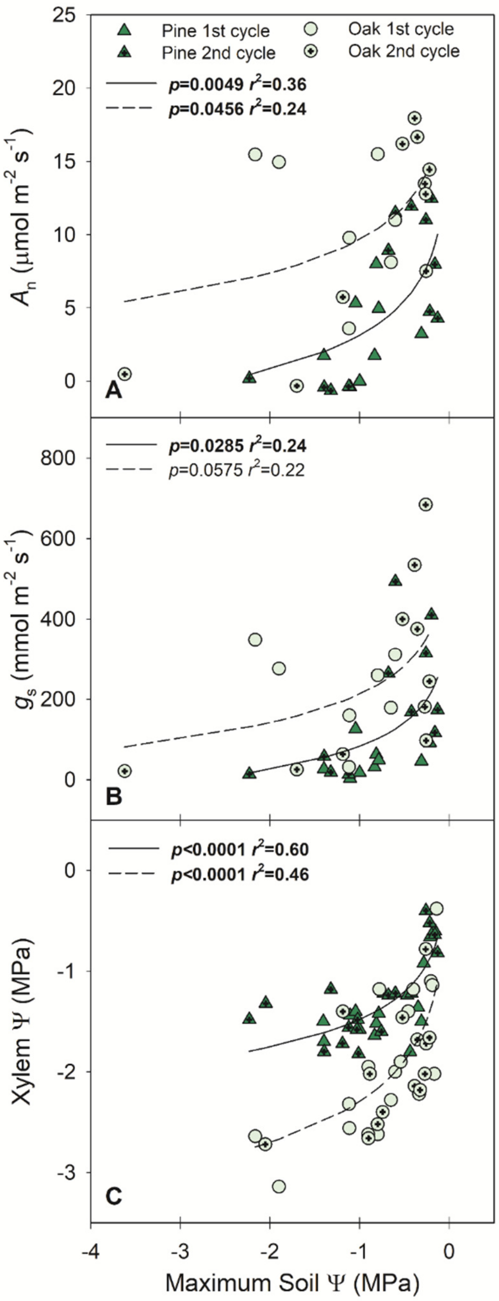

Figure 2. Relationship between maximum soil water potential (Ψ) and (A) net photosynthesis - A_n, (B) stomatal conductance - g_s, and (C) xylem water potential, across the two irrigation cycles. . Light green circles and dashed line, oaks; dark green triangles and solid line, pines. Filled and dotted symbols indicate the first and second cycles, respectively. The regression coefficient (r^2) and significance (p) are represented in each panel. Maximum soil Ψ denotes the highest water potential across soil depths, as a proxy for the most easily available water.

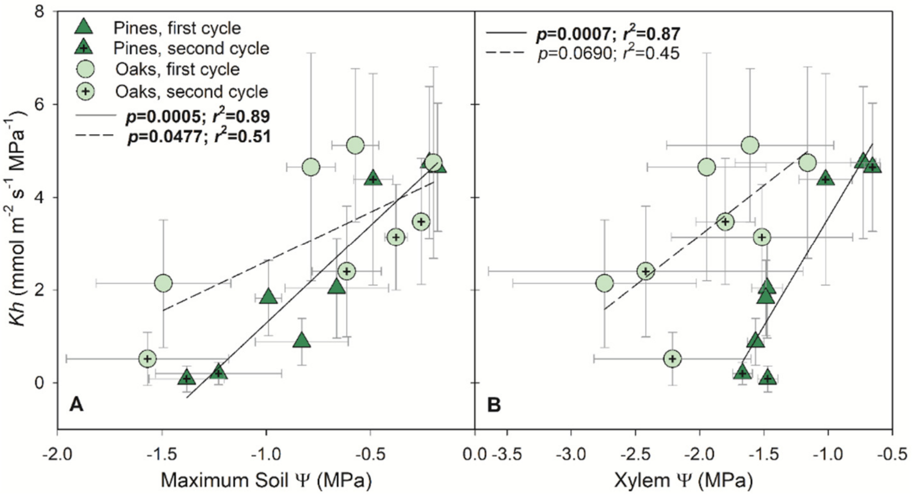

Figure 3. Relationship between leaf-specific hydraulic conductance (K_h) of the whole plant, maximum soil water potential (A) and xylem water potential (B), across the two irrigation cycles. Light green circles and dashed line, oaks; dark green triangles and solid line, pines. Filled and dotted symbols indicate the first and second cycles, respectively. Error bars denote standard errors. The regression coefficient (*r2*) and significance (*p*) are represented in each panel. Maximum soil Ψ denotes the highest water potential across soil depths, as a proxy for the most easily available water.

Isotopic changes: labelling and drought effects

The isotopic composition of soil water followed the isotopic trend of irrigation water, but with a significant offset between irrigation water and soil, depending on the previous soil water signature (Fig. 4). Drainage water collected the first day of watering was also generally close to irrigation water in terms of isotopic composition; thus, we considered that enough water was added to fill or replace all the easily accessible pore water within the soil. On the first irrigation event (13th July), for the pots irrigated with ^{2}H-enriched water (Figs. 4A, 4B), in both species it took about one day to reach the peak values of labelling in xylem water ($p<0.001$ from 13th to 14th July). As expected, the control pots, irrigated with tap water (Figs. 4C, 4D), maintained constant isotopic values (p=1.000). In contrast, in the second irrigation event (20th July, after the drought period), we observed a species-specific time lag between the irrigation event and the peak of xylem labelling. As in the previous irrigation event, oaks showed the isotopic signature of the new irrigation water (either tap water or depleted water) on the next day after irrigation (p=0.020-0.049 from 19th to 21st July) (Figs. 4B, 4D). Conversely, the pines increased their time-lag after the drought period and did not show the full irrigation signature until 3 days after irrigation (p=0.003-0.004, from 19th to 23rd July) (Figs. 4A, 4C).

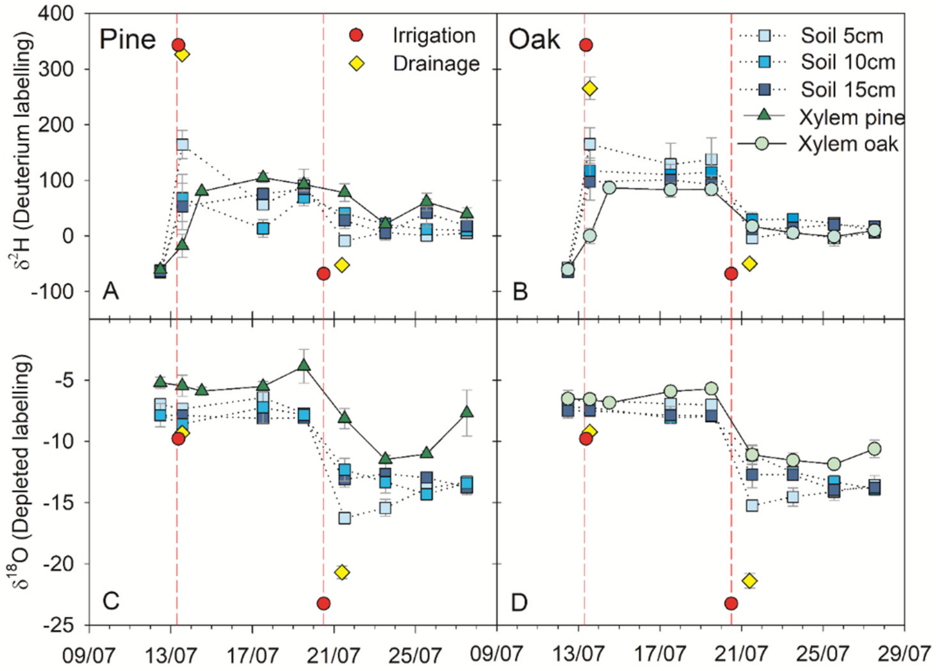

Figure 4. Time evolution of soil (light to dark blue squares), xylem (dark green triangles, pine; light green circles, oak), irrigation (red circles and red-dashed line) and drainage (yellow diamond) water isotopic composition. Pots irrigated with deuterium-enriched water in the first cycle and tap water in the second in pines (A) and in oaks (B); pots irrigated with tap water in the first cycle and depleted water in the second in pines (C) and in oaks (D). Error bars represent standard errors.

Although xylem water tracked changes in soil isotopic composition (with a time lag), it also showed evidence of isotopic enrichment relative to soil water, particularly in pines (Fig. 4, Fig. S7- dual isotope plot for depleted labelling). Considering only the first irrigation with tap water (Fig. 4A, D, Fig. S6 – δ^2H), when changes in soil isotope composition were negligible, enrichment of xylem water (relative to mean soil values) was significant for δ^{18}O and δ^2H in pines (+2.8 ±0.68‰; p=0.003 and +6.8 ±2.41‰; p=0.022, respectively). In oaks, isotopic enrichment was smaller, and only significant for δ^{18}O (+1.0 ±0.29‰; p=0.006 and -0.1 ±2.00‰; p=0.959, respectively). The other irrigation events caused dynamic changes in both soil and xylem water, preventing a precise estimation of soil to xylem fractionation, but deviations overall were still consistent with evaporative fractionation. For example, after the second irrigation with depleted water (Fig. 4C, D), deviations become more positive than with tap water (e.g., for δ^{18}O, +5.4 ±0.97‰ in pines; +3.4 ±1.29‰ in oaks). Conversely, after the first irrigation with deuterated water (Fig. 4A, B), deviations for δ^{18}O were similar to those found for tap water (+2.3 ±0.35‰ in pines; +1.6 ±0.19‰ in oaks) but became negative for δ^2H (-30.1 ±29.63‰ in pines; -61.4 ±22.83‰ in oak), in agreement with the strong departure from equilibrium between water vapour and deuterated xylem water, causing a negative signal through the exchange with atmospheric water vapour (e.g., Cernusak et al. 2022).

Evolution of soil and xylem water content

Despite the changes in soil water content and xylem water potential, xylem water content from twigs in pines was very stable (63 ±7.6%) throughout the experiment (Fig. 5). On the contrary, xylem water content from twigs in oaks was highly variable with time, ranging from maximum values after the first irrigation of 68 ±9.9% to a minimum of 43 ±7.5% at the end of the first drying period. Oak twig water content followed the evolution of soil water content, but with a time lag of three days for recovery after the second irrigation event. Indeed, we observed the minimum values for twig water content in oaks the day after irrigation, despite a clear peak in transpiration rates (see Fig. 1B), indicating that stem refilling happened at a slower rate than the recovery of water transport.

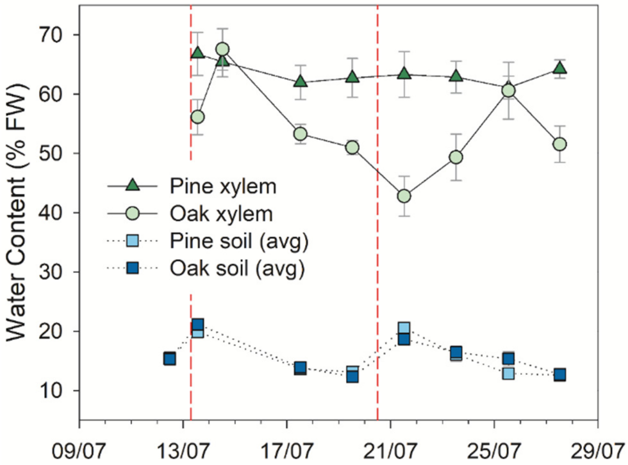

Figure 5. Time evolution of water content in the soil (blue squares) and twig xylem (dark green triangles – pines, light green circles – oaks), calculated by the difference of weights before and after the cryogenic water extraction. A dashed red line represents the days we irrigated. Error bars represent standard errors.

DISCUSSION

Water-use response to drought and recovery in pine and oak potted trees

In agreement to our initial hypothesis (H1), our results showed that pines closed stomata until minimum transpiration rates earlier and at higher water potential (ψ_{xylem} around -1.5MPa) than oaks. Once this minimum water potential was reached in pine trees, and until irrigation, xylem and soil water potential stabilised and sap flow and gas exchange parameters stayed minimal, suggesting a strongly limited physiological activity and water consumption during the last days of drought. This agrees with previous studies on Scots pine, and other pine species, showing a tight stomatal regulation (see e.g., Picon et al. 1996, Irvine et al. 1998, Poyatos et al. 2008, Forner et al. 2014, Klein 2014, Salmon et al. 2015). Conversely, the progressive reduction of ψ_{soil} in oaks, together with the smaller reduction in sap flow rates and gas exchange (particularly during the first drought cycle), indicated that oaks maintained a significant water use despite declining water availability until the last day of the drought treatment, reaching values of ψ_{xylem} around -2.7MPa. Our results agree with previous comparative studies, showing that deciduous oaks are less conservative than pines in terms of stomatal control (e.g., Picon et al. 1996, Poyatos et al. 2005, 2008, Fernández de Uña et al. 2017). This is particularly true for Mediterranean deciduous oaks, that can be regarded as “water spenders”, displaying record values of stomatal conductance within the genus (Gallego et al 1994; Forner et al. 2014; Peguero-Pina et al. 2016; Martín Gomez et al. 2017; Alonso-Forn et al. 2020). Moreover, the significant relationship between ψ_{soil} and g_s, A_n and K_h in pines, points out that photosynthesis and whole tree hydraulic conductance was largely constrained during drought by stomatal closure in this species, consistent with previous works (see e.g., Poyatos et al. 2008, Salmon et al. 2015). The water-saver strategy of pines was also displayed in response to VPD, during well-watered conditions (Fig. S4), compensating VPD changes to prevent excessive water loss in the afternoon, as previously reported in the Mediterranean Aleppo pine (Tatarinov et al. 2016) and, to a lesser extent, the evergreen holm oak (Sancho-Knapik et al. 2022). Conversely, sap flow in well-watered *Q. faginea* increased linearly with VPD during the day (up to >5 kPa in the afternoon), in agreement with the lack of stomatal response to VPD up to 4 kPa, previously reported for this species (Mediavilla and Escudero 2003). However, although it is not possible to isolate soil and VPD effects in our study, our data suggests that *Q. faginea* could become more sensitive to VPD during a persistent drought (Fig. S6B).

At least during the first drought cycle, oak showed negligible changes in gas exchange and K_h, despite much larger variations in ψxylem, maintaining extended physiological activity during drought episodes, in agreement with its water-spender reputation (Gallego et al. 1994, Picon et al. 1996, Poyatos et al. 2008, Klein et al. 2013, Martín-Gómez et al. 2017). Furthermore, this strategy allowed the oaks to keep nearly constant water potential gradients from the soil to the leaves, which would agree with an isohydrodynamic response, as postulated by Franks et al. (2007). Although the drought response in oaks was generally weaker than in pines, the oaks revealed a more significant decline in K_h with respect to ψsoil during the second drying cycle. This eventual loss of conductivity in oaks after the first drought would be partly supported by the incomplete recovery in sap flow observed in oaks after the second irrigation (see e.g. the differences in K_h between the two cycles in Fig. S6). However, it should be noted that, as shown in Fig. S3, leaf area decreased over time due to twig sampling (up to *ca*. 20% at the end of the experiment). Although this was accounted for in our calculations, it could be partly responsible for the incomplete recovery in sap flow and water-use estimates in both species. Unlike in oaks, pines showed a full recovery of water use determined from weight loss, but not in sap flow. This divergence between sap flow (canopy above the sensor) and weight loss (whole plant) could reflect the different exposure of upper and lower canopy, which would be much more evident in pine seedlings than in oaks, due to their denser canopy. Alternatively, we cannot rule out potential dampening effects and stress signaling associated to injuries caused by sap flow sensors (Peters et al. 2018 New Phytologist) and/or twig sampling, which could have proportionally a larger effect in the smaller oak canopy.

Additionally, our experiments also suggested different behavior in response to irrigation after the drought treatment for both species. Contrary to our initial hypothesis (H2), xylem water potential recovery was faster in pines than in oaks, i.e., pines reached their maximum ψ_{xylem} on the next day after watering, whereas oaks required two days. These results agree with previous studies describing the more opportunistic strategy of pines, in comparison to oaks (see e.g., Picon et al. 1996, Poyatos et al. 2008, Klein et al. 2013, Forner et al. 2014, Matheny et al. 2014, Rodríguez-Robles et al. 2015). For example, Poyatos et al. (2008) reported a faster increase in transpiration after the first rain events in Scots pine compared to pubescent oak (*Quercus humilis*), despite the larger sap flow restrictions in the preceding summer drought for this species. Sometimes however, oaks can exhibit a faster recovery after drought than pines, putatively associated with the access to deeper (and wetter) soil layers (Barbeta et al. 2015, del Castillo et al. 2016, Grossiord et al. 2016, Martín-Gómez et al. 2017).

In the pot experiment described here, however, root growth could have been restricted by the size of the containers. Although generally oak roots expand deeper than pines, this did not seem to be exploited by the oaks in our experiment. Indeed, we did not find significant differences in root biomass between the two species (Table S1), and according to the evolution of SWC, oaks dried mostly the soil at the intermediate soil depth sampled (10 cm, see appendix A1 for Figure 5). Nevertheless, visual inspection after plant harvest did not show clear symptoms of confinement (e.g., accumulation of roots in the deepest part of the pots). Definitely, oak recovery capacity in this experiment relied basically on their stomatal response and internal storage use. In this regard, our results would be more representative of poor sites with limited soil depth, where submediterranean oaks (e.g. *Q. faginea* and *Q. subpyrenaica*) have shown to be particularly susceptible to drought, being affected by periodic episodes of tree dieback (Corcuera et al. 2004; Peguero-Pina et al. 2015). Considering that recent literature (Jiang et al., 2020; Matheny et al. 2017) suggest that plant water strategies can shift within a

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species due to variation in rooting depth and root hydraulics, further research should be conducted to assure the full applicability of our results to natural ecosystems with no-constriction of the rooting system (Kannenberg et al. 2022). Hereof, ecological modelling including whole plant hydraulic traits (leaf, stem and root level) could be very useful to solve uncertainties and create a new theoretical and complete theoretical frame of water-use strategies (Matheny et al. 2017, Kannenberg et al. 2022).s

Labelling and stem water dynamics reveal species-specific differences in water uptake, use and storage

We found here that the time for ψ_{xylem} recovery after irrigation was not directly linked to the time required to fully label stem water with irrigation water. In opposition to our first hypothesis (H3), the labelling speed was faster in pines despite the lower transport capacity in relation to oaks. Specifically, when irrigating after drought, the isotopic composition of xylem water in pines took about 3 days after watering to stabilise and reach 'maximum' labelling, despite ψ_{xylem} recovering pre-drought levels almost instantaneously. Conversely, oaks reached maximum labelling signature on the next day after irrigation, despite their physiological performance (ψ_{xylem} stomatal conductance and transpiration rates) being not completely restored until the next two days. The time taken to reach the maximum labelling decreases with labelling speed (sap flow) and increases with the residence time of the label in the xylem (storage capacity). In this regard, there is a general trade-off between the speed of labelling and the time that the label remains into the xylem (residence time), frequently associated to species-specific differences in wood density and wood capacitance (see e.g., James et al. 2003, Meinzer et al. 2003, 2004, 2006; Ferrio et al. 2018). A slower labelling speed and higher residence time has been found for conifers compared to tropical angiosperms (Borchert and Pockman 2005, Meinzer et al. 2009, Mcculloh et al. 2012, Jupa et al. 2016).

Interestingly, this difference in labelling speed between species was less evident when the labelling was performed before the first drought period (first irrigation event), suggesting that the larger reductions in whole-plant hydraulic conductance (K_h) and stomatal conductance in pines, together with a higher residence time, could have reduced their ability to refill their xylem tissues with new water (Brodribb and Cochard 2009). A higher residence time in pines could also explain the greater evaporative fractionation observed in this species (Martín-Gómez et al 2016). According to previous studies, evaporative fractionation in stem water is only visible if stem evaporative losses are high, relative to water transport rates, and hence has been associated to limited transpiration rates and relatively long residence times in the stem (Dawson and Ehleringer 1993, Cernusak et al. 2005, Ellsworth and Sternberg 2014, Martín-Gómez et al. 2016). Additional causes for the observed isotopic enrichment in the twigs could be the mixture of xylem water with enriched water from the leaf through back diffusion (Brandes et al. 2007, Ellsworth and Williams 2007, Dawson and Ehleringer 1993, Farquhar and Lloyd 1993), or the water exchange between xylem and phloem tissues (Cernusak et al. 2005, Brandes et al. 2007); however, with the results obtained in this experiment we could not differentiate the relative contribution of the mentioned processes in the observed isotopic enrichment of twigs.

Xylem water content in pine twigs was very stable (around 65% of fresh weight, Fig. 5) throughout the experiment, despite the variability of soil water content in the pots. For oaks, we observed large fluctuations of xylem water content from twigs going from 65% at well-watered conditions to 45% after the drought treatments. These results are in accordance with previous studies showing distinct water storage patterns in pines and oaks. For example, Sobrado et al. (1992) found small variations in xylem relative water content in twigs (maximum 10%) for *P. sylvestris* during rewetting after experimental dehydration. Similarly, in *Pinus contorta,* Running (1979) reported small variations in sapwood relative water content during the summer season, remaining between 60 to 70%. In a study comparing the tropical semi-arid *Pinus cembroides* and *Quercus potosina*, Rodríguez-Robles et al. (2020) reported significant variations in stem water content for both species, but much larger, and tightly linked to changes in stem diameter, in oak trees. Furthermore, isotope labelling revealed larger storage capacity in the oaks and found evidence for the use of stored water during the dry season. Steppe and Lemeur (2007) also estimated a relatively higher capacitance in *Quercus robur* than in *Fagus sylvatica*, which was attributed to the higher elasticity of the stem storage tissue including bark in oak trees. This species-specific divergence in twig water content can be explained by hydraulic strategies and differences in xylem anatomy. Trunk storage provides a buffer to water demands during transpiration across daily and seasonal time frames (Hao et al. 2013, Matheny et al. 2015, Oliva Carrasco et al. 2015, Jupa et al. 2016). In accordance with Matheny et al. (2015), we observed that the withdrawal of storage water in pines is expected to buffer the diurnal water requirements of transpiration on wet and dry days (showing an early peak of sap flow rates early in the morning), whereas oaks used stored water during drought and do not refill completely until non-limiting conditions (Fig S4). In this regard, our results suggest that there is a connection between leaf water strategy and stem water storage dynamics, which means that plant hydraulic traits related to leaf water strategy are associated with stem water storage and use patterns. On the one hand, water-saver pines can maintain constant twig water content during drought by rapidly closing stomata, and as the stem tissues do not dehydrate, they can easily recover their water potential rapidly once soil water content increases and stomata open again (Tardieu and Simonneau 1998, McDowell et al. 2008). On the other hand, the water-spender oaks become more dehydrated to maintain relatively higher transpiration rates continuously lowering xylem water potential (e.g., Borchert and Pockman 2005; Rodriguez-Robles et al. 2020) consequently they cannot recover water potential levels rapidly to previous non-stressed values until the xylem water content in twigs is fully restored. Consequently, it takes longer to restore xylem water potential in oaks than in pines during rewatering, at least after short-term droughts typical of our study.

The release of storage water to the transpiration stream is also linked to xylem anatomy (see Fig S8). Woody organs represent important sites for plant water storage (Holbrook 1995), and during periods of high transpiration, this water storage can be released by a combination of three mechanisms (Tyree et al. 1992, Tyree and Zimmermann 2002): capillarity (from intercellular spaces and cracks), elasticity (from living parenchyma cells) and embolism (from vessels and tracheids). The release of capillary water (at water potentials close to zero) and elastic storage water (at more negative water potentials) is assumed to prevent hydraulic dysfunction (Gartner and Meinzer 2005, Scholz et al. 2007, Meinzer et al. 2009); the abundance of capillary and elastic storage tissues should therefore be a good indicator of stem capacitance and the ability to buffer changes in water potential. In this regard, *Q. faginea* has a ring-porous xylem with wide and long vessels and a relatively large volume of non-conductive fibres and parenchyma cells (ray and axial) (Fig. S8 A, B; 26.3 ±12.4% for angiosperms, around 35% in *Quercus robur*; Morris et al. 2016). Conversely, the sapwood of *P. sylvestris* includes mainly tracheids that combine both transport and mechanical functions (Fig. S8 C, D; according to Morris et al. 2016, 7.6 ±2.6% are parenchyma cells in conifers). These differences would support the idea that reversible changes in twig water content observed in oak trees, might be explained by a loss of water from capillaries and elastic parenchymatous tissues, delivering water to the xylem under tension, to maintain higher transpiration rates during drought (Rodríguez-Robles et al. 2020). Conversely, the rigid structure of the pine wood, mainly composed of tracheids, would be less able to compensate for water loss through cell shrinking, and thus losses in xylem water content would be likely associated with irreversible cavitation events (see e.g., Rehschuh et al. 2020). Hence, despite Scots pine having a higher total mass of water compared to oak, this water might be mainly located in the tracheid lumen and thus not used as storage water; in contrast, parenchyma cells in oak seem to have a predominant role in water storage and release during decreasing water potential (Scholz et al. 2007, Pfautsch et al. 2015).

The role of storage tissues in preventing cavitation, however, is still a matter of debate. For example, recent studies using micro computed tomography in living and excised stems (Knipfer et al. 2019; Yazaki et al. 2020) point out that cavitation in the large xylem vessels of ring-porous *fagacea* (*Castanea serrata* and *Quercus serrata*) may occur before, or at least simultaneously, to the release of water from fibres and other tissues in the xylem matrix. In this regard, the loss of water observed in our study, if mainly driven by vessel cavitation, would be tightly linked to xylem function (sap flow, K_h), both during drought and recovery, and this was not clearly the case, at least during the first drying cycle. Indeed, in our study we found significant changes in K_h for oak only during the second drying cycle, whereas after the first cycle, the recovery in sap flow preceded the recovery in xylem water content in twigs by 2-3 days. This fast recovery of sap flow is hardly compatible with a significant cavitation, unless we assume a fast refilling of cavitated vessels. Although xylem refilling has been reported for some species (Salleo et al. 1996, Hacke & Sperry 2003, Taneda & Sperry 2008, Knipfer et al. 2019), it seems mainly associated to periods with optimal soil water availability (>-0.5 MPa) and nearly absent transpiration rates. These conditions contrast with those in our study, where soil water potential dropped well below -1 MPa during drought, and VPD ranged from 3 to 5 kPa. Consequently, even during recovery the soil-stem gradient in oaks remained around 1 MPa, putatively incompatible with refilling.

In our study, we did not find significant differences in K_h between the initial values (watering 1) and those reached during recovery (watering 2), neither for pines (from 4.8 ±1.63 to 4.7 ±1.38 mmol m^2 s^{-1} MPa^{-1}, p=0.953) nor for oaks (from 4.7 ±2.07 to 3.5 ±1.36 mmol m^2 s^{-1} MPa^{-1}, p=0.786) (see Fig. 3). Nevertheless, the response of K_h to ψ_{soil} was stronger during the second drying cycle, particularly for the oaks (Fig. S6), so we cannot rule out certain loss of conductivity. Conversely, we observed an increase in the time lag for the uptake of the new labelled water in the pine, but not in oak. This in turn suggests certain loss of conductivity in the pine, although not so strong to be detectable within the error margins of calculated whole-plant K_h. The water potential found in our experiment (-1.5 MPa) is still far from that causing 50% loss of conductivity (P_{50}) in *P. sylvestris* (e.g., -2.5 in Cochard 1992), but cavitation events may occur from -1.5 MPa (Perks et al. 2004). On the other hand, Esteso-Martínez et al. (2006) reported a P_{50} of -3.9 MPa for *Q. faginea*, showing a loss of conductivity starting from -3 MPa. In this regard, although we cannot rule out some level of cavitation in oaks at the water potentials achieved during the experiment (-2.3 MPa), it was certainly not enough to impose a strong limit to transpiration, at least during the first drying cycle, due to the high transport capacity of the remaining vessels.

In Figure 6 we summarise the different patterns of storage, isotope labelling, and water use observed in pines and oaks, during drought and recovery. During the initial, well-watered conditions (Watering 1), both species showed high transpiration rates, and similarly high twig water contents, acquiring the isotope signal of labelled water (represented in blue) within one day. During the first drying cycle (Drying 1), the water potential of pines reached a plateau at -1.5MPa, nearly stopping water flow, whereas the oak kept transpiring actively, achieving lower water potentials in the soil. This resulted in a progressive drying of the stem in oaks, which was almost negligible in the pine. According to the anatomical differences, we attribute this to the loss of water from elastic tissues in the oak, to maintain transpiration. In the recovery phase (Watering 2), the pines showed a slower capacity to replace "old" water with new labelled water (in green), suggesting some loss of conductivity, but not so strong as to prevent a recovery in water use. In this regard, peak labelling values in pine were not reached until 3 days after watering (Drying 2). On the contrary, during recovery (Watering 2) the oaks showed a rapid

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replacement of the isotope signature in the stem, in line with the increase in water use, but kept low twig water contents. We interpret that the water loss in oak reflected the release of storage water, without a reduction in the transport capacity of oaks. This storage water was progressively recovered, requiring about 3 days to reach pre-drought values (Drying 2).

CONCLUSIONS

In this experiment we showed an interesting link between the leaf-level response and the water uptake and storage patterns during drought and recovery for pine and oak young potted trees. Water-saver species such as pines can keep a rather constant stem water status during drought, avoiding dehydration through rapidly closing stomata. Consequently, if the drought period is not very long or extreme in temperature to cause damage to living tissues, water-saver species are able to easily recover their water potential once soil water content increases and stomata open. Nevertheless, the increased time-lag between soil and stem isotope labelling after drought demonstrate certain limitations in whole-plant hydraulic conductance. Conversely, the water-spender oaks withstand more dehydration to maintain high transpiration rates during drought, causing lower xylem water potentials. This dehydration is putatively mediated through the release of water from elastic storage tissues in the stem since the transport function of the xylem remained unaffected under the moderate drought conditions of the experiment. Indeed, drought did not affect the time-lag in isotope labelling between the soil and stem, allowing a quick replacement of water in the highly conductive xylem vessels. Nevertheless, oaks required a longer time period to fully restore their xylem water potential after drought, because of the need to refill storage tissues. Overall, we show that the combined use of water stable isotopes, together with hydraulic and xylem water content measurements, can provide a more complete and comprehensive understanding of plant water use and storage strategies during drought and recovery conditions and provide insight on variations in hydraulic strategies between different tree species.

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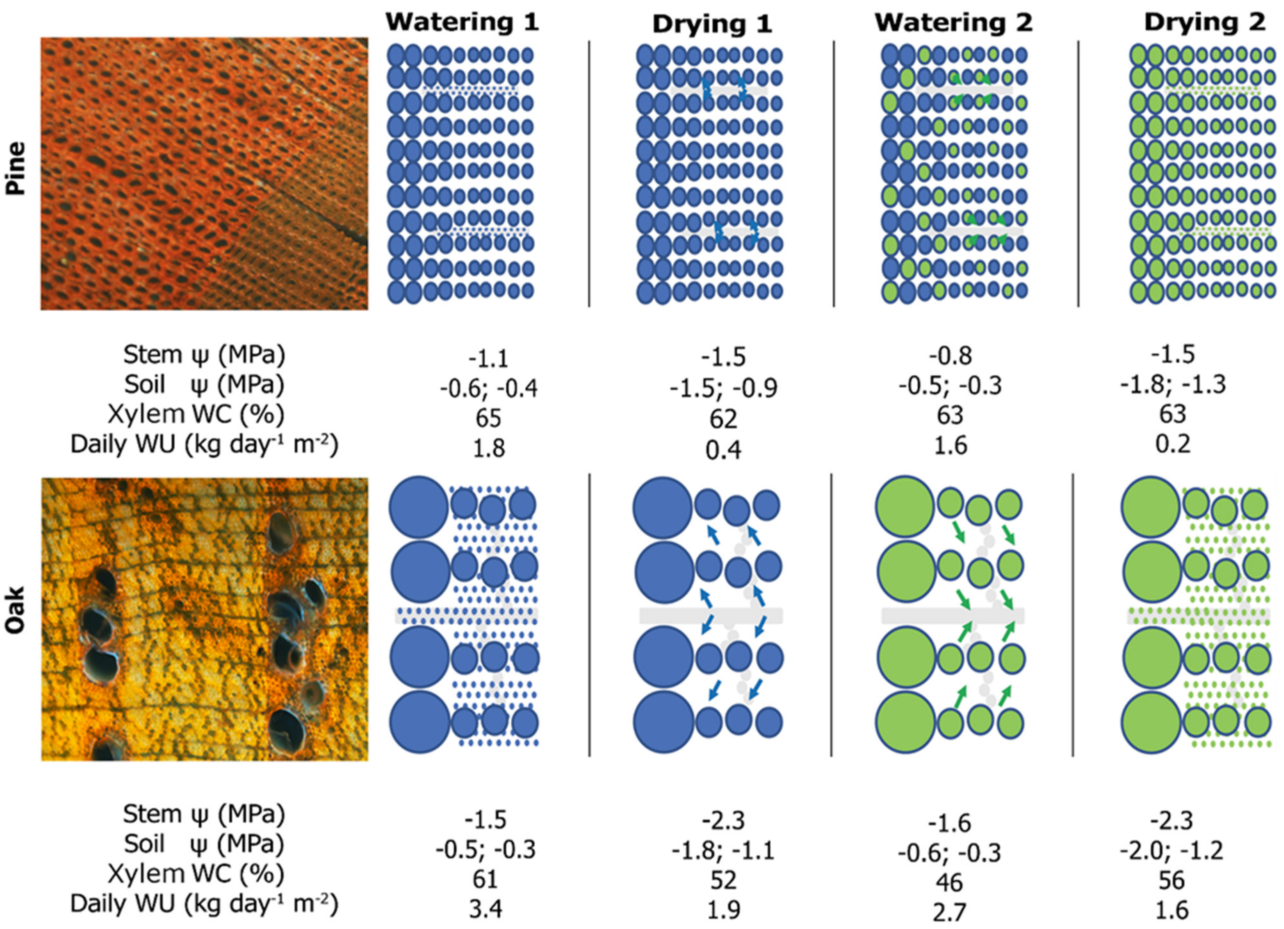

Pine	Watering 1	Drying 1	Watering 2	Drying 2
Stem ψ (MPa)	-1.1	-1.5	-0.8	-1.5
Soil ψ (MPa)	-0.6; -0.4	-1.5; -0.9	-0.5; -0.3	-1.8; -1.3
Xylem WC (%)	65	62	63	63
Daily WU (kg day^{-1} m^{-2})	1.8	0.4	1.6	0.2

Oak	Watering 1	Drying 1	Watering 2	Drying 2
Stem ψ (MPa)	-1.5	-2.3	-1.6	-2.3
Soil ψ (MPa)	-0.5; -0.3	-1.8; -1.1	-0.6; -0.3	-2.0; -1.2
Xylem WC (%)	61	52	46	56
Daily WU (kg day^{-1} m^{-2})	3.4	1.9	2.7	1.6

Figure 6. Schematic representation of the storage and water transport processes explaining the observed evolution of isotope tracers, water use and xylem water content from twigs during drought and recovery. Coloured circles represent the lumen of the main transport tissue (tracheids in pine; earlywood and latewood vessels in oak). Blue and green colour denote the distinct isotope signature of the first and second irrigation water, respectively. Arrows indicate the presumed water flow from and to storage tissues (ray parenchyma in the pine; ray parenchyma, paratracheal and apotracheal parenchyma in oaks). The dotted pattern in tracheids and vessels during drying phases denote the increasing water tension in the xylem, larger in oaks than in pines. ψ_{xylem}, and ψ_{soil}, mean water potential in the xylem, and in the driest and wettest soil depths during each phase, respectively; Xylem WC, mean absolute water content (% of fresh weight) in bark-removed twig samples; Daily WU, mean daily water use per unit of leaf area, derived from sap flow.

DATA AND MATERIALS AVAILABILITY

Data that support the findings of this study will be made available upon request.

SUPPLEMENTARY DATA

Supplementary figures, tables and Appendices for this article are available at Tree Physiology Online.

CONFLICT OF INTEREST

None declared.

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AUTHORS' CONTRIBUTIONS

P.M.-G., J.P.F. and U.R.-R. planned and designed the research, collected and analysed the data, J.V.S.S. realised the wood anatomical photos, P.M.-G., J.P.F. and U.R.-R. wrote the manuscript with contribution from the other authors.

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SUPPLEMENTARY MATERIAL

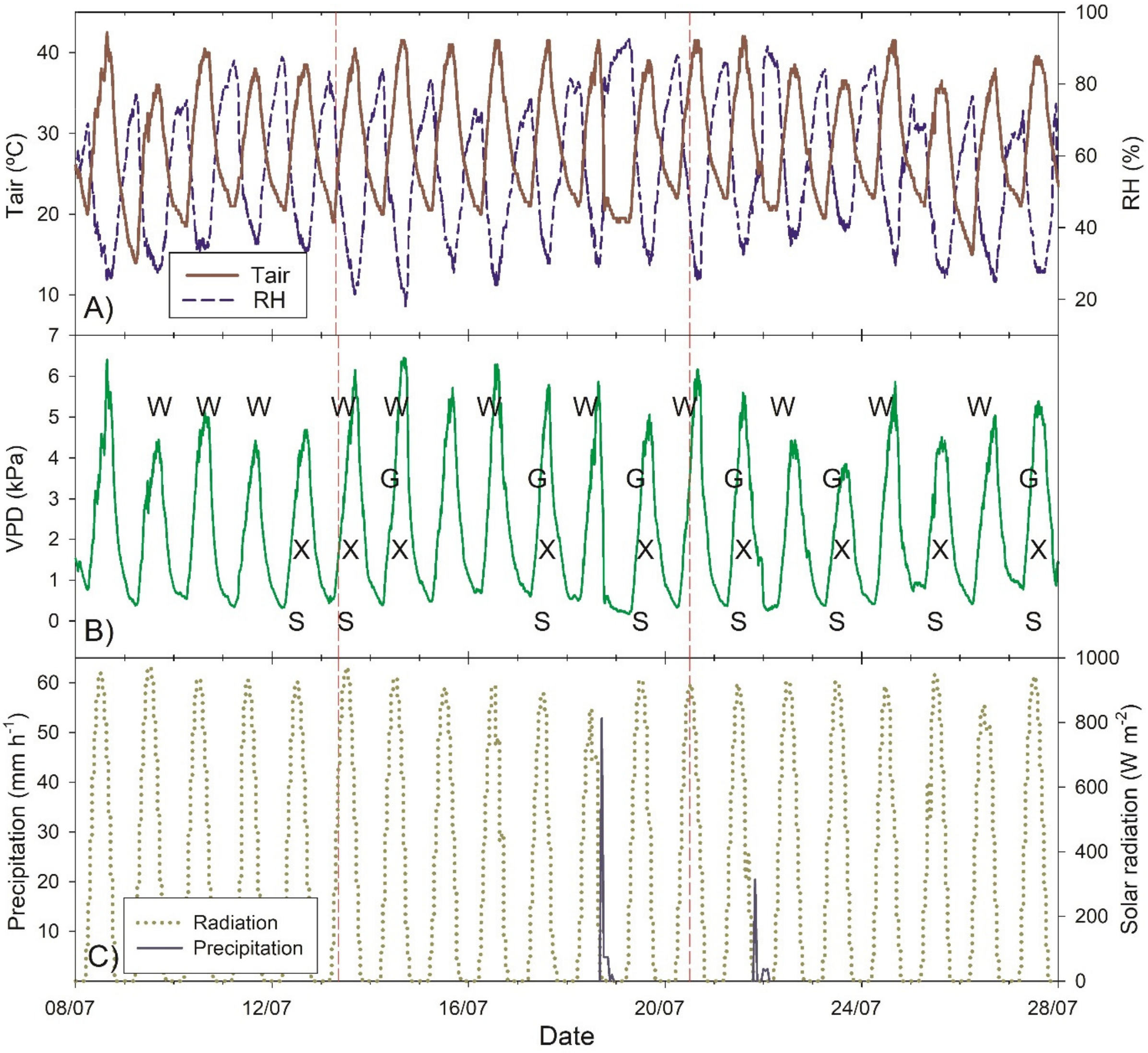

Figure S1. Evolution of main meteorological variables and measurement plan during the experiment. A) Air temperature (Tair, ºC) and relative humidity (RH, %). B) Atmospheric vapour pressure deficit (VPD). C) Hourly accumulated precipitation (mm h-1) and solar radiation (W m-2). Tair, RH and VPD were monitored in situ, precipitation and radiation retrieved from a nearby meteorological station (Raimat, UTMX 287655, UTMY4617757, 286m.a.s.l.). For reference, the timing of the two irrigation events (red dashed vertical lines), and the key sampling and measurement campaigns is shown: S and X, soil and xylem sampling, respectively; G, gas exchange measurements; W, weight of pots.

Table S1. Summary of biomass traits. The data corresponds to the trees used for sap flow monitoring, harvested at the end of the experiment. Mean ±SE, mean and standard error for each species; *p*-value indicates the significance of a two-tailed t-test.

Species	TreeID	Leaves (g)	Stem + branches (g)	Coarse roots (g)	Fine roots (g)	Root/shoot
Oak	O03	15.7	56.2	29.0	15.3	0.62
	O10	10.1	66.1	17.3	55.2	0.95
	O02	13.2	91.0	14.9	34.4	0.47
	O01	8.2	56.6	32.7	54.9	1.35
	O08	14.5	65.0	42.1	26.6	0.86
	O09	9.5	45.3	24.9	7.9	0.60
	Mean ± SE	*11.9 ±1.2*	*63.4 ±6.3*	*26.8 ±4.1*	*32.4 ±8.1*	*0.81 ±0.13*
Pine	P01	98.4	164.8	12.5	38.3	0.19
	P02	62.8	119.2	9.4	21.1	0.17
	P03	67.5	147.3	20.1	23.2	0.20
	P09	53.6	144.9	15.8	26.6	0.21
	P08	128.4	253.1	37.6	49.9	0.23
	P10	59.8	110.6	11.8	28.2	0.23
	Mean ± SE	*78.4 ±11.9*	*156.7 ±20.9*	*17.9 ±4.2*	*31.2 ±4.5*	*0.21 ±0.01*
	p-value	**0.003**	**0.005**	0.160	0.902	**0.006**

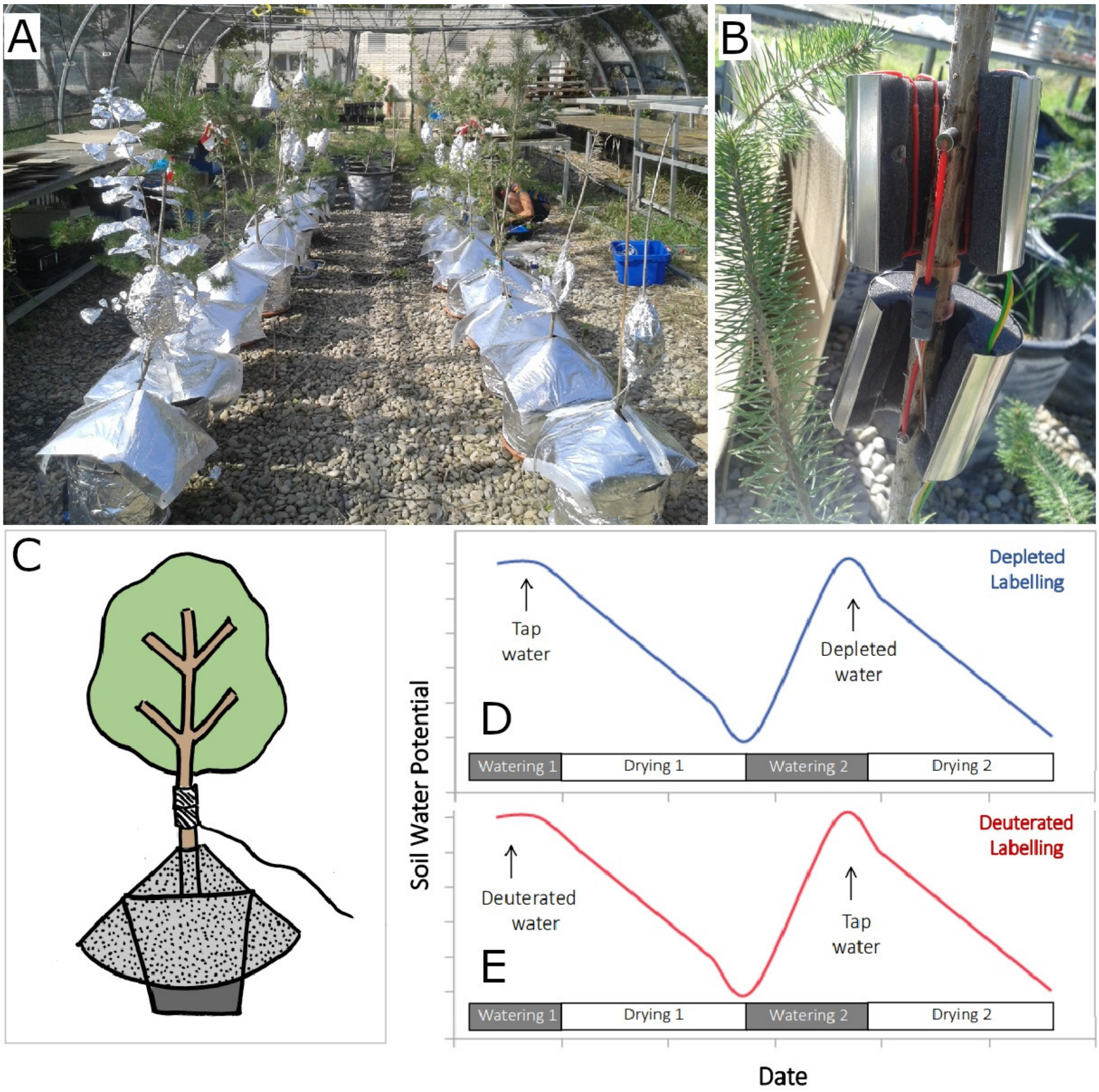

Figure S2. Experimental set-up. A) General view of the experiment. B) Detail of the disposition of the sap flow sensors. C) schematic diagram for the system installed in the tree pots to avoid precipitation inputs and soil evaporation. D, E) Irrigation schedule used in the experiment (b, c). Plants were divided into two groups, 8 plants for "Depleted labelling" (tap water + depleted water (D) and 8 plants for "Deuterium labelling" (deuterium-enriched water + tap water (E). All trees were irrigated in the day 1 of the experiment, dried for the next 8 days; irrigated again but with different water and dried for the next 8 days.

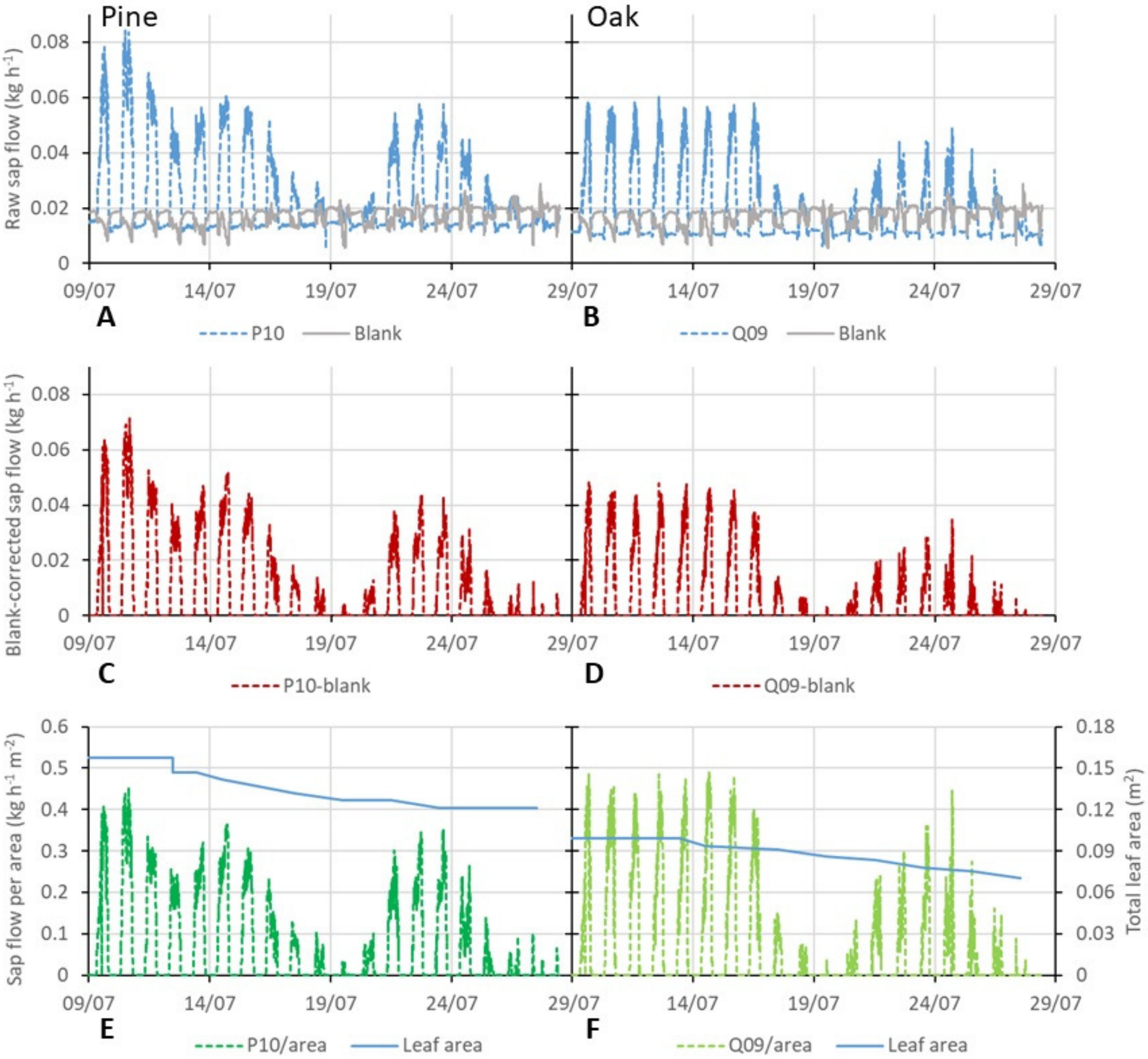

Figure S3. Example of the steps in the calculation of sap flow per area in one pine (A, C, E) and one oak (B, D, F). A, B) evolution over time of 5-min records of raw sap flow (kg h-1) and apparent sap flow observed in defoliated trees (blank), used to correct for thermal gradients. To prevent overcorrection, we followed a conservative approach to calculate blank values: for each defoliated tree, we determined the lower bound of the 95% confidence interval of a 30-min moving average (*N*=6) of apparent sap flow, then we took the minimum value across the two defoliated trees. C, D) Blank-corrected sap flow, calculated as the difference between measured sap flow and the blank, and assuming sap flow = 0 whenever sap flow < blank, which generally occurred overnight. E, F) evolution of total leaf area above the sensor, and calculated sap flow per area, evidencing the higher transpiration rates per area of the oak. Notice that accounting for changes in leaf area over time flattened the difference between sap flow maxima during pre-drought and recovery phases.

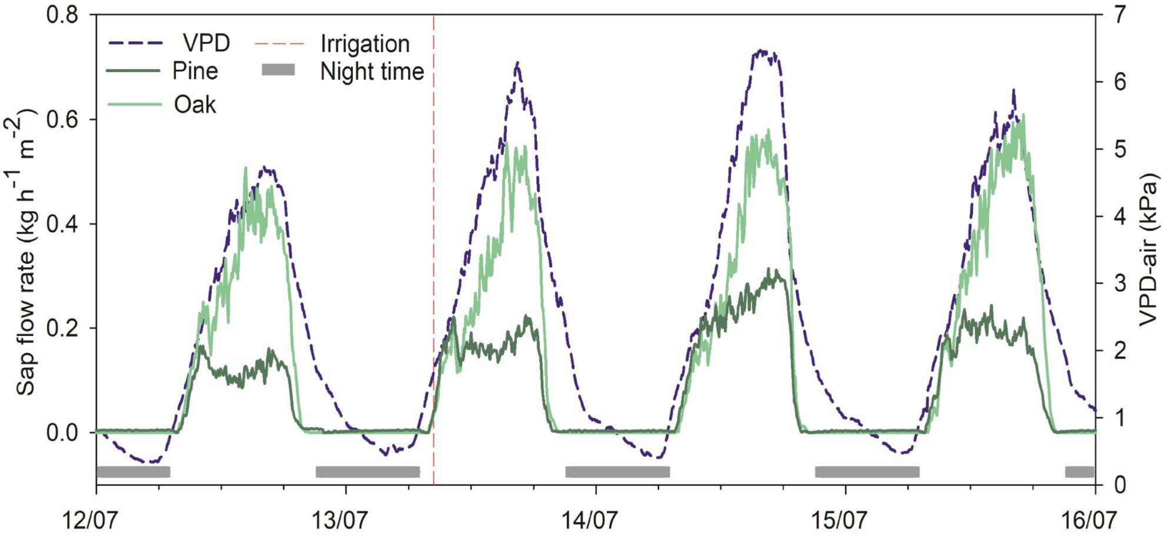

Figure S4. Detail for the time evolution of average sap flow for pines (dark green) and oaks (light green) and VPD (blue dotted line) during four days with well-watered conditions. The grey boxes in the time axis denote night periods.

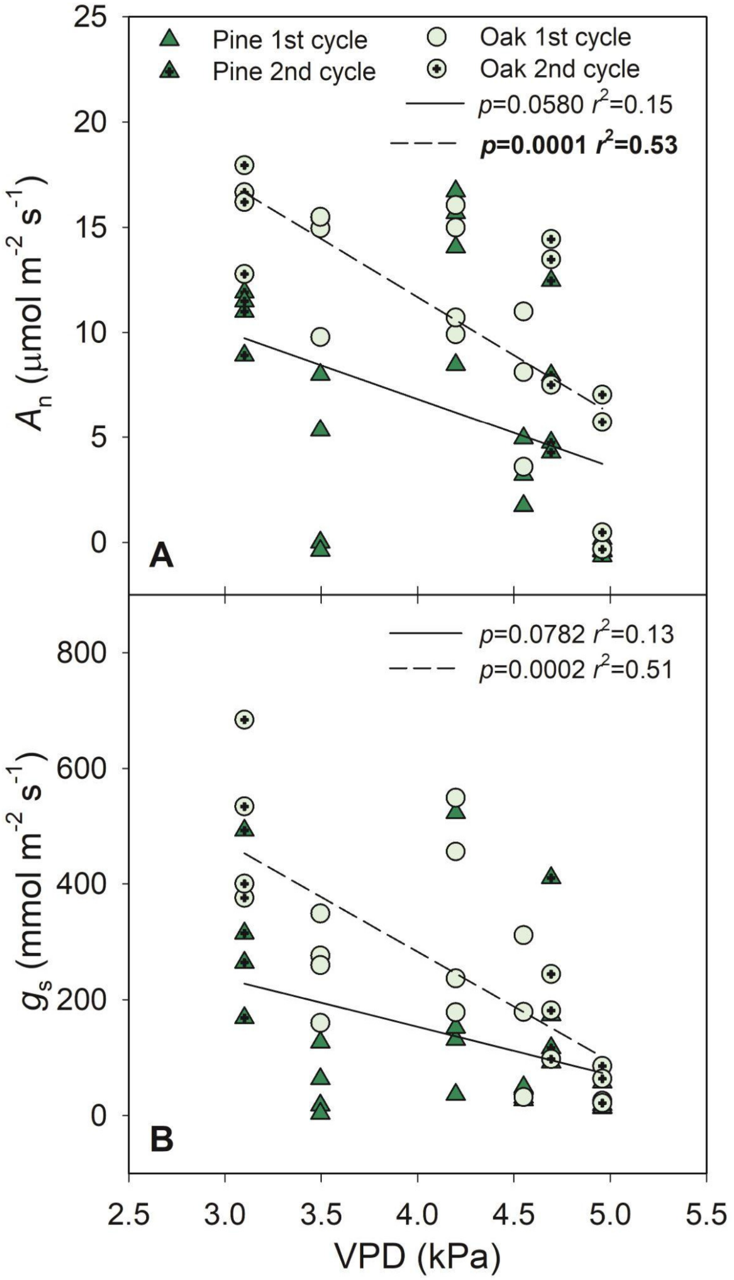

Figure S5. Relationship between VPD and gas exchange variables: A) net photosynthesis (A_n), and B) stomatal conductance(g_s). Light green circles and dashed line, oaks; dark green triangles and solid line, pines. Filled and crossed symbols denote the first and second cycles, respectively. The regression coefficient (r^2) and significance (p) are represented in each panel.

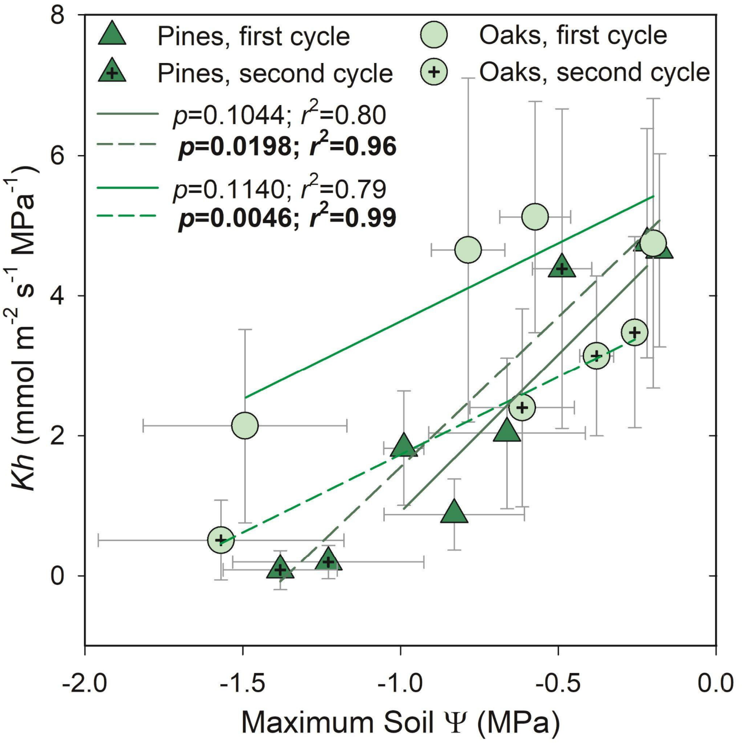

Figure S6. Relationship between leaf-specific hydraulic conductance (*Kh*) of the whole plant and maximum soil water potential, separately for the first and the second irrigation cycles. Symbols and regression lines in light green for oaks, in dark green for pines. Filled and dotted symbols indicate the first and second cycles, respectively. Regression lines are solid for the first cycle and dashed for the second cycle. Error bars denote standard errors. The regression coefficient (r^2) and significance (p) are represented. Maximum soil Ψ denotes the highest water potential across soil depths, as a proxy for the most easily available water.

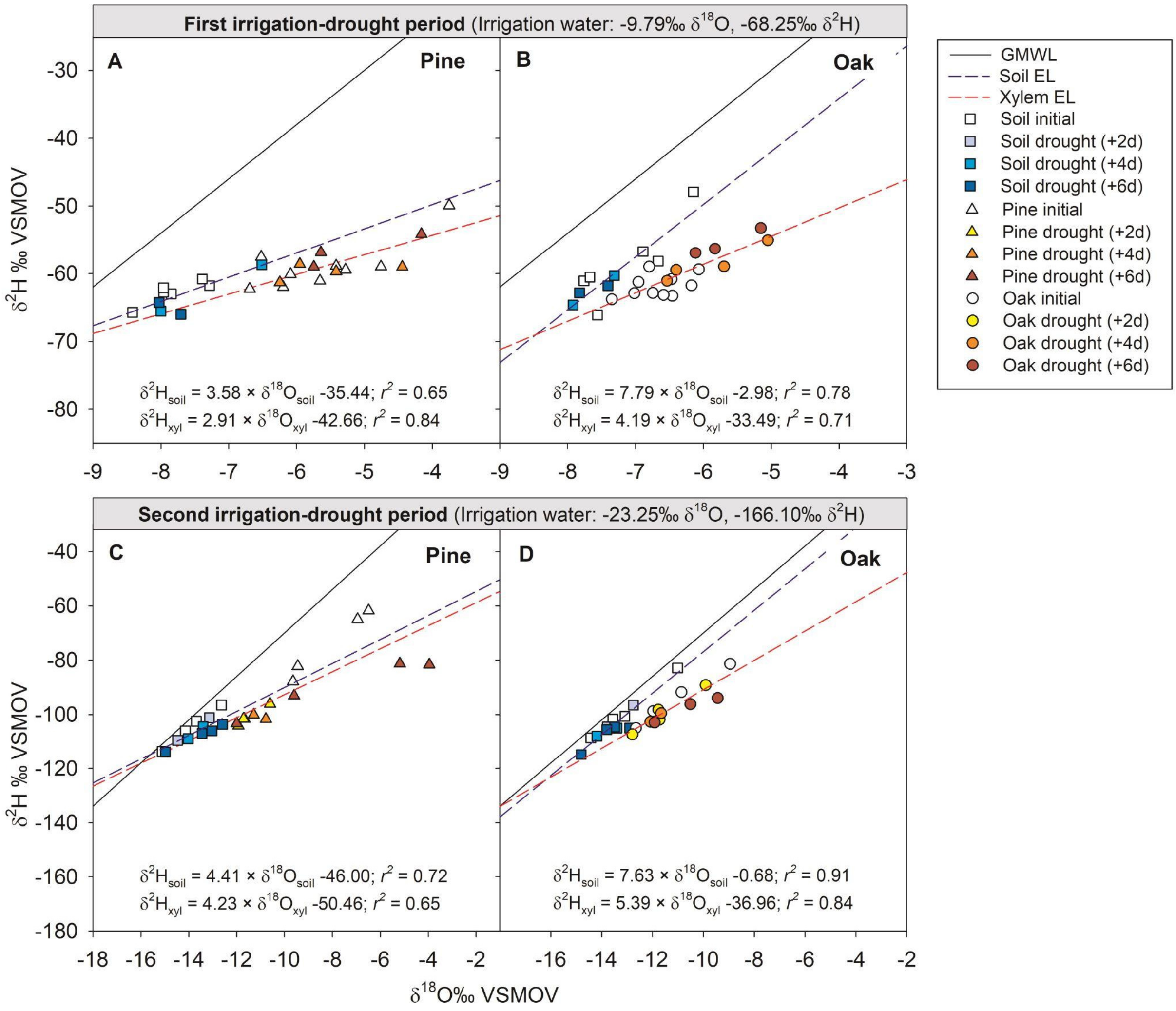

Figure S7. Dual isotope plot ($\delta^{18}O$ and $\delta^{2}H$) with the time evolution of soil (white and light to dark blue squares) and xylem (white and yellow to red triangles – for pines: A, C panels; or circles – for oaks: B, D panels) water isotopic composition, for the "Depleted labelled" plants. Global meteoric water line (GMWL) and soil and xylem evaporative lines are represented in black, blue dashed and red dashed lines respectively. First cycle of irrigation with tap water (-9.79 ± 0.24‰ for $\delta^{18}O$ and -68.25 ± 3.42‰ for $\delta^{2}H$ VSMOW) followed by 4 and 6 days of drought, in A for pines and B for oaks. Second cycle of irrigation with depleted water (-23.25‰ for $\delta^{18}O$ and -166.102‰ for $\delta^{2}H$) followed by 2, 4 and 6 days of drought, in C for pines and D for oaks. Initial conditions were measured some hours after the irrigation event in the 13th July (first cycle) and 21st July (second cycle) and are represented in white for soil and xylem samples.

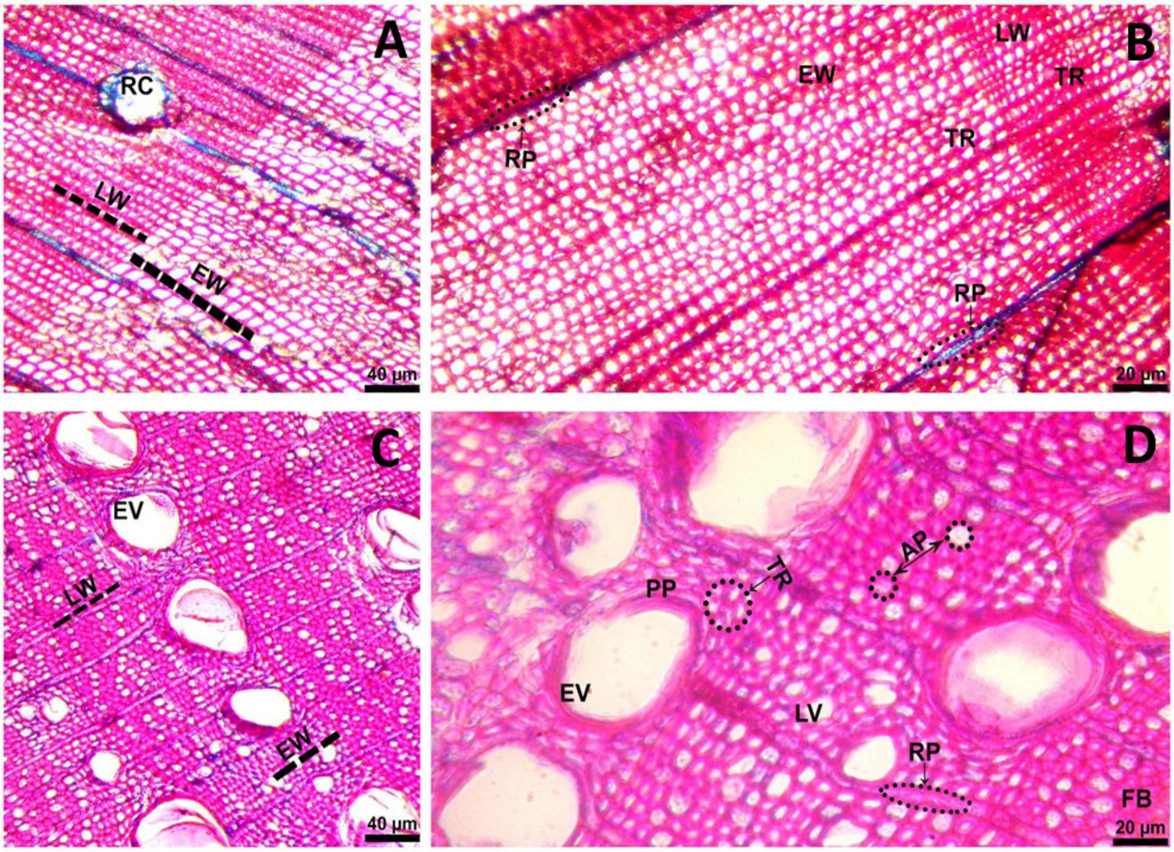

Figure S8. Example of the main anatomical features of sapwood in branches of *Pinus sylvestris* (A, B) and *Quercus faginea* (C, D). Fresh cut 10 µm-thickness slices, stained with 0.2% SafraBlue solution for 5 minutes. EW, earlywood; LW, latewood; RC, resin canal, TR, tracheids; EV and LV, earlywood, and latewood vessels, respectively; RP, AP and PP, ray, apotracheal and paratracheal parenchyma, respectively; FB, fibres.

Appendix A1. Support Statistics for Figures

FIGURE 1

Daytime (Rad>500 W m-2) hourly Sap Flow (kg h-1 m-2) (FIG. 1AB)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
         Sum Sq   Mean Sq NumDF   DenDF  F value Pr(>F)
Sp      0.000101 0.0001010     1    8.04   1.6641 0.2329
Date    0.107979 0.0063517    17 1121.64 104.6473 <2e-16 ***
Sp:Date 0.015489 0.0009111    17 1121.15  15.0111 <2e-16 ***
```

ANOVA (lme with AR1)

```
            numDF denDF   F-value p-value
(Intercept)     1  1058 239.53579  <.0001
Sp              1     8   3.10560   0.116
Date           17  1058  95.09588  <.0001 ***
Sp:Date        17  1058  22.77207  <.0001 ***
```

Date effect and p-value; daily enmeans and S.E. (g h-1 m-2) for Pine and Oak; Oak-Pine effect and p-value(lmer)

Date	p-value		PineSF_enmean	PineSF_SE	OakSF_enmean	OakSF_SE	Oak-Pine	p-value	
11/07/2015	1.0000		43.8	3.52	51.4	3.52	7.64100	0.1185	
12/07/2015	0.0000	***	29.3	3.49	46.1	3.49	16.80400	0.0003	***
13/07/2015	**0.9257**		**43.4**	**4.81**	**35**	**4.81**	**-8.39900**	**0.0024**	******
14/07/2015	0.6303		44.6	3.49	48	3.49	3.38900	0.0957	.
15/07/2015	0.0638	.	40.4	3.49	48.6	3.54	8.19220	0.8335	
16/07/2015	0.0000	***	30.7	3.52	54.8	3.59	24.13100	0.0000	***
17/07/2015	0.0000	***	20.2	3.52	31.8	3.59	11.60000	0.1467	
18/07/2015	0.0000	***	21.1	3.52	33.1	3.59	11.92100	0.1167	
19/07/2015	0.0000	***	19	3.49	20.3	3.55	1.31600	0.0165	*
20/07/2015	**0.0000**	*******	**17.2**	**3.49**	**23.9**	**3.52**	**6.69770**	**0.7164**	
21/07/2015	0.0000	***	30.7	3.52	32.8	3.52	2.14900	0.0373	*
22/07/2015	0.0007	***	37.6	3.49	32.2	3.49	-5.37900	0.0000	***
23/07/2015	0.0000	***	31.4	3.52	29.4	3.54	-2.04100	0.0003	***
24/07/2015	0.0000	***	29	3.49	33.4	3.55	4.38700	0.2172	
25/07/2015	0.0000	***	18.4	3.49	31.1	3.55	12.69300	0.0555	.
26/07/2015	0.0000	***	18.9	3.49	25.9	3.55	7.01380	0.8119	
27/07/2015	0.0000	***	15.8	3.49	16.9	3.55	1.10600	0.0133	*
28/07/2015	0.0000	***	23.6	4.11	16.9	4.3	-6.72900	0.0005	***

Note: data after irrigation events are highlighted in bold

Degrees-of-freedom method: kenward-roger Confidence level used: 0.95

Daily WU (accumulated sap flow, kg day-1 m-2) (FIG. 1AB)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
         Sum Sq Mean Sq NumDF   DenDF F value    Pr(>F)    
Sp        1.315  1.3145     1   7.978  5.0422   0.05504 .  
Date    152.913  7.6456    20 134.063 29.3270 < 2.2e-16 ***
Sp:Date  26.934  1.3467    20 134.063  5.1656 2.245e-09 ***
```

ANOVA (lme with AR1)

```
            numDF denDF  F-value p-value
(Intercept)     1   134 83.68695  <.0001
Sp              1     8  9.17560  0.0163 *
Date           20   134 14.91281  <.0001 ***
Sp:Date        20   134  4.78869  <.0001 ***
```

Daily WU (accumulated flow, kg day-1 m-2) (FIG. 1AB)
Multipe range test(lmer)

```
Sp      Date   emmean    SE   df lower.CL upper.CL .group
 P  08/07/2015  2.761 0.393 16.9  1.93195    3.591            A     GH          ST     Zabcdefghi
 P  09/07/2015  2.592 0.393 16.9  1.76220    3.421           0A    FGH         RSTUVWXYZabcdefghi
 P  10/07/2015  3.285 0.393 16.9  2.45569    4.115                   H           T          efghi
 P  11/07/2015  1.905 0.393 16.9  1.07531    2.734    3    890A  DEFG IJKLMNOPQRS UVWXYZabcd
 P  12/07/2015  1.246 0.393 16.9  0.41691    2.076  123456789  BCDE   IJKLMNOPQ
 P  13/07/2015  1.770 0.393 16.9  0.94058    2.600    3    890A  DEFG IJKLMNOPQRS UVWXYZabcd
 P  14/07/2015  2.475 0.393 16.9  1.64553    3.305          90A   EFGH    MNOPQRSTUVWXYZabcdefghi
 P  15/07/2015  1.895 0.393 16.9  1.06526    2.724    3    890A  DEFG IJKLMNOPQRS UVWXYZabcd
 P  16/07/2015  0.832 0.393 16.9  0.00233    1.661  12345678   BCD    IJKL
 P  17/07/2015  0.382 0.393 16.9 -0.44742    1.212  12 4567    BC
 P  18/07/2015  0.364 0.393 16.9 -0.46546    1.194  12 4567    BC
 P  19/07/2015  0.362 0.393 16.9 -0.46751    1.192  12 4567    BC
 P  20/07/2015  0.439 0.393 16.9 -0.39065    1.268  12 4567    BC
 P  21/07/2015  1.383 0.393 16.9  0.55326    2.212  1234567890 BCDEF  IJKLMNOPQR  UVWXY
 P  22/07/2015  1.862 0.393 16.9  1.03286    2.692    3    890A  DEFG IJKLMNOPQRS UVWXYZabcd
 P  23/07/2015  1.572 0.393 16.9  0.74198    2.401   23   7890ABCDEFG IJKLMNOPQRS UVWXYZabcd
 P  24/07/2015  0.970 0.393 16.9  0.14020    1.799  12345678   BCD    IJKL
 P  25/07/2015  0.197 0.393 16.9 -0.63260    1.027  1  456
 P  26/07/2015  0.193 0.393 16.9 -0.63703    1.022  1  456
 P  27/07/2015  0.178 0.393 16.9 -0.65131    1.008  1  456
 P  28/07/2015  0.147 0.393 16.9 -0.68269    0.976  1  456
 Q  08/07/2015  3.103 0.393 16.9  2.27358    3.933                     JKL NOPQ   UVWXYZabcdefghi
 Q  09/07/2015  2.681 0.393 16.9  1.85190    3.511       6      C     IJKLMNOPQRSTUVWXYZabcdefghi
 Q  10/07/2015  3.665 0.393 16.9  2.83585    4.495                            Q      XY   cd   hi
 Q  11/07/2015  3.035 0.393 16.9  2.20557    3.865                     JKL NOPQ   UVWXYZabcdefghi
 Q  12/07/2015  3.258 0.393 16.9  2.42893    4.088                      KL  OPQ    VWXY abcd fghi
 Q  13/07/2015  3.346 0.393 16.9  2.51621    4.175                       L   PQ     WXY  bcd  ghi
 Q  14/07/2015  3.666 0.393 16.9  2.83609    4.495                            Q      XY   cd   hi
 Q  15/07/2015  3.866 0.411 20.0  3.00787    4.724                                    Y    d    i
 Q  16/07/2015  3.666 0.411 20.0  2.80791    4.524                            Q      XY   cd   hi
 Q  17/07/2015  2.086 0.440 25.6  1.18147    2.990      56     BCDEFGHIJKLMNOP RSTUVW  Zab  efg
 Q  18/07/2015  1.782 0.440 25.6  0.87712    2.686     4567890ABCDEFGHIJK MNO  RSTUV   Za   ef
 Q  19/07/2015  0.702 0.440 25.6 -0.20220    1.607  12345 7890AB DEFGH
 Q  20/07/2015  1.657 0.440 25.6  0.75249    2.561     4567890ABCDEFGHIJ  MN   RSTU    Z    e
 Q  21/07/2015  2.614 0.440 25.6  1.70905    3.518       6      C     IJKLMNOPQRSTUVWXYZabcdefghi
 Q  22/07/2015  2.251 0.440 25.6  1.34617    3.155      56     BCDEFGHIJKLMNOPQRSTUVWX Zabc efgh
 Q  23/07/2015  2.073 0.440 25.6  1.16892    2.978      56     BCDEFGHIJKLMNOP RSTUVW  Zab  efg
 Q  24/07/2015  1.801 0.440 25.6  0.89680    2.706     4567890ABCDEFGHIJK MNO  RSTUV   Za   ef
 Q  25/07/2015  1.953 0.440 25.6  1.04833    2.857     4567890ABCDEFGHIJKLMNOP RSTUVW  Zab  efg
 Q  26/07/2015  1.312 0.440 25.6  0.40725    2.216  1234567890ABCDEFGHI   M    RST
 Q  27/07/2015  0.334 0.440 25.6 -0.57005    1.239  1234  7890A
 Q  28/07/2015 -0.331 0.440 25.6 -1.23553    0.573  123
```

Note: data after irrigation events are highlighted in bold

Degrees-of-freedom method: kenward-roger Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 42 estimates significance level used: alpha = 0.05
NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.

Daily WU (from weight loss, kg day-1 m-2) (FIG. 1AB)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
        Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)    
Sp       0.252  0.2519     1 10.013  0.6629    0.4345    
Date    93.452 11.6815     8 79.029 30.7426 < 2.2e-16 ***
Sp:Date 24.708  3.0885     8 79.029  8.1280 5.978e-08 ***
```

ANOVA (lme with AR1)

```
            numDF denDF   F-value p-value
(Intercept)     1    79 176.71619  <.0001
Sp              1    10   0.36995  0.5566
Date            8    79  37.89947  <.0001 ***
Sp:Date         8    79   7.49627  <.0001 ***
```

Multipe range test(lmer)

```
Sp      Date    emmean    SE   df lower.CL upper.CL .group         
 P  09/07/2015  4.667 0.395 23.4    3.850     5.48              C E
 P  10/07/2015  4.062 0.395 23.4    3.245     4.88           0ABCDE
 P  11/07/2015  1.990 0.395 23.4    1.173     2.81   23  67        
 P  14/07/2015  3.280 0.395 23.4    2.463     4.10     45  890AB D 
 P  16/07/2015  2.056 0.395 23.4    1.239     2.87   234 6789      
 P  18/07/2015  0.582 0.413 27.2   -0.265     1.43  1              
 P  22/07/2015  3.740 0.395 23.4    2.923     4.56      5    0ABCDE
 P  24/07/2015  2.862 0.395 23.4    2.045     3.68    34567890AB D 
 P  26/07/2015  1.391 0.395 23.4    0.574     2.21  12             
 Q  09/07/2015  3.626 0.395 23.4    2.809     4.44       6789 ABCDE
 Q  10/07/2015  3.721 0.395 23.4    2.904     4.54        7 9  BCDE
 Q  11/07/2015  2.427 0.395 23.4    1.610     3.24  123456 8 0A    
 Q  14/07/2015  4.246 0.395 23.4    3.429     5.06               DE
 Q  16/07/2015  4.108 0.395 23.4    3.291     4.92        7 9  BCDE
 Q  18/07/2015  1.705 0.395 23.4    0.888     2.52  12345          
 Q  22/07/2015  2.840 0.395 23.4    2.023     3.66   234567890ABC  
 Q  24/07/2015  2.907 0.395 23.4    2.090     3.72   234567890ABC  
 Q  26/07/2015  2.329 0.395 23.4    1.512     3.15  12345    0     
```

Note: data after irrigation events are highlighted in bold

```
Degrees-of-freedom method: kenward-roger Confidence level used: 0.95 
P value adjustment: tukey method for comparing a family of 18 estimates significance level used: alpha = 0.05 
NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.
```

Xylem WP (MPa) (FIG. 1CD)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
         Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)    
Sp       5.2030  5.2030     1 12.513  43.710 2.034e-05 ***
Date    10.3961  1.2995     8 49.471  10.917 1.046e-08 ***
Sp:Date  2.0397  0.2550     8 49.471   2.142   0.04897 *  
```

ANOVA (lme with AR1)

```
            numDF denDF  F-value p-value
(Intercept)     1    46 902.4205  <.0001
Sp              1    14  37.7099  <.0001 ***
Date            8    46  13.5595  <.0001 ***
Sp:Date         8    46   2.2879  0.0374 .
```

Multipe range test(lmer)

```
Sp      Date    emmean    SE   df lower.CL upper.CL .group 
 P  12/07/2015 -1.488 0.184 59.4    -1.86   -1.119    34567
 P  13/07/2015 -0.701 0.213 60.0    -1.13   -0.275       67
 P  14/07/2015 -0.911 0.150 59.1    -1.21   -0.612      567
 P  17/07/2015 -1.552 0.184 59.4    -1.92   -1.184   23456 
 P  19/07/2015 -1.498 0.184 59.4    -1.87   -1.129   234567
 P  21/07/2015 -0.642 0.184 59.4    -1.01   -0.274        7
 P  23/07/2015 -1.033 0.184 59.4    -1.40   -0.664     4567
 P  25/07/2015 -1.654 0.213 60.0    -2.08   -1.229   23456 
 P  27/07/2015 -1.470 0.130 57.2    -1.73   -1.210    3456 
 Q  12/07/2015 -1.587 0.184 58.9    -1.96   -1.219   234567
 Q  13/07/2015 -1.180 0.184 58.9    -1.55   -0.812     4567
 Q  14/07/2015 -1.566 0.150 59.2    -1.87   -1.266    3456 
 Q  17/07/2015 -1.965 0.184 58.9    -2.33   -1.597  1234   
 Q  19/07/2015 -2.720 0.184 58.9    -3.09   -2.352  1      
 Q  21/07/2015 -1.836 0.213 59.8    -2.26   -1.410  12345  
 Q  23/07/2015 -1.495 0.184 58.9    -1.86   -1.127   234567
 Q  25/07/2015 -2.440 0.184 58.9    -2.81   -2.072  12     
 Q  27/07/2015 -2.223 0.164 59.1    -2.55   -1.894  123    
```

Note: data after irrigation events are highlighted in bold

```
Degrees-of-freedom method: kenward-roger Confidence level used: 0.95 
P value adjustment: tukey method for comparing a family of 18 estimates significance level used: alpha = 0.05 
NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.
```

Soil minimum WP (MPa), log-transformed (FIG. 1CD)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
        Sum Sq Mean Sq NumDF  DenDF F value Pr(>F)
Sp       0.015  0.0147     1 14.684  0.0805 0.7805
Date    43.747  6.2496     7 59.281 34.3070 <2e-16 ***
Sp:Date  1.994  0.2848     7 59.281  1.5635 0.1642
```

ANOVA (lme with AR1)

```
            numDF denDF  F-value p-value
(Intercept)     1    56 16.79327  0.0001
Sp              1    14  0.05738  0.8142
Date            7    56 36.09813  <.0001 ***
Sp:Date         7    56  1.63787  0.1438
```

Multipe range test(lmer)

```
 Date       response   SE   df    lower.CL upper.CL .group
 12/07/2015    0.848 0.1350 69.7    0.617    1.165      3
 13/07/2015    0.293 0.0329 67.4    0.234    0.366   1
 17/07/2015    1.296 0.2064 69.7    0.943    1.780     34
 19/07/2015    1.803 0.2871 69.7    1.312    2.477      4
 21/07/2015    0.382 0.0443 67.8    0.303    0.481   12
 23/07/2015    0.687 0.1094 69.7    0.500    0.944    23
 25/07/2015    1.224 0.1949 69.7    0.891    1.682     34
 27/07/2015    1.863 0.2163 67.8    1.477    2.348      4
```

Note: data after irrigation events are highlighted in bold

```
Results are averaged over the levels of: Sp Degrees-of-freedom method: kenward-roger Confidence level used: 0.95
Intervals are back-transformed from the log scale
P value adjustment: tukey method for comparing a family of 8 estimates Tests are performed on the log scale
significance level used: alpha = 0.05
NOTE: If two or more means share the same letter,we cannot show them to be different. But we also did not show them to be the same.
```

Soil maximum WP (MPa), log-transformed (FIG. 1CD)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
        Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)
Sp       0.012  0.0124     1 14.680  0.1070 0.74824
Date    46.656  6.6652     7 59.505 57.3447 < 2e-16 ***
Sp:Date  1.916  0.2737     7 59.505  2.3548 0.03423 *
```

ANOVA (lme with AR1)

```
            numDF denDF   F-value p-value
(Intercept)     1    56 142.88851  <.0001
Sp              1    14   0.04947  0.8272
Date            7    56  69.20032  <.0001 ***
Sp:Date         7    56   2.97518  0.0100 *
```

Multipe range test(lmer)

```
Sp     Date    response     SE   df lower.CL upper.CL .group
 P  12/07/2015    0.562 0.1046 69.1    0.388    0.815    3456
 P  13/07/2015    0.215 0.0285 62.0    0.165    0.281  12
 P  17/07/2015    0.725 0.1348 69.1    0.500    1.050     4567
 P  19/07/2015    0.987 0.1836 69.1    0.681    1.430      567
 P  21/07/2015    0.178 0.0235 62.0    0.136    0.231  1
 P  23/07/2015    0.460 0.0855 69.1    0.317    0.666   2345
 P  25/07/2015    1.132 0.2106 69.1    0.781    1.641      567
 P  27/07/2015    1.303 0.1729 62.0    1.000    1.699        7
 Q  12/07/2015    0.553 0.1030 68.9    0.381    0.802    345
 Q  13/07/2015    0.195 0.0259 62.0    0.150    0.255  1
 Q  17/07/2015    0.752 0.1400 68.9    0.518    1.090     4567
 Q  19/07/2015    1.400 0.2608 68.9    0.966    2.031       67
 Q  21/07/2015    0.257 0.0363 64.2    0.193    0.341  123
 Q  23/07/2015    0.372 0.0694 68.9    0.257    0.540  1234
 Q  25/07/2015    0.536 0.0998 68.9    0.370    0.777    345
 Q  27/07/2015    1.340 0.1897 64.2    1.010    1.778        7
```

Note: data after irrigation events are highlighted in bold

Results are averaged over the levels of: Sp Degrees-of-freedom method: kenward-roger Confidence level used: 0.95
Intervals are back-transformed from the log scale
P value adjustment: tukey method for comparing a family of 16 estimates Tests are performed on the log scale
significance level used: alpha = 0.05
NOTE: If two or more means share the same letter,we cannot show them to be different. But we also did not show them to be the same.

FIGURE 2

An (µmol m-2 s-1)
***vs* Maximum Soil WP (MPa) (FIG. 2A)**

```
                Df Sum Sq Mean Sq F value    Pr(>F)
Sp               1 327.84  327.84 16.4242 0.0002897 ***
log(-SWPmax)     1 274.38  274.38 13.7459 0.0007653 ***
Sp:log(-SWPmax)  1   0.03    0.03  0.0013 0.9714050
Residuals       33 658.70   19.96
```

gs (mmol m-2 s-1)
***vs* Maximum Soil WP (MPa) (FIG. 2B)**

```
                Df Sum Sq Mean Sq F value  Pr(>F)
Sp               1 135946  135946  6.2632 0.01746 *
log(-SWPmax)     1 209419  209419  9.6483 0.00388 **
Sp:log(-SWPmax)  1   2101    2101  0.0968 0.75768
Residuals       33 716278   21705
```

Xylem WP (MPa)
vs Maximum Soil WP (MPa) (Fig. 2C)

```
                Df Sum Sq Mean Sq F value    Pr(>F)
Sp               1 5.2416  5.2416 41.8702 3.239e-07 ***
log(-SWPmax)     1 4.3998  4.3998 35.1455 1.505e-06 ***
Sp:log(-SWPmax)  1 0.0809  0.0809  0.6463    0.4276
Residuals       31 3.8808  0.1252
```

FIGURE S5

An (µmol m-2 s-1)
***vs* VPD (kPa) (FIG. S5A)**

```
           Df Sum Sq Mean Sq F value   Pr(>F)
Sp          1 253.81  253.81  12.103 0.001186 **
VPDhour     1 390.34  390.34  18.614 9.51e-05 ***
Sp:VPDhour  1  27.34   27.34   1.304 0.259953
Residuals  42 880.74   20.97
```

gs (mmol m-2 s-1)
***vs* VPD (kPa) (Fig S5B)**

```
           Df Sum Sq Mean Sq F value    Pr(>F)
Sp          1 164462  164462  8.4402  0.005832 **
VPDhour     1 376715  376715 19.3329 7.341e-05 ***
Sp:VPDhour  1  58553   58553  3.0049  0.090348 .
Residuals  42 818397   19486
```

Alternative models for gas exchange, including VPD*SWP interaction

An (µmol m-2 s-1)

_vs_ Maximum Soil WP (MPa) & VPD (kPa)

```
                        Df Sum Sq Mean Sq F value    Pr(>F)    
Sp                       1 327.84  327.84 42.8457 3.607e-07 ***
log(-SWPmax)             1 274.38  274.38 35.8588 1.645e-06 ***
VPDhour                  1 336.53  336.53 43.9812 2.860e-07 ***
Sp:log(-SWPmax)          1   2.33    2.33  0.3048  0.585102    
Sp:VPDhour               1  19.53   19.53  2.5523  0.120974    
log(-SWPmax):VPDhour     1  14.44   14.44  1.8874  0.180021    
Sp:log(-SWPmax):VPDhour  1  64.00   64.00  8.3643  0.007184 ** 
Residuals               29 221.90    7.65                      
```

gs (mmol m-2 s-1)

_vs_ Maximum Soil WP (MPa) & VPD (kPa)

```
                        Df Sum Sq Mean Sq F value    Pr(>F)    
Sp                       1 135946  135946 12.0775 0.0016267 ** 
log(-SWPmax)             1 209419  209419 18.6048 0.0001699 ***
VPDhour                  1 318245  318245 28.2729 1.051e-05 ***
Sp:log(-SWPmax)          1     16      16  0.0014 0.9703890    
Sp:VPDhour               1  47141   47141  4.1880 0.0498715 *  
log(-SWPmax):VPDhour     1  25522   25522  2.2674 0.1429355    
Sp:log(-SWPmax):VPDhour  1   1026    1026  0.0911 0.7649253    
Residuals               29 326429   11256
```

FIGURE 3 & S6

Kh (mmol m-2 s-1 MPa-1)

vs Maximum Soil WP (MPa) (FIG. 3A)

```
          Df  Sum Sq Mean Sq F value   Pr(>F)
Sp         1  3.4015  3.4015  3.5368 0.084503 .
SWPmax     1 29.5574 29.5574 30.7335 0.000127 ***
Sp:SWPmax  1  3.5660  3.5660  3.7079 0.078178 .
Residuals 12 11.5408  0.9617
```

vs Xylem WP (MPa) (FIG. 3B)

```
          Df  Sum Sq Mean Sq F value    Pr(>F)
Sp         1  3.4015  3.4015  3.1278 0.1023538
XWP        1 26.9055 26.9055 24.7409 0.0003231 ***
Sp:XWP     1  4.7088  4.7088  4.3300 0.0595323 .
Residuals 12 13.0499  1.0875
```

Alternative models for Kh, including drought cycle

Kh (mmol m-2 s-1 MPa-1)

vs Maximum Soil WP (MPa) & cycle (FIG. S6)

```
                  Df  Sum Sq Mean Sq F value    Pr(>F)
Sp                 1  3.4015  3.4015  7.4748  0.025686 *
SWPmax             1 29.5574 29.5574 64.9524 4.141e-05 ***
Cycle              1  2.4420  2.4420  5.3663  0.049185 *
Sp:SWPmax          1  2.9334  2.9334  6.4461  0.034769 *
Sp:Cycle           1  6.0812  6.0812 13.3633  0.006442 **
SWPmax:Cycle       1  0.0039  0.0039  0.0086  0.928270
Sp:SWPmax:Cycle    1  0.0059  0.0059  0.0129  0.912403
Residuals          8  3.6405  0.4551
```

vs Xylem WP (MPa) & cycle

```
                Df  Sum Sq Mean Sq F value    Pr(>F)
Sp               1  3.4015  3.4015  3.7022 0.0905384 .
XWP              1 26.9055 26.9055 29.2843 0.0006372 ***
Cycle            1  3.1861  3.1861  3.4678 0.0995948 .
Sp:XWP           1  5.9488  5.9488  6.4748 0.0344654 *
Sp:Cycle         1  0.9573  0.9573  1.0419 0.3372504
XWP:Cycle        1  0.1992  0.1992  0.2168 0.6538818
Sp:XWP:Cycle     1  0.1172  0.1172  0.1275 0.7302333
Residuals        8  7.3502  0.9188
```

FIGURE 4

Xylem d2H (‰) in Deuterium-labelled plants (FIG. 4AB)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
        Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)    
Sp        5207  5207.1     1    45 11.2250  0.001641 ** 
Date    121214 15151.7     8    45 32.6625 2.819e-16 ***
Sp:Date  11366  1420.8     8    45  3.0627  0.007711 ** 
```

ANOVA (lme with AR1)

```
            numDF denDF   F-value p-value
(Intercept)     1    36 150.39862  <.0001
Sp              1     9   6.59643  0.0303 *
Date            8    36  31.77588  <.0001 ***
Sp:Date         8    36   3.01967  0.0106 *
```

Multipe range test(lmer)

```
Sp      Date    emmean   SE  df lower.CL upper.CL .group
 P  12/07/2015 -60.736 15.7 45  -92.291   -29.18  1    
 P  13/07/2015 -17.595 10.8 45  -39.285     4.09  12   
 P  14/07/2015  79.963 10.8 45   58.273   101.65      56
 P  17/07/2015 104.651 10.8 45   82.961   126.34       6
 P  19/07/2015  92.445 10.8 45   70.755   114.13      56
 P  21/07/2015  77.846 10.8 45   56.156    99.54     456
 P  23/07/2015  21.362 10.8 45   -0.328    43.05   234  
 P  25/07/2015  61.351 10.8 45   39.661    83.04    3456
 P  27/07/2015  39.220 10.8 45   17.531    60.91   2345 
 Q  12/07/2015 -60.782 15.5 45  -92.083   -29.48  1     
 Q  13/07/2015  -0.025 10.8 45  -21.715    21.66  12    
 Q  14/07/2015  86.522 10.8 45   64.832   108.21      56
 Q  17/07/2015  82.905 10.8 45   61.215   104.59      56
 Q  19/07/2015  83.792 12.4 45   58.747   108.84      56
 Q  21/07/2015  17.355 10.8 45   -4.335    39.05   23   
 Q  23/07/2015   5.491 10.8 45  -16.199    27.18  123   
 Q  25/07/2015  -1.295 12.6 45  -26.598    24.01  12    
 Q  27/07/2015   9.019 22.3 45  -35.887    53.93  12345 
```

Note: data after irrigation events are highlighted in bold

```
Degrees-of-freedom method: kenward-roger Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 18 estimates significance level used: alpha = 0.05
NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.
```

Xylem d18O (‰) in Depleted-labelled plants (FIG. 4CD)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
         Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)    
Sp       22.600  22.600     1    46  4.4224   0.04097 *  
Date    289.497  36.187     8    46  7.0812 4.649e-06 ***
Sp:Date  10.981   1.373     8    46  0.2686   0.97298    
```

ANOVA (lme with AR1)

```
            numDF denDF  F-value p-value
(Intercept)     1    37 598.3380  <.0001
Sp              1     9   3.8283  0.0821 .
Date            8    37   6.6110  <.0001 ***
Sp:Date         8    37   0.2892  0.9653
```

Multipe range test(lmer)

```
Sp      Date    emmean   SE df lower.CL upper.CL .group
 P  12/07/2015  -5.20 1.64 46    -8.50    -1.90  123
 P  13/07/2015  -5.45 1.32 46    -8.11    -2.80  123
 P  14/07/2015  -5.90 1.13 46    -8.17    -3.62  123
 P  17/07/2015  -5.51 1.13 46    -7.79    -3.24   23
 P  19/07/2015  -3.87 1.13 46    -6.14    -1.59    3
 P  21/07/2015  -8.14 1.13 46   -10.41    -5.86  123
 P  23/07/2015 -11.48 1.13 46   -13.76    -9.21  1
 P  25/07/2015  -8.04 1.32 46   -10.70    -5.38  123
 P  27/07/2015  -7.69 1.13 46    -9.96    -5.41  123
 Q  12/07/2015  -6.52 1.64 46    -9.82    -3.23  123
 Q  13/07/2015  -6.55 1.13 46    -8.83    -4.28  123
 Q  14/07/2015  -6.84 1.13 46    -9.12    -4.57  123
 Q  17/07/2015  -5.92 1.13 46    -8.20    -3.65  123
 Q  19/07/2015  -5.70 1.31 46    -8.33    -3.07  123
 Q  21/07/2015 -11.11 1.13 46   -13.39    -8.83  12
 Q  23/07/2015 -11.55 1.13 46   -13.82    -9.27  1
 Q  25/07/2015  -9.24 1.32 46   -11.89    -6.58  123
 Q  27/07/2015  -8.82 1.13 46   -11.10    -6.55  123
```

Note: data after irrigation events are highlighted in bold

Degrees-of-freedom method: kenward-roger Confidence level used: 0.95

P value adjustment: tukey method for comparing a family of 18 estimates significance level used: alpha = 0.05

NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.

Xylem d2H (‰) in Depleted-labelled plants (FIG. 4CD)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
         Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)    
Sp        125.7  125.68     1    46  0.6693    0.4175    
Date    15819.9 1977.48     8    46 10.5309 3.027e-08 ***
Sp:Date   826.1  103.26     8    46  0.5499    0.8126    
```

ANOVA (lme with AR1)

```
            numDF denDF   F-value p-value
(Intercept)     1    37 1750.7756  <.0001
Sp              1     9    0.6979  0.4251
Date            8    37   10.3629  <.0001 ***
Sp:Date         8    37    0.5536  0.8082
```

Multipe range test(lmer)

```
Sp      Date   emmean   SE df lower.CL upper.CL .group
 P  12/07/2015  -60.0 9.94 46    -80.0    -40.0  1234
 P  13/07/2015  -55.9 8.00 46    -72.0    -39.7   234
 P  14/07/2015  -60.6 6.85 46    -74.4    -46.9   234
 P  17/07/2015  -59.7 6.85 46    -73.4    -45.9   234
 P  19/07/2015  -53.0 6.85 46    -66.8    -39.2     4
 P  21/07/2015  -74.2 6.85 46    -88.0    -60.4  1234
 P  23/07/2015 -100.9 6.85 46   -114.6    -87.1  1
 P  25/07/2015  -80.1 8.00 46    -96.2    -64.0  1234
 P  27/07/2015  -89.8 6.85 46   -103.6    -76.0  123
 Q  12/07/2015  -63.2 9.92 46    -83.2    -43.3  1234
 Q  13/07/2015  -61.3 6.85 46    -75.1    -47.5   234
 Q  14/07/2015  -61.6 6.85 46    -75.4    -47.8   234
 Q  17/07/2015  -58.6 6.85 46    -72.4    -44.9   234
 Q  19/07/2015  -55.5 7.91 46    -71.4    -39.6    34
 Q  21/07/2015  -94.2 6.85 46   -108.0    -80.4  12
 Q  23/07/2015  -99.2 6.85 46   -113.0    -85.4  1
 Q  25/07/2015  -82.2 8.00 46    -98.3    -66.0  1234
 Q  27/07/2015  -84.2 6.85 46    -98.0    -70.4  1234
```

Note: data after irrigation events are highlighted in bold

```
Degrees-of-freedom method: kenward-roger Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 18 estimates significance level used: alpha = 0.05
NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.
```

FIGURE 5

Xylem WC (%)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
        Sum Sq Mean Sq NumDF  DenDF F value    Pr(>F)    
Sp      1787.5 1787.54     1 15.945 28.6988 6.491e-05 ***
Date    1523.4  190.43     8 88.112  3.0572  0.004393 ** 
Sp:Date 1268.4  158.55     8 88.112  2.5455  0.015254 *  
```

ANOVA (lme with AR1)

```
            numDF denDF  F-value p-value
(Intercept)     1    97 6072.625  <.0001
Sp              1    14   31.204  0.0001 ***
Date            1    97    9.419  0.0028 **
Sp:Date         1    97    3.578  0.0615 .
```

Multipe range test(lmer)

```
Sp      Date   emmean   SE df lower.CL upper.CL .group
 P  12/07/2015   59.4 5.69 97     48.1     70.7  1234
 P  13/07/2015   66.8 3.00 97     60.8     72.7     4
 P  14/07/2015   65.4 3.24 97     59.0     71.9    34
 P  17/07/2015   61.9 2.80 97     56.4     67.5   234
 P  19/07/2015   62.7 3.00 97     56.8     68.7   234
 P  21/07/2015   63.3 3.24 97     56.9     69.7   234
 P  23/07/2015   62.9 2.80 97     57.3     68.4   234
 P  25/07/2015   61.1 2.80 97     55.5     66.6   234
 P  27/07/2015   64.2 3.00 97     58.2     70.1   234
 Q  12/07/2015   59.4 3.99 97     51.5     67.4  1234
 Q  13/07/2015   56.1 2.99 97     50.2     62.1  1234
 Q  14/07/2015   67.5 2.80 97     62.0     73.1     4
 Q  17/07/2015   53.3 3.00 97     47.3     59.2  1234
 Q  19/07/2015   51.0 2.80 97     45.4     56.5  123
 Q  21/07/2015   42.7 3.56 97     35.7     49.8  1
 Q  23/07/2015   49.3 2.99 97     43.4     55.3  12
 Q  25/07/2015   60.6 3.56 97     53.5     67.6  1234
 Q  27/07/2015   51.5 3.56 97     44.5     58.6  1234
```

Note: data after irrigation events are highlighted in bold

```
Degrees-of-freedom method: kenward-roger Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 18 estimates significance level used: alpha = 0.05
NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.
```

Soil WC (%)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
        Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)    
Sp        0.33   0.330     1 14.341  0.1746 0.68229    
Date    864.31 123.472     7 59.206 65.2706 < 2e-16 ***
Sp:Date  31.99   4.571     7 59.206  2.4162 0.03027 *  
```

ANOVA (lme with AR1)

```
            numDF denDF  F-value p-value
(Intercept)     1    68 5925.516  <.0001
Sp              1    14    0.002  0.9638
Date            1    68   34.805  <.0001 ***
Sp:Date         1    68    0.050  0.8238
```

Multipe range test(lmer)

```
Sp      Date  emmean    SE   df lower.CL upper.CL .group
 P  12/07/2015   15.4 0.741 69.4     14.0     16.9  1234 
 P  13/07/2015   19.9 0.525 64.4     18.8     20.9      5
 P  17/07/2015   13.7 0.741 69.4     12.2     15.1  123  
 P  19/07/2015   13.1 0.741 69.4     11.6     14.6  123  
 P  21/07/2015   20.6 0.525 64.4     19.5     21.6      5
 P  23/07/2015   16.0 0.741 69.4     14.6     17.5   234 
 P  25/07/2015   12.9 0.741 69.4     11.4     14.4  123  
 P  27/07/2015   12.6 0.525 64.4     11.5     13.6  1    
 Q  12/07/2015   15.4 0.741 69.2     13.9     16.8  123  
 Q  13/07/2015   21.2 0.525 64.4     20.1     22.2      5
 Q  17/07/2015   13.8 0.741 69.2     12.3     15.3  123  
 Q  19/07/2015   12.4 0.741 69.2     10.9     13.8  12   
 Q  21/07/2015   18.7 0.561 66.0     17.6     19.8     45
 Q  23/07/2015   16.5 0.741 69.2     15.0     18.0    34 
 Q  25/07/2015   15.3 0.741 69.2     13.8     16.8  123  
 Q  27/07/2015   12.4 0.561 66.0     11.3     13.5  1    
```

Note: data after irrigation events are highlighted in bold

```
Degrees-of-freedom method: kenward-roger Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 16 estimates significance level used: alpha = 0.05
NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.
```

Alternative model including the soil profile WC (%)

ANOVA (lmer)

```
Type III Analysis of Variance Table with Satterthwaite's method
               Sum Sq Mean Sq NumDF   DenDF  F value    Pr(>F)
Sp               1.50    1.50     1  12.745   0.5703   0.46389
Depth           19.15    9.57     2 177.365   3.6452   0.02809 *
Date          2349.50  335.64     7 177.330 127.7847 < 2.2e-16 ***
Sp:Depth        12.40    6.20     2 177.365   2.3613   0.09725 .
Sp:Date         95.33   13.62     7 177.330   5.1848 2.158e-05 ***
Depth:Date      39.29    2.81    14 177.365   1.0683   0.38928
Sp:Depth:Date   13.50    0.96    14 177.365   0.3671   0.98225
```

Multipe range test(lmer)

```
Sp Depth emmean    SE   df lower.CL upper.CL .group
 P  05      15.5 0.379 30.4     14.7     16.3  12
 P  10      15.3 0.379 30.4     14.6     16.1  12
 P  15      15.6 0.379 30.4     14.8     16.4  12
 Q  05      16.5 0.379 30.4     15.7     17.3   2
 Q  10      15.2 0.379 30.4     14.4     16.0  1
 Q  15      15.7 0.379 30.4     14.9     16.5  12

Degrees-of-freedom method: kenward-roger Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 6 estimates significance level used: alpha = 0.05
NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.
```

```
 Sp     Date    emmean    SE    df lower.CL upper.CL .group
  P  12/07/2015   15.0 0.636 113.3     13.7     16.2  12345
  P  13/07/2015   19.8 0.447  52.8     18.9     20.7       67
  P  17/07/2015   13.7 0.565  88.3     12.5     14.8  1234
  P  19/07/2015   13.1 0.565  88.3     12.0     14.2  123
  P  21/07/2015   20.6 0.447  52.8     19.7     21.5       67
  P  23/07/2015   16.1 0.565  88.3     14.9     17.2     45
  P  25/07/2015   12.9 0.565  88.3     11.7     14.0  12
  P  27/07/2015   12.7 0.447  52.8     11.8     13.6  12
  Q  12/07/2015   16.1 0.636 113.3     14.8     17.3    345
  Q  13/07/2015   21.2 0.447  52.8     20.3     22.1        7
  Q  17/07/2015   13.7 0.565  88.3     12.6     14.8  1234
  Q  19/07/2015   12.4 0.565  88.3     11.3     13.6  1
  Q  21/07/2015   18.7 0.447  52.8     17.8     19.6       6
  Q  23/07/2015   16.6 0.565  88.3     15.5     17.7      5
  Q  25/07/2015   15.2 0.565  88.3     14.1     16.3   2345
  Q  27/07/2015   12.5 0.447  52.8     11.6     13.4  1
```

Note: data after irrigation events are highlighted in bold

```
Degrees-of-freedom method: kenward-roger Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 16 estimates significance level used: alpha = 0.05
NOTE: If two or more means share the same letter, we cannot show them to be different. But we also did not show them to be the same.
```