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# Short-term dynamics of evaporative enrichment of xylem water in woody stems: implications for ecohydrology

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**SUMMARY**

In ecohydrology, it is generally assumed that xylem water reflects the water source used by plants. Several studies have reported isotopic enrichment within woody tissues, particularly during dormancy periods or after long periods of inactivity. However, little is known about the short-term dynamics of this process. Here we assessed the magnitude and dynamics of xylem isotopic enrichment in suberized twigs of pines and oaks. We performed a series of laboratory experiments, in which we monitored hourly changes in water content and isotopic composition under two contrasting scenarios of sap flow restriction. Firstly, we simulated the effect of extreme hydraulic failure by excising twigs to restrict sap flow, while sealing the wounds to ensure that water loss took place only through the leaves or bark, as would be the case of evaporation in attached stems. Secondly, we studied the effect of reduced leaf transpiration by darkening with aluminium foil all the leaves of healthy, well-watered saplings growing in pot conditions. We found evidence of fast evaporative enrichment in metabolically-active stems, as a consequence of a temporal decline in sap flow rates, and not necessarily linked to a traceable decline in stem water content. The excision experiments showed significant isotopic changes (*ca.* +1 ‰ in oxygen) appearing in less than one hour. Similarly, the pot experiment showed a progressive increase in isotope composition (up to +8‰ in oxygen in three days-cycle) when the leaves were covered, and a rapid recovery to initial values when sap flow rates were re-established (Fig. 4). We conclude that evaporative enrichment of xylem water in stems is a highly dynamic process that may cause significant effects even during short periods of restricted water flow. This has important implications for the study of plant water uptake, as well as for ecosystem- and global-scale hydrological models.

**Keywords:** deuterium excess, drought, evaporative enrichment, humidity, oxygen isotope composition, sap flow, transpiration, source water.

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**INTRODUCTION**

The analysis of the isotopic composition of xylem water has been extensively applied to determine the source of water used by plants, providing an useful insight into many ecohydrological processes (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. For example, the different contribution of seasonal precipitation to soils, streams and groundwater lead to substantial isotopic differences among these water pools (see e.g. Gat 1996, Tang and Feng 2001, Máguas et al. 2011). Additionally, the preferential loss of light isotopes during evaporation causes a progressive isotopic enrichment of the liquid phase at the site of evaporation (Craig and Gordon 1965), and creates strong isotopic gradients along the soil profile during dry periods (Allison et al. 1983). Taking advantage of this variability, isotopic tracing has revealed the use of contrasting water sources among adjacent plants, both at the inter-specific (e.g. Sternberg and Swart 1987, Filella and Peñuelas 2004, Máguas et al. 2011) and intra-specific level (Dawson 1993, Voltas et al. 2015). Isotopic studies have also shown that water uptake is a highly dynamic process (Brandes et al. 2007, Máguas et al. 2011, Ellsworth and Sternberg 2014, Bertrand et al. 2014), often involving complex ecological interactions like competition (Dawson 1993, Comas et al. 2015) or facilitation through hydraulic redistribution (Filella and Peñuelas 2004, Prieto et al. 2012).

Isotopic tracing of plant water relies on two important working premises (Ehleringer and Dawson 1992). First of all, it is generally accepted that there is no fractionation during the uptake process by roots, except for some xerophytic and halophytic species (Lin et al. 1993, Ellsworth and Williams 2007). The second basic assumption is that there is no fractionation during the transport of water along the xylem, from the roots to the upper-canopy stems, as it is mainly a mass flow movement. In the leaves, the magnitude of isotopic enrichment is a function of 1) the humidity gradient between the site of evaporation and the atmosphere and 2) the isotopic composition of atmospheric water vapour and 3) transpiration rates (Dongmann et al. 1974, Farquhar and Lloyd 1993, for a recent review see Cernusak et al. 2016). Similarly, if water loss occur through the bark, stems would display an evaporative enrichment of source water proportional to the humidity gradient, and counteracted by the extent of transpiration flow (Dawson and Ehleringer 1993). In this sense, Dawson and Ehleringer (1993) clearly proved the existence of xylem isotopic enrichment for green, unsuberized stems. Similarly, some authors have also described evaporative enrichment in suberized stems of deciduous plants during leafless periods, when a long-lasting water stagnation in the xylem leads to partial desiccation (Phillips and Ehleringer 1995, Treydte et al. 2014, Bertrand et al. 2014, del Castillo et al. 2016). Only recently, Ellsworth and Sternberg (2014) tested the effect of manipulative defoliation in the evergreen oak *Quercus virginiana*: they observed a significant enrichment in xylem water 4 weeks after defoliation, diverging from soil values until the new leaves were formed. However, the short-term (hourly) dynamic of evaporative enrichment in active, leaved branches is still unknown. This information is crucial to provide a proper interpretation of daily, and even seasonal changes in water uptake patterns, based on isotope measurements (see e.g. Filella and Peñuelas 1999, Bertrand et al. 2014, Cernusak et al. 2016, Voltas et al. 2015).

Besides bark transpiration, it has been suggested that an additional cause for xylem enrichment and xylem-soil decoupled observations may be the mixture of xylem water with enriched water from the leaf (Brandes et al. 2007, Ellsworth and Williams 2007). This may occur directly through the back-diffusion of enriched water from the leaf veins to the twig xylem (Dawson and Ehleringer 1993, Farquhar and Lloyd 1993), or by means of water

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exchange between xylem and phloem tissues (Cernusak et al. 2005, Brandes et al. 2007). In this regard, manipulative experiments in leaved branches are necessary to characterize not only the short-term dynamic of evaporative enrichment during the growing cycle, but also to disentangle the role of leaves in stem isotopic enrichment.

The general aim of the current study was to determine the short-term dynamics of isotopic enrichment of xylem water under conditions of limited sap flow in active, suberized and leaved stems. For this purpose, we conducted a series of laboratory experiments, representative of two alternative scenarios of limited sap flow. On the one hand, twig excision experiments were used to emulate the effect of severe hydraulic failure (i.e. source-limited), therefore associated to progressive branch dehydration. On the other, we tested the effect of sap flow restrictions under stomatal-limited transpiration (i.e. shadowed leaves or drought limitation), by temporarily covering the leaves of well-watered saplings. Three species were selected as representative of conifer and broadleaves: the Scots pine (*Pinus sylvestris*), and two closely-related species of marcescent oak (*Quercus faginea* and *Quercus subpyrenaica*). Our first objective was to determine the magnitude and timing of the isotopic enrichment in suberized stems. Considering stem water flow as a highly dynamic process, we predicted evaporative enrichment to fluctuate rapidly following a short-term sap flow restriction. Our second objective was to define the processes behind isotopic enrichment in the stem, particularly the relative contribution of stem evaporation and back diffusion of leaf water. Finally, we aimed to provide recommendations for future sampling, assessing the effect of different variables that could affect isotopic enrichment, such as twig diameter or xylem water content, and to discuss the implications of evaporative enrichment of xylem water for the interpretation of ecohydrological processes.

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## METHODS

### Twig excision experiments

In order to assess evaporative enrichment under hydraulic limitation of sap flow, we monitored the evolution of isotope composition in excised twigs. After cutting the twigs, the input of water is instantly restricted, leading to a progressive dehydration of the xylem tissues, with the subsequent loss of conductivity and downregulation of stomatal conductance (Sperry et al. 2002). Directly after excision, the cut was immediately sealed with silicon to prevent water loss through the wound. In this way, we aimed to emulate the effect of severe hydraulic failure in attached stems, while allowing to register weight losses and changes in water content.

Twig samples were collected in summer 2013 and 2014 from adult trees growing in a pine-oak mixed stand located in the Spanish Pre-Pyrenees (Boalar Forest - Jaca - Huesca, 30T 693606 4714041). The species sampled were Scots pine (*Pinus sylvestris* L.) and a Mediterranean marcescent oak (*Quercus subpyrenaica* Villar). After sealing the cut, twigs were left drying under ambient conditions for different periods of time, and then a piece of xylem (1.75 ± 0.43 mm in diameter) was sampled as described below (see section "sample collection and water extraction"). In 2013 (from now on referred as "medium-term excision experiment") the drying process ranged from 1.5 to 68 hours, whereas in 2014 ("short-term excision experiment") we focused on the early stages of hydraulic limitation (from 0.5 to 8.75 hours). In order to assess the potential effect of back-diffused leaf water on xylem isotopic enrichment, we applied three different treatments to the twigs in the medium-term experiment: 1) twigs with uncovered leaves; 2) twigs with leaves covered with aluminium foil to restrict transpiration and 3) defoliated twigs. In the latter, leaf scars were sealed with silicon immediately after defoliation. In the short-term experiment, we only applied the two first treatments (leaves covered and leaves uncovered), in order to increase the level of replication. Percentage of water loss (% weight loss) was calculated as the difference between the initial fresh weight, i.e. after completing the treatment preparation, and the fresh weight at the time of xylem sampling. During the experiments, temperature and relative humidity were continuously monitored using EL-USB-2 datalogger from EasyLog (Lascar Electronics Ltd).

### Pot experiment

The effect of restricted sap flow rates as a consequence of limited transpiration was assessed during summer 2014 in a pot experiment located at the Experimental Fields of the Universitat de Lleida. Commercial saplings of Scots pine and a marcescent oak (*Quercus faginea* L.) were used for this experiment. Three saplings per species (3 and 5 years old, for oaks and pines, respectively) were originally cultivated in nursery containers with standard substrate. Six months before the experiment, they were transplanted to 20-liter pots filled with a forest loamy soil, collected in the same area of the field campaigns. To minimize isotopic fractionation due to soil evaporative enrichment, the pots were kept near field capacity throughout the experiment, and the soil surface was covered with aluminium foil. A separate test with defoliated trees (one per species) in aluminium-covered pots showed negligible water losses after 12 consecutive days without watering (less than 0.8% weight loss). Well-watered, leaved trees kept under the same environmental conditions showed *ca.* 2-3% daily water loss, i.e. 30-45 fold larger than in defoliated trees. Tap water with nearly constant isotopic signature was used for irrigation (-9.59 ± 0.19 for $\delta^{18}O$ and -65.92 ± 1.52 for $\delta^{2}H$, measured from June to September 2014). During the experiment, we followed a 3-day cycle. In the first cycle (reference), plants remained uncovered until the third night, when all the leaves

were covered with aluminium foil. The leaves remained covered until the third night, when the aluminium foil was removed. After this, sampling continued for three days (recovery). Twigs were sampled twice everyday (at predawn and midday), leaves were sampled simultaneously from the last reference day to the end of the experiment. Soil cores (15 mm diameter × *ca*. 200 mm height) were sampled during the last day of each experimental phase, pooling the soil from the whole profile, after removing the upper 2 cm. Throughout the experiment, sap flow was monitored with "baby gauges" SF62, coupled to the Sap flow meter T4.2 (EMS Brno, Brno, Czech Republic). These data were downloaded and analyzed with Mini32 software ver.403.34 (EMS Brno, Brno, Czech Republic). Xylem diameter (without bark and phloem) was about 1.50 ± 0.59 mm. Climatic data (temperature and relative humidity) was obtained from a nearby (14 km) public meteorological station (Raimat - Lleida).

**Sample collection and water extraction**

For xylem sampling, bark and phloem were removed and the peeled xylem was immediately placed in air-tight glass tubes (Duran GL-18). The tubes were frozen on dry ice (twig excision experiment) or liquid nitrogen (pot experiment) directly after sampling, and kept frozen until processing. Water extraction was performed by cryogenic vacuum distillation (Dawson and Ehleringer 1993) at the Dept. of Crop and Forest Sciences, Universitat de Lleida (Spain). Xylem and soil water was 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™ 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 were performed to ensure complete distillation (see e.g. Martín-Gómez et al. 2014; Palacio et al. 2014, Orlowski et al. 2016). All xylem samples were weighted before and after distillation, in order to calculate xylem water content (Xylem WC), and measured to determine mean twig diameter. In a preliminary test with 13 soil and 69 xylem samples, we compared the weight just after distillation, and after oven-drying at 60ºC for 24h. 93% of the samples gained weight in the oven-drier, indicating that they were dry enough to rehydrate under such conditions, with an average significant increase of 1.33±0.18% ($P$<0.0001) in xylem samples, and a marginally significant gain in soils (0.24±0.11%; $P$=0.062). For a subset of the soil samples used in this study, we also tested alternative distillation times, showing no significant differences in WC between the samples distilled at 120°C for 2h and 5h (13.0±1.4% and 13.3±2.6%, respectively; N=9).

**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). The analyser was coupled to a high-precision vaporiser (A0211) through a Micro-Combustion Module™ (MCM), integrated in-line between the vaporiser and the analyser. The MCM removes the contaminants through oxidation, in a way that only pure water arrives to the analyser (Picarro 2012). For each sample, six replicates of 1 µl were injected into the vaporizer, keeping the last three injections for calculation, which showed negligible memory effects and rather homogeneous values (average within-sample standard deviation was 0.16‰ for $\delta^{18}O$ and 0.67‰ for $\delta^{2}H$). The estimated precision for the L2120-*i*, based on the repeated analysis of 4 reference water samples was 0.10‰ and 0.40‰, for $\delta^{18}O$ and $\delta^{2}H$, respectively. After

calibration with three internal standards, 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). Deuterium excess (D-ex) was calculated as D-ex = $\delta^{2}H - 8 \times \delta^{18}O$, and it reflects the slower movement of the $H_2{}^{18}O$ molecule during diffusion, leading to a relative enrichment of the $H^2HO$ molecules in the less strongly bound phase (e.g. in the gas phase during the evaporation of water) and thus, it can be considered as a good indicator of evaporative processes in water (Craig and Gordon 1965, Gat 1996).

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. As a quality assessment of the level of contamination among our samples, we compared the results with and without post-processing correction ($\delta_{corr}$ and $\delta_{raw}$, respectively). We found a very strong correlation between corrected and uncorrected values ($\delta^{18}O_{raw}$=-0.33+0.9997*$\delta^{18}O_{corr}$, $R^2$=0.998; $\delta^{2}H_{raw}$=-2.60+1.0035*$\delta^{2}H_{corr}$, $R^2$=0.989 for $\delta^{2}H$; $N$=369). The offset between raw and corrected values was significant, but small, and the slope of the relationship between raw and corrected values did not differ significantly from unity. Due to the low level of contamination, and for consistency we used module raw results for all samples.

**Statistical analyses**

In the two excision experiments, differences in isotope composition, % weight loss and xylem WC were initially assessed with full factorial models of time, species and treatment, again including xylem diameter as a covariable. After discarding treatment effects, we further assessed changes over time with a simplified model for each species, including initial values (i.e. before treatment). Subsequently, % weight loss and xylem WC were included as covariables in a full factorial of species and time, in order to assess their contribution to changes in isotope composition. In the pot experiment, the relationship between xylem isotope composition and either transpiration rates or leaf water isotopic composition was assessed by means of pair-wise Pearson correlations and linear regressions. At this point, it should be noted that we found two outlier values for pine xylem WC, likely associated to weighting errors, in the medium-term excision experiment (time = 7.75 hours). These values caused a highly unbalanced error distribution, and increased dramatically the error term in the models. Therefore, we decided to exclude the 7.75 hours-time in all the statistical analyses in which xylem WC was involved.

In all cases, generalized linear mixed models were based on Restricted Maximum Likelihood - REML ($\alpha$ = 0.05). Differences among levels of a given factor were tested by Least Square Mean (LSM) contrast ($\alpha$ = 0.05). All the statistical analyses were performed with JMP Pro 11 (SAS Inc., Cary, NC, USA).

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## RESULTS

### General trends in isotopic values

As shown in Fig. 1, we found isotopic enrichment in xylem water both under hydraulic and stomatal-limited sap flow (excision experiments and pot experiment, respectively). All samples followed a similar evaporative water line, which varied slightly depending on the species and the particular environmental conditions (temperature and relative humidity). The three experiments were conducted during summer, under warm and relatively dry conditions. However, in the excision experiments the conditions were less variable, and on average slightly warmer and drier (Fig. 1a,b) than in the pot experiment (Fig. 1c). Mean temperature was of 27.8ºC and 24.5ºC, and relative humidity of 45.9% and 54.0%, for medium-term and short-term experiments, respectively. In the pot experiment, temperature ranged from 13.0 to 32.0 ºC (mean: 22.3ºC) and humidity from 34.0 to 96.0% (mean: 69.9%). The magnitude of evaporative enrichment was greater at the day scale ($\delta^{18}O$ changes up to +7.4‰ for pine and +12.2‰ for oak in the medium-term excision experiment, Fig. 2a,c) but was also significant at the hourly scale ($\delta^{18}O$ changes up to 0.92‰ for pine and 1.98‰ for oak in short-term excision experiment, Fig. 2b,d). In the long-term experiment, first significant changes emerged after 7.75h for $\delta^{18}O$ (Fig. 2b) in both species, but after 28.25h for $\delta^{2}H$ (Fig. 2a). In the short-term experiment, the pines only showed a significant enrichment for $\delta^{18}O$ after 8.75h (Fig. 2d), whereas in the oaks first significant differences appeared after 0.5h (Fig. 2b,d).

### Water loss and isotopic changes under hydraulic limitation of sap flow

In the excision experiments, isotopic enrichment was largely explained by time after cut (Table 1). Xylem diameter was not significant as a co-variable for isotopic results ($\delta^{18}O$, $\delta^{2}H$, D-ex) but it explained part of the variability in % weight loss and xylem WC in the short-term excision experiment (Table 1). We found significant differences between species in isotopic values, % weight loss and xylem WC. In contrast, we only found a significant effect of the treatment for % weight loss (Table 1). In the medium-term experiment, LSM contrasts revealed significant changes in $\delta^{18}O$ from time=7.75 h, and in $\delta^{2}H$ from time=28.75 h, regardless of the species. In the short-term experiment, significant differences in $\delta^{18}O$ and $\delta^{2}H$ appeared after 0.5 hours in oaks and (only for $\delta^{18}O$) after 8.75 hours in pines. There were also significant changes in % weight loss with time, up to 31 % for pines and 43 % for oaks in the medium-term excision experiment (Fig. 3a), and up to 10 % in the short-term experiment (Fig. 3b). Statistical contrasts showed significant differences in % weight loss in the medium-term experiment from 50.25 and 28.25 hours, for pine and oak, respectively (Fig. 3a), and from 1.25 hours in the short-term experiment (Fig. 3b). Xylem WC content was initially higher in pines than in oaks, and showed larger changes in the medium-term (Fig. 3c) than in the short-term experiment (Fig. 3d).

Table 2 shows the output of the models explaining isotopic composition by means of % weight loss and xylem WC in the two excision experiments. We did not find significant effects of xylem WC and % weight loss on $\delta^{18}O$ and $\delta^{2}H$. However, we found that xylem WC partially explained the observed changes in % weight loss.

### Sap flow and isotopic changes under stomatal-limited transpiration

In the pot experiment, the reduction in sap flow was gradual, reaching its minimum on the third day after the leaves were covered (Fig. 4a,b), whereas xylem isotopic enrichment was already visible from 6 hours after covering (Fig. 4c-f). Un-covering produced a gradual recovery

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of sap flow and isotopic values, with first visible changes appearing after 12 hours, and reaching initial isotopic values in 36 hours. It should be noted that, due to the progressive reduction in total leaf area with time (as a consequence of twig sampling) and the changing environmental conditions, we did not observe a complete recovery of initial daily sap flow values after un-covering. In uncovered periods, we generally observe more enriched values at predawn than at midday (Fig. 4c-f). Unlike for the excision experiments, in the pot experiment we did not find significant changes in xylem WC, remaining stable throughout the experiment (60.3 $\pm$0.62 % for pines, 49.6 $\pm$0.64 % for oaks). Besides, in relation to leaf water, after covering the leaves with aluminium foil, a depletion in $\delta^{18}O$ and $\delta^{2}H$ in leaf water is seen (Fig. 4c-f). To test whether isotope mixing between xylem and leaf water could explain this pattern, we compared xylem and leaf isotopic values during the covered period. Isotopic changes in leaf water were not significantly correlated with those of xylem water either for $\delta^{18}O$ ($R^2$=-0.47, $P$=0.079 for pine and $R^2$=-0.30, $P$=0.272 for oak, $N$=15) or $\delta^{2}H$ ($R^2$=-0.28, $P$=0.272 for pine and $R^2$= +0.31, $P$=0.265 for oak, $N$=15). Conversely, the decline in sap flow rates from initial values to the end of the covered period was significantly associated with an enrichment in the isotopic composition of xylem water for both species (Fig. 5).

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## DISCUSSION

### *Experimental evidence of fast evaporative enrichment*

Our results show that evaporative enrichment of xylem water takes place in fully-developed, suberized stems shortly after sap flow is limited, regardless of the cause of this limitation. Firstly, the excision experiments showed that the isotopic composition of xylem water increases over time directly after the cut, with significant isotopic changes appearing in less than one hour. Secondly, the pot experiment showed that isotopic enrichment in xylem water could also appear in healthy, well-watered trees when stem flow is limited by leaf-level stomatal closure (e.g. in response to shadowing or low humidity), but evaporative demand is high enough to keep a significant stem transpiration. Similar to our results, Ellsworth and Sternberg (2014) also found a departure from original values in the xylem of suberized stems after artificial defoliation to emulate sap flow limitation in deciduous species during leafless periods. Notably, the magnitude of the change observed in our pot experiment after only three days of sap flow restriction was in the range of that found by Ellsworth and Sternberg (2014) about one month after defoliation (ca. +4‰ in $\delta^{18}O$; +20‰ in $\delta^{2}H$). They interpreted that evaporation from the stem was minimal, but the period between defoliation and the first sampling was long enough to cause significant effects on the xylem water. However, our results indicate that this process is much faster than originally expected. Ellsworth and Sternberg (2014) also reported a rather slow recovery of the initial values (>50 days), linked to the development of new leaves. In line with this, Phillips and Ehleringer (1995) realised that the xylem water of winter-deciduous trees departed from the meteoric water line during leafless periods, returning to it only after complete leaf flushing. In our case, after uncovering the leaves, the xylem recovered to non-evaporated soil values in a few hours, proving that in fully active plants evaporative enrichment in the xylem is a reversible, highly dynamic process.

### *Mechanisms causing isotopic enrichment in the xylem*

It is generally assumed that water loss through the stem surface in suberized stems is minimal (see e.g. Schönherr 1982). However, some studies have measured significant bark and lenticular transpiration (e.g. Stöhr and Lösch 2004, Catinon et al. 2012) pointing to the need to test this assumption for an extensive amount of species and environmental conditions. The outcome of the three experiments strongly supports a major effect of stem transpiration on the isotopic enrichment of xylem water. When leaf transpiration is limited, the gradient in water potential between the stem and the leaves is smaller, and hydraulic flow decreases (Sperry et al. 1993); as a consequence, water storage may increase to mitigate xylem cavitation. Under such conditions, the limited stem flow increases water turnover time, reducing the input of fresh, unenriched xylem water, and allowing for accumulative evaporative enrichment. The progressive enrichment in xylem water followed a typical evaporation line, i.e. with flatter slope in the $\delta^{18}O/\delta^{2}H$ bi-plot than the meteoric water line (see e.g. Craig and Gordon 1965, Gat 1996). In all cases, the slope of the evaporative line fell within the expected range for soil evaporation in the area (ca. 3-4; see e.g. Gibson et al. 2008), hence being compatible with evaporation in a porous media (Allison et al. 1983). Evaporative enrichment would also increase with time, until a limiting threshold when further evaporation no longer results in isotope enrichment of the remaining water (Skrzypek et al. 2015). In our study, the magnitude of the deviation of δ-values from original, non-evaporated, xylem water was a function of the time since transpiration was stopped/limited in the two excision experiments, as well as in the pot experiment.

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Unlike in the excision experiments, the strong changes in isotope composition in the pot experiment were not associated to a net loss of water in the xylem. Apparently, covering with aluminium foil strongly reduced leaf transpiration, but kept a significant residual flow, presumably through stem transpiration. Estimated daily water use after covering the leaves was still 20 $\pm$ 4.5% and 30 $\pm$ 9.5% of that with uncovered leaves, for pines and oaks, respectively. Hence, even a short, moderate limitation of the transpiration flow (e.g. under mild drought stress, or during cloudy days) may be enough to create significant evaporative gradients within the stem, without causing a measurable dehydration. Although absolute sap flow rates in saplings should be taken with caution due to the strong effect of temperature gradients on heat balance sensors, particularly under limited flow (Do and Rocheteau 2002), our findings support previous works suggesting that water losses through the stem surface are not negligible (Stöhr and Lösch 2004, Catinon et al. 2012).

Isotopic enrichment in the stem might be also explained through the contribution of enriched leaf water (either through backward diffusion, or indirectly through the phloem) to changes in the xylem (Dawson and Ehleringer 1993, Bertrand et al. 2014). However, we did not find significant differences between defoliated and non-defoliated stems in the excision experiment, suggesting a minor effect of leaf processes on xylem enrichment. Furthermore, in the pot experiment, leaf and xylem water were not significantly correlated during the covering period, contrary to what would be expected if back-diffusion of leaf water were the main source for changes in the xylem. On the other hand, phloem water is also enriched (see e.g. Adar et al. 1995, Cernusak et al. 2005), and is known to exchange with xylem water under certain conditions (Nardini et al. 2011). However, due to the smaller amount of water in the phloem, xylem-phloem exchange is likely to have greater effect on phloem values than on the xylem (Cernusak et al. 2005).

*Defining the most suitable sampling strategy*

Ellsworth & Williams (2007) observed that mature stems with fully developed bark had a lower proportion of water in the phloem, and explained the difference between young and old stems through changing proportions of xylem, phloem and bark water. Unlike in this work (and others, e.g. Dawson and Ehleringer 1993, Phillips and Ehleringer 1995), we peeled the branches before sampling, in order to avoid direct contamination from phloem water. Despite this, if the relative proportion of water in the phloem decreases with age, sampling older branches would reduce the potential effect of xylem-phloem exchange on xylem water. Besides, increasing branch diameter would also minimize the effect of stem evaporation due to the lower surface/volume ratio. In our experiments, twig diameter explained part of the variability in stem water loss, but was not associated with isotopic composition. Regarding the sampling time, Dawson and Ehleringer (1993) reported that evaporative enrichment was still present at predawn but was lower than at midday, so they proposed to collect xylem at predawn to avoid evaporation. However, our results highlight that predawn xylem water might suffer substantial evaporative enrichment due to temporary water stagnation. In our pot experiment, after uncovering the leaves, xylem water remained unchanged (i.e. enriched) at predawn, and did not reflect the input of new source water until midday. This suggests that predawn sampling, which in principle implies more limited sap flow rates, may not be appropriate if night-time or previous-day conditions favoured evaporative losses (see e.g. Resco de Dios et al. 2013). Therefore, we recommend to sample medium-size twigs at the time of maximum transpiration, avoiding pre-dawn measurements and afternoon depression of stomatal conductance during drought stress.

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## CONCLUSIONS

In our study, we report evidence of fast evaporative enrichment in metabolically active stems as a consequence of a temporal decline in sap flow rates. In other words, xylem water isotopic composition does not only reflect source water, but also stem hydraulic processes. Hence, observed seasonal fluctuations, e.g. in response to drought (Bertrand et al. 2014, Voltas et al. 2015), or even daily variations (Filella and Peñuelas 1999) might not necessarily reflect changes in source water, as previously assumed, but a confounding effect of xylem evaporative enrichment under limited sap flow (see e.g. del Castillo et al. 2016). Although stem evaporative enrichment can be seen as a handicap for water-sourcing studies, once evaporative effects are constrained (e.g. through the use of $^{17}$O-excess; Landais et al. 2006) it could provide a new insight into xylem water dynamics. Beyond the tree scale, our findings also highlight the need to assess the contribution of stem transpiration to tree water balance, and its potential effect on the isotopic partition of water fluxes at the ecosystem level (Wang and Yakir 2000, Dubbert et al. 2013).

Evaporation through the stem surface appears to be the main driver of xylem isotopic enrichment during periods of limited sap flow. In particular, we did not find evidence of a feedback effect of leaf water on stem values. On the other hand, the dynamics of isotopic enrichment were similar, regardless of the original cause for sap flow reductions (i.e. limited leaf transpiration or stem hydraulic restrictions), further supporting a physical rather than a physiological regulation of this process. Nevertheless, additional studies addressing specifically isotopic variations in leaf xylem and phloem water might help to disentangle the potential role of leaf-derived water pools in evaporative enrichment.

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### Author contributions

P.M.G., J.P.F. and L.S. planned and designed the research, collected and analysed the data, and wrote the manuscript.

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## REFERENCES

Adar E, Gev I, Lip J, Yakir D, Gat J (1995) Utilization of oxygen-18 and deuterium in stream flow for the identification of transpiration sources: soil water versus groundwater in sand dune terrain. In: Adar E, Leibundgut C (eds) Application of Tracers in Arid Zone Hydrology, Int. Assoc., pp 329–338.

Allison GB, Barnes CJ, Hughes MW (1983) The distribution of deuterium and $^{18}O$ in dry soils 2. Experimental. J Hydrol 64:377–397.

Bertrand G, Masini J, Goldscheider N, Meeks J, Lavastre V, Celle-Jeanton H, Gobat J-M, Hunkeler D (2014) Determination of spatiotemporal variability of tree water uptake using stable isotopes ($\delta^{18}O$, $\delta^{2}H$) in an alluvial system supplied by a high-altitude watershed, Pfyn forest, Switzerland. Ecohydrology 7:319–333.

Brandes E, Wenninger J, Koeniger P, Schindler D, Rennenberg H, Leibundgut C, Mayer H, Gessler A (2007) Assessing environmental and physiological controls over water relations in a Scots pine (*Pinus sylvestris* L.) stand through analyses of stable isotope composition of water and organic matter. Plant Cell Environ 30:113–127.

Catinon M, Ayrault S, Boudouma O, Asta J, Tissut M, Ravanel P (2012) Atmospheric element deposit on tree barks: The opposite effects of rain and transpiration. Ecol Indic 14:170–177.

Cernusak LA, Barbour MM, Arndt SK, Cheesman AW, English NB, Feild TS, Helliker BR, Holloway-Phillips MM, Holtum JAM, Kahmen A, McInerney FA, Munksgaard NC, Simonin KA, Song X, Stuart-Williams H, West JB, Farquhar GD (2016) Stable isotopes in leaf water of terrestrial plants. Plant Cell Environ 39:1087–1102.

Cernusak LA, Farquhar GD, Pate JS (2005) Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. Tree Physiol 25:129–146.

Comas C, del Castillo J, Voltas J, Ferrio JP (2015) Point processes statistics of stable isotopes: Analysing water uptake patterns in a mixed stand of Aleppo pine and Holm oak. For Syst 24(1):e009, doi:http://dx.doi.org/10.5424/fs/2015241-05846.

Craig H, Gordon LI (1965) Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. In: Tongiorgi E (ed) Proceedings of a conference on stable isotopes in oceanographic studies and paleotemperatures. Laboratory of Geology and Nuclear Science, Pisa, pp 9–130.

Dawson TE (1993) Water sources of plants as determined from xylem-water isotopic composition: perspectives on plant competition, distribution, and water relations. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon-water relations. Academic Press, Inc., New York, pp 465–496

Dawson TE, Ehleringer JR (1993) Isotopic enrichment of water in the 'woody' tissues of plants: Implications for plant water source, water uptake, and other studies which use the stable isotopic composition of cellulose. Geochim Cosmochim Acta 57:3487–3492.

del Castillo J, Comas C, Voltas J, Ferrio J.P (2016) Dynamics of competition over water in a mixed oak-pine Mediterranean forest: Spatio-temporal and physiological components. Forest Ecology and Management 382:214-224.

Do F, Rocheteau A (2002) Influence of natural temperature gradients on measurements of xylem sap flow with thermal dissipation probes. 2. Advantages and calibration of a noncontinuous heating system. Tree Physiol 22:649–654.

Dongmann G, Nürnberg HW, Förstel H, Wagener K (1974) On the enrichment of $H_2{}^{18}O$ in the leaves of transpiring plants. Radiat Environ Biophys 11:41–52.

Dubbert M, Cuntz M, Piayda A, Máguas C, Werner C (2013) Partitioning evapotranspiration – Testing the Craig and Gordon model with field measurements of oxygen isotope ratios of evaporative fluxes. J Hydrol 496:142–153.

Ehleringer JR, Dawson TE (1992) Water uptake by plants: perspectives from stable isotope composition. Plant Cell Environ 15:1073–1082.

Ellsworth PZ, Sternberg LSL (2014) Seasonal water use by deciduous and evergreen woody species in a scrub community is based on water availability and root distribution. Ecohydrology 8:538–551.

Ellsworth PZ, Williams DG (2007) Hydrogen isotope fractionation during water uptake by woody xerophytes. Plant Soil 291:93–107.

Farquhar GD, Lloyd J (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon–water relations. Academic Press, San Diego, pp 47–70

Filella I, Peñuelas J (1999) Altitudinal differences in UV absorbance, UV reflectance and related morphological traits of *Quercus ilex* and *Rhododendron ferrugineum* in the Mediterranean region. Plant Ecol 145:157–165.

Filella I, Peñuelas J (2004) Indications of hydraulic lift by Pinus halepensis and its effects on the water relations of neighbour shrubs. Biol Plant 47:209–214.

Gat J (1996) Oxygen and hydrogen isotopes in the hydrologic cycle. Annu Rev Earth Planet Sci 24:225–262.

Gibson JJ, Birks SJ, Edwards TWD (2008) Global prediction of $\delta_A$ and $\delta^2H$- $\delta^{18}O$ evaporation slopes for lakes and soil water accounting for seasonality. Global Biogeochem Cycles 22:1–12.

Landais A, Barkan E, Yakir D, Luz B (2006) The triple isotopic composition of oxygen in leaf water. Geochim Cosmochim Acta 70:4105–4115.

Lin GH, Sternberg LDL, Ehleringer JR, Hall AE, Farquhar GD (1993) Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In: J.R. Ehleringer, A.E. Hall and G.D. Farquhar (eds) Stable isotopes and plant carbon–water relations. Academic Press, San Diego, pp 497–510

Máguas C, Rascher KG, Martins-Loução A, Carvalho P, Pinho P, Ramos M, Correia O, Werner C (2011) Responses of woody species to spatial and temporal ground water changes in coastal sand dune systems. Biogeosciences 8:3823–3832.

Martín-Gómez P, Barbeta A, Voltas J, Peñuelas J, Dennis K, Palacio S, Dawson TE, Ferrio JP (2015) Isotope-ratio infrared spectroscopy: a reliable tool for the investigation of plant-water sources? New Phytol 207:914–927.

Nardini A, Salleo S, Jansen S (2011) More than just a vulnerable pipeline: xylem physiology in the light of ion-mediated regulation of plant water transport. J Exp Bot 62:4701–4718.

Phillips SL, Ehleringer JR (1995) Limited uptake of summer precipitation by bigtooth maple (*Acer grandidentatum* Nutt) and Gambel's oak (*Quercus gambelii* Nutt). Trees Struct Funct 9:214–219.

Orlowski N, Breuer L, McDonnell JJ (2016) Critical issues with cryogenic extraction of soil water for stable isotope analysis. Ecohydrology 9:3-10.

Palacio S, Azorín J, Montserrat-Martí G, Ferrio JP (2014) The crystallization water of gypsum rocks is a relevant water source for plants. Nature Communications 5: 4660.

Picarro (2012) Micro-Combustion Module$^{TM}$ (MCM): Elimination of organics datasheet.

Prieto I, Armas C, Pugnaire FI (2012) Water release through plant roots: New insights into its consequences at the plant and ecosystem level. New Phytol 193:830–841.

Resco de Dios V, Díaz-Sierra R, Goulden ML, Barton CVM, Boer MM, Gessler A, Ferrio JP, Pfautsch S, Tissue DT (2013) Woody clockworks: Circadian regulation of night-time water use in *Eucalyptus globulus*. New Phytol 200:743–752.

Schönherr J (1982) Resistance of plant surfaces to water loss: transport properties of cutin, suberin and associated lipids. In: Lange O, Nobel P, Osmond C, Ziegler H (eds) Physiological Plant Ecology II. Springer-Verlag: Berlin, pp 153–179.

Skrzypek G, Mydłowski A, Dogramaci S, Hedley P, Gibson JJ, Grierson PF (2015) Estimation of evaporative loss based on the stable isotope composition of water using Hydrocalculator. J Hydrol 523:781–789.

Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hyraulic conductance on stomatal conductance and xylem cavitation. J Exp Bot 44:1075–1082.

Sperry JS, Hacke UG, Oren R, Comstock JP (2002) Water deficits and hydraulic limits to leaf water supply. Plant, Cell Environ 25:251–263.

Sternberg LDSL, Swart PK (1987) Utilization of freshwater and ocean water by coastal plants of southern Florida. Ecology 68:1898–1905.

Stöhr A, Lösch R (2004) Xylem sap flow and drought stress of Fraxinus excelsior saplings. Tree Physiol 24:169–180.

Tang K, Feng X (2001) The effect of soil hydrology on the oxygen and hydrogen isotopic compositions of plants' source water. Earth Planet Sci Lett 185:355–367.

Treydte K, Boda S, Graf Pannatier E, Fonti P, Frank D, Ullrich B, Saurer M, Siegwolf R, Battipaglia G, Werner W, Gessler A (2014) Seasonal transfer of oxygen isotopes from precipitation and soil to the tree ring: Source water versus needle water enrichment. New Phytol 202:772–783.

Voltas J, Lucabaugh D, Chambel MR, Ferrio JP (2015) Intraspecific variation in the use of water sources by the circum-Mediterranean conifer *Pinus halepensis*. New Phytol 208:1031–41.

Wang XF, Yakir D (2000) Using stable isotopes of water in evapotranspiration studies. Hydrol Process 14:1407–1421.

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## TABLES AND FIGURES

**Table 1** Summary statistics of the mixed models to test for time and treatment effects on xylem isotopic composition and water content during the excision experiments. Models include only samples after treatment, i.e. excluding initial, reference values. $\delta^{18}O$, $\delta^{2}H$, oxygen and hydrogen isotopic composition, respectively; D-ex, Deuterium excess; % Weight loss, relative weight loss of twigs between excision and xylem sampling; Xylem WC, water content (%) in the xylem, determined gravimetrically from pre- and post-distillation weights. *P*-values are presented only for significant factors ($P<0.05$), otherwise denoted as non-significant (n.s.). For xylem WC in the medium-term excision experiment, $N=24$ (see methods for details).

| Factor | $\delta^{18}O$ | $\delta^{2}H$ | D-ex | % Weight loss | Xylem WC |
|---|---|---|---|---|---|
| *Medium-term excision experiment (N=30)* | | | | | |
| Xylem diameter | n.s. | n.s. | n.s. | n.s. | n.s. |
| Species (oak, pine) | 0.0105 | n.s. | 0.0033 | 0.001 | <0.0001 |
| Treatment | n.s. | n.s. | n.s. | 0.0011 | n.s. |
| Time | 0.0062 | 0.0131 | 0.0052 | 0.0357 | 0.0042 |
| Species × treatment | n.s. | n.s. | n.s. | n.s. | n.s. |
| Species × time | 0.0182 | n.s. | 0.0046 | n.s. | n.s. |
| Treatment × time | n.s. | n.s. | n.s. | n.s. | n.s. |
| *Short-term excision experiment (N=74)* | | | | | |
| Xylem diameter | n.s. | n.s. | n.s. | 0.0049 | 0.0005 |
| Species (oak, pine) | 0.0053 | n.s. | <0.0001 | n.s. | <0.0001 |
| Treatment | n.s. | n.s. | n.s. | <0.0001 | n.s. |
| Time | <0.0001 | n.s. | <0.0001 | <0.0001 | n.s. |
| Species × treatment | n.s. | n.s. | n.s. | n.s. | n.s. |
| Species × time | n.s. | n.s. | 0.0207 | n.s. | n.s. |
| Treatment × time | n.s. | n.s. | n.s. | 0.0254 | n.s. |
| Species x treatment × time | n.s. | n.s. | n.s. | 0.0197 | n.s. |

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**Table 2** Summary statistics of the mixed models relating changes in water content during the excision experiments with xylem isotopic composition ($\delta^{18}O$, $\delta^{2}H$, oxygen and hydrogen isotopic composition, respectively; D-ex, Deuterium excess). Models combine all treatments and include initial, reference vaules. % Weight loss, relative weight loss of twigs between excision and xylem sampling; Xylem WC, water content (%) in the xylem, determined gravimetrically from pre- and post-distillation weights. *P*-values are presented only for significant factors ($P<0.05$), otherwise denoted as non-significant (n.s.). For xylem WC in the medium-term excision experiment, $N=34$ (see methods for details).

| Factor | $\delta^{18}O$ | $\delta^{2}H$ | D-ex | % Weight loss |
|---|---|---|---|---|
| *Medium-term excision experiment (N=40)* | | | | |
| % Weight Loss | n.s. | n.s. | n.s. | |
| Species | 0.0036 | n.s. | 0.0001 | |
| Time | <0.0001 | <0.0001 | <0.0001 | |
| Species × time | 0.0001 | 0.0483 | <0.0001 | |
| Xylem WC | n.s. | n.s. | n.s. | 0.0423 |
| Species | n.s. | n.s. | n.s. | n.s. |
| Time | <0.0001 | <0.0001 | <0.0001 | 0.0017 |
| Species × time | 0.0142 | n.s. | 0.0018 | n.s. |
| *Short-term excision experiment (N=114)* | | | | |
| % Weight Loss | n.s. | n.s. | 0.0362 | |
| Species | 0.0242 | <0.0001 | <0.0001 | |
| Time | <0.0001 | n.s. | <0.0001 | |
| Species × time | <0.0001 | 0.0116 | 0.0002 | |
| Xylem WC | n.s. | n.s. | 0.0003 | n.s. |
| Species | n.s. | n.s. | n.s. | n.s. |
| Time | <0.0001 | 0.0063 | <0.0001 | <0.0001 |
| Species × time | <0.0001 | 0.0095 | 0.0009 | n.s. |

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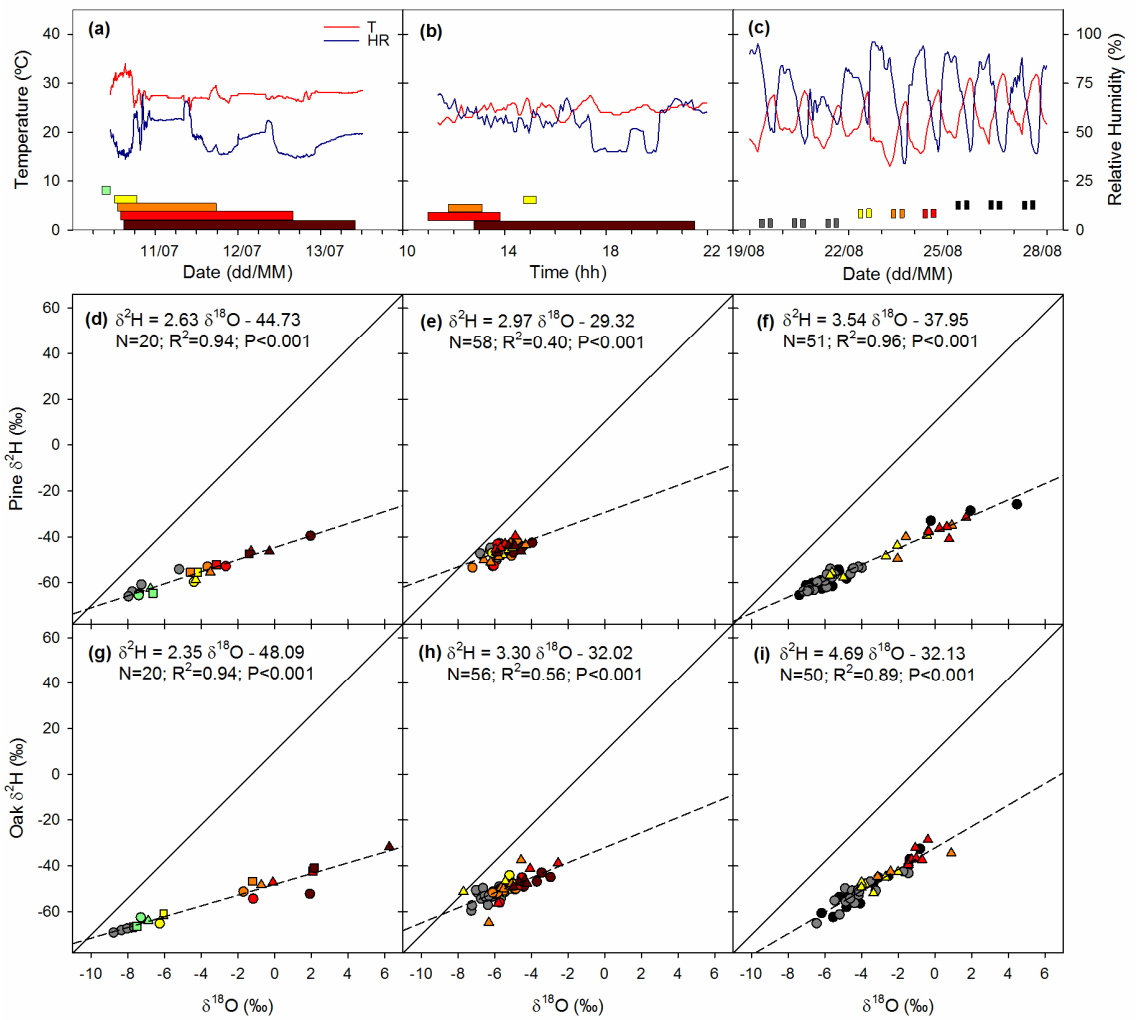


**Fig. 1** Time-frame and isotopic composition ($\delta^{18}O$ vs. $\delta^{2}H$) of pine and oak xylem water in the medium-term and short-term excision experiments (left and middle panels, respectively), and in the pot experiment (right panels). Upper panels (a, b and c) display the evolution of Temperature (in red) and Relative Humidity (in blue) and the sampling schedule. Coloured boxes in the excision experiments (a, b) indicate the drying time of twigs between excision and xylem sampling. For the pot experiment (c), boxes indicate the sampling times. In excision experiments (panels d, g, e, h) grey symbols represent samples collected immediately after excision, while green, yellow, orange, red and brown symbols indicate different times after excision. Circles represent non-covered twigs, triangles twigs covered with aluminium foil and squares defoliated twigs. In the pot trial (f, i), yellow, orange and red triangles stand for the three consecutive days of aluminium foil covering; grey and black circles show the values of uncovered samples, before and after the treatment, respectively. Global Meteoric Water Line (GMWL) is shown with a black continuous line and the linear regression between $\delta^{18}O$ and $\delta^{2}H$ for twig xylem water (evaporation line) with a dashed line. Linear regression equations, number of samples (*N*), determination coefficient ($R^2$) and *P*-values (P) are also presented.

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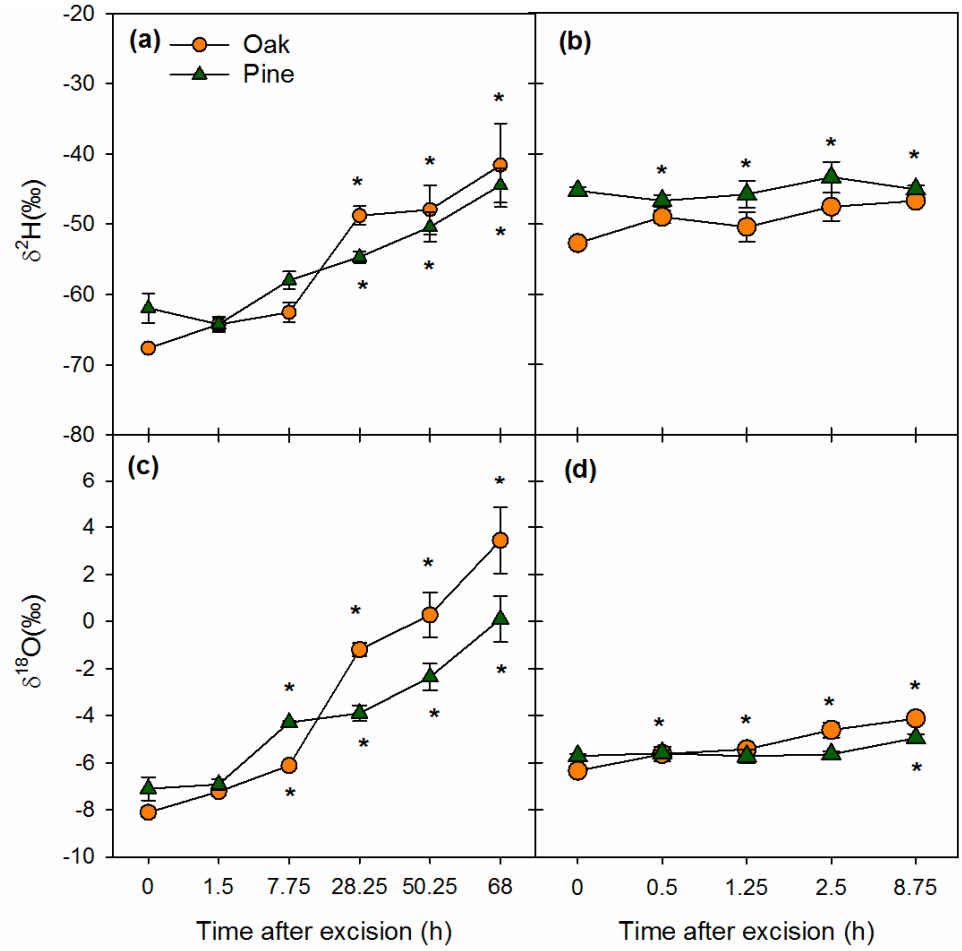


**Fig. 2** Temporal evolution of hydrogen ($\delta^2H$; a,b) and oxygen ($\delta^{18}O$; c,d) isotope composition in the long-term (a,c) and short-term (b,d) excision experiments. Triangles and circles are used for pines and oaks, respectively. Error bars, ± SE. Differences were tested by Least Square Mean Contrast (α=0.05) on the model including species, time and xylem water content as co-variable (see Table 2). Asterisks (*) indicate significant differences respect to initial time for each species; up, oak; down, pine. Note that the time axis is categorical.

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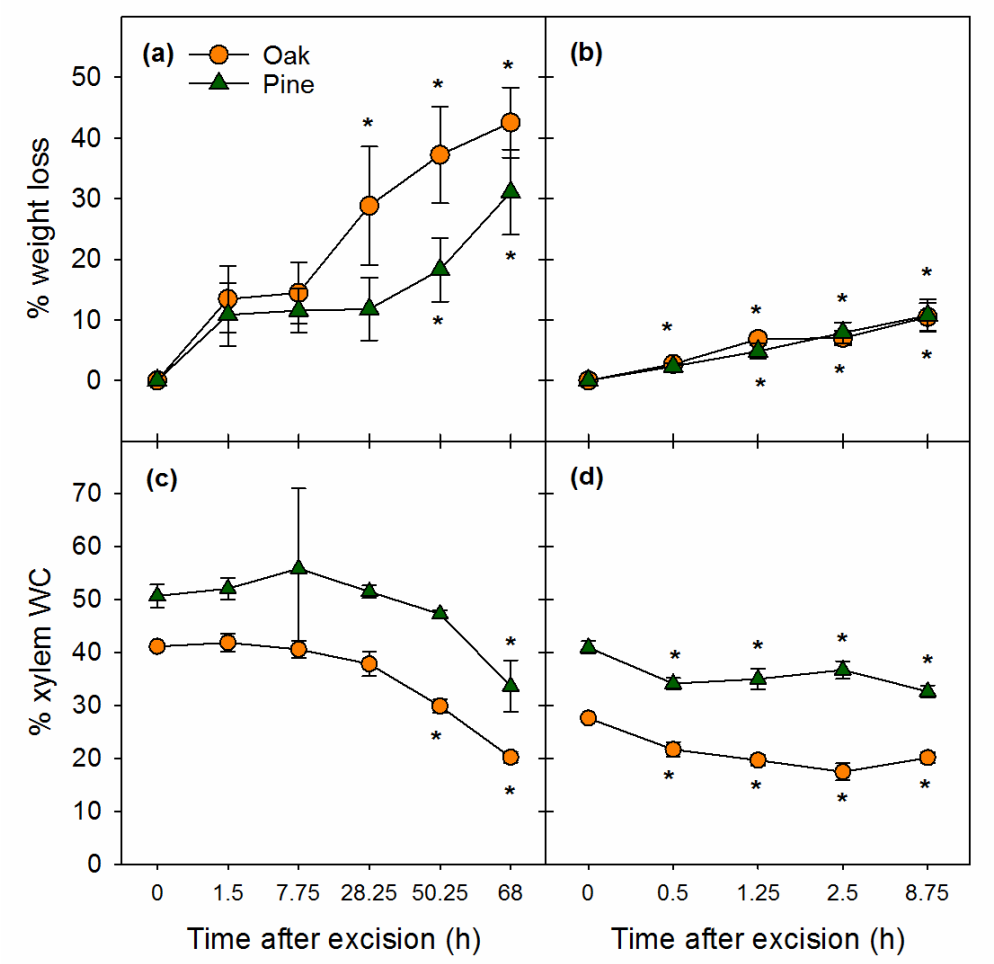


**Fig. 3** Temporal evolution of water content during the excision experiments: a,b) relative weight loss of twigs (%) between excision and xylem sampling (% Weight loss); c,d) water content (%) in the xylem, determined gravimetrically from pre- and post-distillation weights (Xylem WC). Left panels (a,c), medium-term experiment; right panels (b,d), short-term experiment. Triangles and circles are used for pines and oaks, respectively. Error bars, ± SE. Asterisks denote significant differences by Least Square Mean (α=0.05), including species and time as crossed factors, and xylem diameter as a co-variable. For xylem WC in the medium-term experiment (c), the model excluded the values at 7.75 h. When overlapping, up, oak; down, pine. Note that the time axis is categorical.

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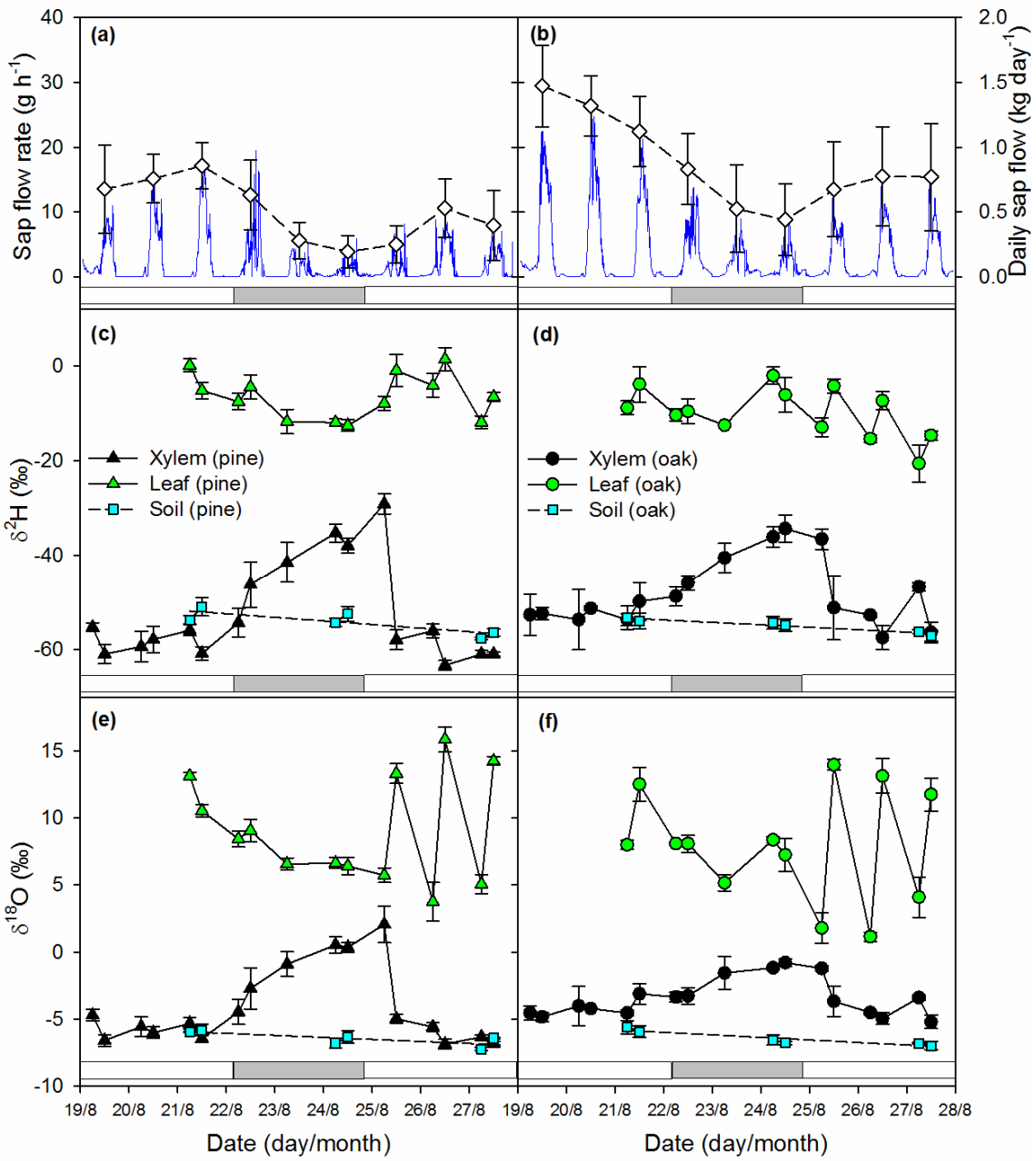


**Fig. 4** Temporal evolution of sap flow, and isotopic composition ($\delta^{18}O$, $\delta^{2}H$ in ‰) in soil, xylem and leaf water during the pot trial. White and grey boxes denote uncovered and covered periods, respectively. In sap flow panels (a,b) the blue line show mean sap flow rate (g $h^{-1}$, *N*=3), and white diamonds show accumulated daily values (kg $day^{-1}$). In isotope panels (c,d,e,f) triangles and circles are used for pines and oaks, respectively: green symbols, leaf water; black symbols, xylem water. Blue squares stand for soil values. Error bars, ± SE.

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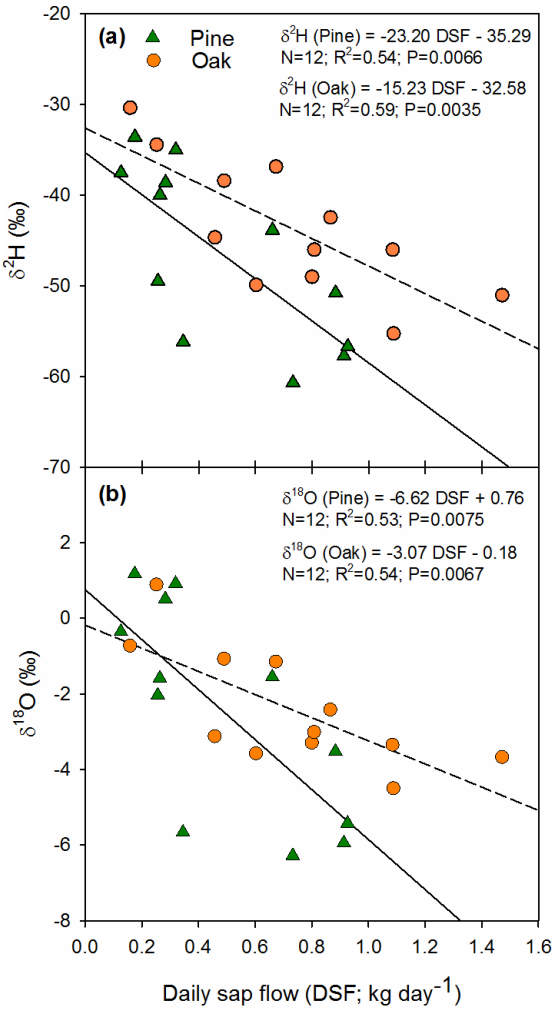


**Fig. 5** Linear regression between accumulated daily sap flow (kg day$^{-1}$) and daily means of hydrogen (a) and oxygen (b) isotopic composition ($\delta^2$H and $\delta^{18}$O, respectively). Values for each individual, from the last day before aluminium foil covering to the end of the covered period. Triangles and circles are used for pines and oaks, respectively. Regressions are plotted with continuous lines for pines, and dashed lines for oaks.