Seasonal transfer of oxygen isotopes from precipitation and soil to the tree ring: source water versus needle water enrichment

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Summary

- For accurate interpretation of oxygen isotopes in tree rings (δ¹⁸O), it is necessary to disentangle the mechanisms underlying the variations in the tree’s internal water cycle and to understand the transfer of source versus leaf water δ¹⁸O to phloem sugars and stem wood.
- We studied the seasonal transfer of oxygen isotopes from precipitation and soil water through the xylem, needles and phloem to the tree rings of Larix decidua at two alpine sites in the Lötschental (Switzerland). Weekly resolved δ¹⁸O records of precipitation, soil water, xylem and needle water, phloem organic matter and tree rings were developed.
- Week-to-week variations in needle-water ¹⁸O enrichment were strongly controlled by weather conditions during the growing season. These short-term variations were, however, not significantly fingerprinted in tree-ring δ¹⁸O. Instead, seasonal trends in tree-ring δ¹⁸O predominantly mirrored trends in the source water, including recent precipitation and soil water pools. Modelling results support these findings: seasonal tree-ring δ¹⁸O variations are captured best when the week-to-week variations of the leaf water signal are suppressed.
- Our results suggest that climate signals in tree-ring δ¹⁸O variations should be strongest at temperate sites with humid conditions and precipitation maxima during the growing season.

Introduction

Oxygen isotopes in tree rings (δ¹⁸O_{tree-ring}) have been used to reconstruct past atmospheric conditions such as the isotopic composition of precipitation (Saurer et al., 1997b; Anderson et al., 1998; Robertson et al., 2001; Danis et al., 2006), air temperature (Libby et al., 1976; Burk & Stuiver, 1981; Saurer et al., 2000; Rebetez et al., 2003; Edwards et al., 2008), precipitation amount (Masson-Delmotte et al., 2005; Treydte et al., 2006, 2007; Reynolds-Henney et al., 2007, 2008; Saurer et al., 2008), relative air humidity (Ramesh et al., 1986; Saurer, 1997b; Robertson et al., 2001; Edwards et al., 2008), and even atmospheric circulation patterns (Welker et al., 2005; Miller et al., 2006; Roden & Ehleringer, 2007; Brien et al., 2012; Liu et al., 2012; Saurer et al., 2012). This diversity of reconstructions illustrates the versatility of tree-ring-based oxygen isotope measurements, but also hints at the challenge of understanding the complexity of the climatic and biological systems responsible for isotopic fractionation. Although the sign of the trees’ response to these variables (positive/negative correlation) may be robust among species, sites and regions (Treydte et al., 2007; Saurer et al., 2008) and tree ring δ¹⁸O seems to be unaffected by air pollution (Rinne et al., 2010), the strength of the climate signal can vary strongly. Several environmental parameters may be represented with near equal fidelity (Kress et al., 2010) or may show temporally unstable relationships with δ¹⁸O_{tree-ring} (Reynolds-Henney et al., 2007; Treydte et al., 2007). Uncertainties arise mainly from the complex interplay between signals carried in the source water taken up by the roots and those produced by evaporative enrichment and physiological (post-)photosynthetic processes at the leaf level and during downstream metabolism (Offermann et al., 2011; Gessler et al., 2013).

Through the roots, trees take up source water, which in the optimum case for climate reconstruction is predominantly precipitation, carrying a specific atmospheric δ¹⁸O signal (Rozanski et al., 1992). However, this precipitation signal can be damped, lagged or even masked, depending on the temporal variation of the amount, mixture, and isotopic composition of infiltrated water (e.g. rainfall, snow melt water, or surface water), and further effects such as evaporative enrichment of soil water or the influence of ground water (Ehleringer & Dawson, 1992). To date it has been assumed that no measurable oxygen isotope...
fractionation should occur when water is transported from the soil into the xylem of roots, stem, and branches (Dawson & Ehleringer, 1991, 1993).

At the leaf level, processes affecting lamina leaf water $\delta^{18}$O are reasonably well described with mechanistic models. These processes are evaporative enrichment (Dongmann et al., 1974) and the modification of leaf water enrichment by the advection of unenriched source water to the leaf evaporative sites. Lamina leaf water $\delta^{18}$O is on the one hand determined by the evaporative enrichment at the sites of evaporation (Dongmann et al., 1974) and the diffusion of this $^{18}$O-enriched water back to the leaf lamina. This diffusion process is, on the other hand, opposed by the advective transport of unenriched xylem water through the leaf lamina to the evaporation sites via the transpiration stream. The net effect of these advective versus diffusive transport processes is called the Péclet effect (Farquhar & Lloyd, 1993; Barbour et al., 2001; Farquhar & Cernusak, 2005; Barbour, 2007; Barnard et al., 2007; Cuntz et al., 2007; Gessler et al., 2007). Furthermore, it is known that the $\delta^{18}$O of the leaf water (i.e. the water in which the chemical reactions of carbon assimilation occur) is imprinted on the newly assimilated organic matter. The carbonyl group is enriched in $^{18}$O by 27‰ compared with the lamina leaf water (DeNiro & Epstein, 1981; Sternberg et al., 1986; Yakir & Deniro, 1990). Yet, knowledge of the physiological mechanisms of isotope fractionation and oxygen atom exchange between organic matter and reaction water (i.e. the water within which any biochemical reaction occurs) in downstream metabolic processes remains fragmentary, in particular how these processes are influenced by environmental versus plant internal factors (Farquhar et al., 1998a,b; Brandes et al., 2006; Sternberg, 2008).

Many studies have focused on the mechanistic processes behind these fractionation processes and their implications for $\delta^{18}$O tree-ring (Farquhar et al., 1998a,b; Barbour & Farquhar, 2000; Roden et al., 2000, 2005; Barbour et al., 2002; Ogée et al., 2009). Only a few (Gessler et al., 2009; Roden et al., 2009; Offermann et al., 2011), however, specifically related these processes to the preservation of a climate signal in the stem wood at highly resolved time-scales. Moreover, these few studies did not allow general conclusions but were often species specific, although taking into account transport time lags, and the generally acknowledged exchange of 42% of the sucrose oxygen with nonenriched stem water during cellulose synthesis (Gessler et al., 2009); whereas the $\delta^{18}$O signal could be traced from leaf water to the tree ring in Scots pine (Pinus sylvestris L.), the tree-ring oxygen isotopic signal in European beech (Fagus sylvatica L.) was strongly uncoupled from canopy physiology (Offermann et al., 2011). Therefore, a deeper understanding of the contribution of all potential fractionation and exchange steps occurring on the way through the tree to stable isotope fixation in tree rings is a prerequisite for a reliable interpretation of this environmental proxy.

Here, we followed the complete pathway of $\delta^{18}$O from precipitation to the tree ring over one growing season under varying environmental conditions. We present weekly resolved records of xylem and needle water, phloem organic matter and stem wood $\delta^{18}$O of Larix decidua growing at two contrasting elevations (valley and tree line) characterized by cold/moist and warm/dry site conditions in the Lötschental in the Swiss Alps. These high-resolution tree measurements were related to precipitation and soil water $\delta^{18}$O, and to external environmental variables such as air temperature, relative air humidity and vapour pressure deficit, via both statistical and mechanistic modelling approaches. We systematically examined the system across: (1) space, by following the isotope pathway from precipitation and soil water through the xylem up to the canopy and downwards through the phloem into the tree ring; (2) time, by studying the temporal variability in the fixation of the $\delta^{18}$O signal in the tree ring across an entire growing season (2008); and (3) climatic conditions, by investigating trees at both sites.

In particular, we tested the following assumptions: the strength of the signal transfer differs between the two study sites as a result of different temperature and soil moisture conditions; twig xylem water $\delta^{18}$O consistently represents source water $\delta^{18}$O; phloem $\delta^{18}$O is the transmitter of the leaf water signal on the seasonal scale; and, most importantly, both seasonal variations in source water $\delta^{18}$O and needle water $^{18}$O enrichment propagate into the tree ring at similar strengths.

Materials and Methods

Study sites and trees

Our study region is the Lötschental, an inner-alpine dry valley in the Swiss Alps. The sites are located at two contrasting elevations characterized by cold/moist and warm/dry site conditions, respectively: at the upper tree line (2100 m above sea level (asl), 46°23′58″N, 7°44′34″E, south-south-east) of a south-facing slope and at the valley bottom on a rocky hill (1350 m asl, 46°23′29″N, 7°45′38″E, north-north-west). These sites are the end-points of an elevational transect where weekly monitoring of cambial growth and associated instrumental variables (e.g. air temperature, stem temperature, and soil moisture) has been carried out since 2007 (Moser et al., 2010; King et al., 2013). During the 2008 growing season, when the sampling for this study was performed, the mean temperature was 11.6°C at the valley bottom and 8.3°C at the tree line. The valley site is drier than the tree-line site (0.16 m$^3$ m$^{-3}$ volumetric water content (VWC) versus 0.51 m$^3$ m$^{-3}$ VWC, respectively, measured at 10 cm depth; weeks 29–43) as a result of less precipitation (292 mm vs 410 mm, respectively; weeks 25–42) and greater evaporative demand. Soils at both sites are c. 60-cm-deep podzolic cambisols.

At both sites we selected four Larix decidua Mill. trees with an average age of c. 150–200 yr, average height of c. 20 m and average breast-height diameter of 35 cm (valley) and 48 cm (tree line), respectively. The stands were quite open with all individuals exposed to direct sunlight. During the growing season the development of phenological stages was determined by recording the times of bud break and needle maturing, yellowing and fall. We defined the beginning of the growing season by bud break (valley, week 16; tree line, week 20) and the end by needle fall (valley, week 46; tree line, week 44) (Moser et al., 2010).
Sampling of needle water, xylem water, and phloem organic matter

Weekly sampling took place from 7 April 2008 (week 15) until 11 November 2008 (week 46). Samples of xylem water, needle water and phloem organic matter for each tree were obtained by cutting three 15-cm-long twigs from the sun-exposed crown at heights of between 2 and 5 m and separating them into needles, bark and wood. This was done around noon at the tree line and c. 14:00–16:00 h at the valley bottom. Needles and bark-free twigs were put into 18 × 180 mm airtight closed glass tubes. Approximately 1 cm² of bark removed from the twigs was placed in 1.8 ml of deionized water in 2-ml exudation vials. Phloem exudation was conducted for 5 h until an equilibrium between phloem sap and water was reached (Gessler et al., 2004, 2013). All samples (water and twigs) were packed in ice in the field and during transport and later kept in the freezer at −18°C.

Needle and xylem water were obtained by cryogenic vacuum extraction at the Paul Scherrer Institute, according to Ehleringer et al. (2000). Samples in extraction vials were heated to 80°C and evaporating water was trapped in U-tubes, submerged in liquid nitrogen. Extraction was performed under a vacuum of 0.03 hPa for at least 2 h.

Phloem organic matter was obtained from the exudation solutions by successively pipetting and drying the solution at 65°C in silver capsules. To avoid any hygroscopic effects such as adsorptions by successively pipetting and drying the solution at 65°C for at least 2 h. 

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Sample preparation for tree-ring δ¹⁸O

At the end of the growing season, we took tree cores of 10 mm diameter from all sampled trees at breast height. Because of low growth rates and irregular tree ring boundaries, however, one tree per site had to be taken out of the analysis. The 2008 tree rings of the remaining three trees per site were cut into sequences of 20-μm tangential sections with a microtome. These whole-wood sections were dried and packed into silver capsules for isotope measurements. The use of whole wood instead of cellulose is justified by another study at the same sites reporting a relatively constant offset of −4.59‰ at the valley bottom and −4.55‰ at the tree line and no significant differences in the climate responses of the two wood materials during the study period 1982–2011 (Grieder, 2013).

For each 20-μm tangential section, the timing of cell formation was estimated by a model derived from the growth rates of four other representative trees at the same site, for which the number of cells in the enlarging, wall thickening and mature phase respectively, was determined by taking micro cores on a weekly basis (Rossi et al., 2003; Moset et al., 2010). A Gompertz function, relating the cell position in the tree ring to its time of formation, was calculated for the enlarging phase of cell development. The week of cell enlargement of each section was defined as the middle of the period when the cells of the corresponding section enter and exit the enlargement phase.

Sampling of precipitation and soil water

Precipitation was sampled weekly in rain collectors installed at each site. The collectors were placed in PVC pipes to protect the samples from direct solar radiation (Thimonier et al., 2005), and consisted of a funnel with a 314-cm² opening connected by tubing to a 2-l storage bottle. These bottles were buried in the soil to keep the sample cool and minimize evaporation between sampling periods (O’Driscoll et al., 2005). After recording the rain volume of each collector, a 50-ml aliquot was sampled in a plastic bottle and packed in ice, and then later frozen until oxygen isotope analysis.

Soil water was collected via tension lysimetry using glass suction plates at 10 cm depth and ceramic suction cups at 60 cm depth with five replicates per depth. The lysimeters were connected to 250-ml glass bottles, stored in an insulated and light-excluding box. A vacuum of c. 400 hPa was generated and renewed every 5 h to obtain a sample representative of conditions between the weekly samplings. Because there was a lower extracted volume during the summer, soil water was first flushed in a sealed 10-ml glass vial to minimized vapour loss, which would alter the isotopic composition of the remaining water. The overflow was collected in the 250-ml bottle. Both water samples (aliquots and overflow) were collected weekly; dry conditions at the valley site allowed only the collection of mainly spring water.

We tested for potential biases in the isotopic composition of rainwater and soil water resulting from evaporation effects during the sampling period by putting samples of Evian water (Evian-Volvic Suisse SA, Zurich, Switzerland) of known δ¹⁸O values (Spangenberg & Vennemann, 2008) into the rain collector and the lysimeter box for 1 wk. The test was performed twice in August during a warm period (16–28°C). We found no significant difference between the δ¹⁸O values of the Evian water from the original bottle (−10.21‰ ± 0.11‰) and the Evian samples from the rain collector (−10.14‰ ± 0.12‰) and the lysimeter box, respectively (−10.30‰ ± 0.04‰). The soil and rainwater samples were also stored at −18°C, until oxygen isotope measurements.

Isotope measurements

Isotope measurements were carried out at the Swiss Federal Research Institute (WSL), the Paul-Scherrer-Institute (PSI) and the University of Trier. The liquid samples were equilibrated and analysed for δ¹⁸O using either a gas bench–Delta V Advantage mass spectrometer (MS) configuration or a high-temperature conversion/elemental analyser (TC/EA), linked to a Delta Plus XP MS via a Conflo III interface (all from Thermo Fisher Scientific, Bremen, Germany). The latter instrumentation was also used to determine the δ¹⁸O values of phloem organic matter (δ¹⁸Ophloem). Bulk wood samples were analysed with an elemental analyser (EA-1102; Carlo Erba, Milan, Italy), linked to a Delta Plus XL MS via a Conflo III interface (both from Thermo Fisher Scientific).
Fisher Scientific). To ensure comparability of the measurements among the three laboratories, working standards, which were calibrated against the internationally accepted standard, were routinely cross-calibrated. The overall analytical precision was $\pm 0.2\%$ for water and $\pm 0.3\%$ for wood samples. For phloem organic matter measurements with two replicates of the same sample, the standard deviation was $\pm 0.6\%$, probably as a result of inhomogeneity of phloem exudates. All data are referenced to Vienna Standard Mean Ocean Water (VSMOW) and given in $\%$, as deviation from this standard.

### Meteorological data

At both sites, air temperature and relative humidity were measured at 15-min intervals with HOBO pro v2 sensors (U23-002; Onset, MA, USA) in the stands at 2 m height. The vapour pressure deficit (VPD) was calculated as the deficit between the actual vapour pressure and the saturation vapour pressure.

For the correlations between $\delta^{18}$O of plant water or organic matter and climatic variables, mean and maximum values for the sampling days (e.g. $T_{\text{mean}}$ and $T_{\text{max}}$ for temperature) (Fig. 1) and the 7 d preceding and including the sampling day (e.g. $T_{\text{mean week}}$ and $T_{\text{max week}}$ for temperature) were calculated.

### Mechanistic modelling

Mechanistic models were applied to test our statistically derived results and to obtain a better understanding of the main factors and processes regulating needle water evaporative $\delta^{18}$O enrichment and tree-ring $\delta^{18}$O fixation at our sites. Needle water $\delta^{18}$O at the sites of evaporation ($\delta^{18}$ONW) was modelled according to Dongmann et al. (1974) based on the assumptions of Craig & Gordon (1965) and Farquhar & Lloyd (1993):

$$\delta^{18}$ONW = $\delta^{18}$O_{source} + $\varepsilon$ + $\varepsilon_k + (\delta^{18}$O_{vapour} - $\delta^{18}$O_{source})$$

Eqn 1

$\delta^{18}$ONW is thus based on the combined influence of source water $\delta^{18}$O and the evaporation effects in the needles. For $\delta^{18}$O_{source} we used the weekly measured twig xylem water $\delta^{18}$O ($\delta^{18}$O_{xylem}) data in our calculations. $\varepsilon^+$, the equilibrium fractionation factor describing the fractionation due to phase transition from liquid to vapour, is temperature sensitive and is calculated according to Bott inga & Craig (1969):

$$\varepsilon^+ ({}^\circ\text{C}) = 2.664 - 3.206(10^i/T_i) + 1.534(10^j/T_i^2)$$

Eqn 2

where $T_i$ is the needle temperature in Kelvin. According to Barbour et al. (2002), needles of conifers are closely coupled to the atmosphere and thus needle temperature equals air temperature. This is particularly the case for all our coniferous trees growing in relatively open stands. As a consequence, we assumed for our calculations $T_i$ = $T_{\text{air}}$ as previously done for the same stand by Gessler et al. (2013). $\varepsilon_k$ represents the kinetic fractionation during water diffusion through stomata and the leaf boundary layer and was calculated according to Farquhar et al. (1989) with the fractionation factors recalculated by Cappa et al. (2003). Under European summer conditions, the isotopic composition of water vapour in the air ($\delta^{18}$O_{vapour}) can be estimated as the difference between $\delta^{18}$O_{source} and $\varepsilon^+$, assuming that water vapour is in isotopic equilibrium with soil water (Förstel & Hüt zen, 1983). The parameter that controls $\delta^{18}$ONW most strongly is relative humidity, given in Eqn 1 as the ratio of the atmospheric ($\varepsilon_a$) to intercellular ($\varepsilon$) vapour pressure, assuming that the needles’ intercellular spaces are water vapour saturated and therefore $\varepsilon_a$ = $\varepsilon$.

In a second needle water modelling approach, we calculated photosynthesis-weighted average daily $\delta^{18}$ONW values. This approach considers the fact that photosynthesis and organic matter production are usually highest in the morning when leaf water enrichment is relatively low, and in the afternoon assimilation is often lowest, when leaf water is most enriched (Cernusak et al., 2005). For every sampling date we calculated diurnal courses of $\delta^{18}$ONW as well as 15-min-resolved diurnal courses between the ratio between shortwave radiation (SWR) and VPD (SWR/VPD). From our perspective, using the ratio of SWR/VPD is the best way to consider the ratio between the diurnal course of photosynthesis and leaf water enrichment with a maximum before noon in our model. $\delta^{18}$ONW was multiplied by the SWR/VPD index, also at 15-min resolution, and divided by the daily sum of the SWR/VPD index. This results in a daily mean which is weighted towards morning/noon because of higher SWR/VPD values at that time of the day.

The leaf water model can be extended to a wood model by some simple additions, modified after Saurer et al. (1997a):

![Graph](image-url)
\[ \delta^{18}O_{\text{tree-ring}} = \delta^{18}O_{\text{source}} + f(e^+ + e_k + (\delta^{18}O_{\text{vapour}} - \delta^{18}O_{\text{source}} - e_k)\varepsilon_k/\varepsilon_l + e_w + e_p) \]

with \( \delta^{18}O_{\text{tree-ring}} \) being \( \delta^{18}O \) of whole wood in the tree ring. The biochemical fractionation \( (e_w) \) between water and oxygen of the cellulose carbonyl groups is widely assumed to be \( 27\% \) (DeNiro & Epstein, 1981), although this constant value does not consider diurnal, seasonal or climatic variation. \( e_p \) accounts for the difference between cellulose and bulk wood. As values for \( e_p \), we use here constants of \(-4.59\%\) (valley bottom) and \(-4.55\%\) (tree line), derived from \( \delta^{18}O \) measurements of whole wood and cellulose from the same study trees (Grieder, 2013). Furthermore, a damping effect \( f \), with \( 0 < f < 1 \), can be related to needle water inhomogeneity and the exchange of oxygen atoms of sucrose with xylem water during cellulose formation. This damping factor \( f \) has to be calibrated and should reflect the combined influence of physiological modification of needle water and metabolite \( \delta^{18}O \) and that of the source water \( \delta^{18}O \) signal.

**Results**

**Comparison of individual trees**

The \( \delta^{18}O \) values of xylem water (\( \delta^{18}O_{\text{Xylem}} \)), needle water (\( \delta^{18}O_{\text{NW}} \)) and phloem organic matter (\( \delta^{18}O_{\text{Phloem}} \)), respectively, were remarkably coherent among all trees at a site (Fig. 2a–c), with mean inter-series correlations always at \( P < 0.01 \). The \( \delta^{18}O_{\text{tree-ring}} \) patterns showed common variability particularly in seasonal trends, with, notably, lowest but increasing \( \delta^{18}O \) values at the beginning of the growing season at both sites (Fig. 2d). The short-term variations were generally quite modest and not particularly coherent among trees. In addition, the \( \delta^{18}O_{\text{tree-ring}} \) data trended to display offsets between trees.

**Distribution of mean values**

The mean values for all tree tissues at a site averaged over the growing season indicated that \( \delta^{18}O_{\text{Phloem}} \) was by 0.7\%/oo (valley) and 0.6\%/oo (tree line) lower than \( \delta^{18}O_{\text{tree-ring}} \). The offset between \( \delta^{18}O_{\text{Phloem}} \) and \( \delta^{18}O_{\text{NW}} \) was 17.9\%/oo in the valley and 19.7\%/oo at the tree line, compared with an offset between \( \delta^{18}O_{\text{Phloem}} \) and \( \delta^{18}O_{\text{Xylem}} \) of 32.6\%/oo and 30.7\%/oo respectively (Fig. 3b, c).

Mean \( \delta^{18}O_{\text{tree-ring}} \) values were similar at the two sites (25.3\%/oo for the valley and 25.2\%/oo for the tree line) (Fig. 3b). There was also only a small difference of 1.5\%/oo between mean \( \delta^{18}O_{\text{Xylem}} \) values (–8.1\%/oo for the valley and –9.6\%/oo for the tree line) and 1.2\%/oo between mean \( \delta^{18}O_{\text{Phloem}} \) values (24.6\%/oo for the valley and 23.4\%/oo for the tree line). Mean \( \delta^{18}O_{\text{NW}} \), however, showed a more distinct offset of 3.6\%/oo (8.1\%/oo for the valley and 4.5\%/oo for the tree line), and a value of 3.6\%/oo was also found for mean evaporative enrichment of needle water \( \Delta^{18}O_{\text{NW}} \) (16.4\%/oo and 12.8\%/oo respectively). This was calculated by subtracting \( \delta^{18}O_{\text{Xylem}} \) from \( \delta^{18}O_{\text{NW}} \).

**Seasonal patterns at the two sites**

The \( \delta^{18}O_{\text{NW}} \) and \( \Delta^{18}O_{\text{NW}} \) data showed high week-to-week variability, while the \( \delta^{18}O \) values of the other tree tissues were more damped in their weekly variations and displayed more pronounced seasonal trends (Fig. 3a).

In particular, \( \delta^{18}O_{\text{Xylem}} \) at both sites started at high values in early spring and decreased markedly at the beginning of the growing season (Fig. 3a). The minimum was reached shortly before needle maturation in the middle of May/beginning of June (valley, week 22; tree line, week 27). This initial trend was obviously decoupled from the trend in soil water at the same time with strongly depleted \( ^{18}O \) isotope values. We regard this pattern as a delayed physiological signal from winter evaporative enrichment in the twigs and omit all early values before the spring minimum for the comparison with external factors.

At the valley bottom, correlations among almost all the tree internal isotope parameters were significant, although not very high (mean of all \( r \)-values = 0.58; \( P < 0.05 \)), with the strongest correlation being found between \( \delta^{18}O_{\text{Xylem}} \) and \( \delta^{18}O_{\text{NW}} \) \( (r = 0.67; P < 0.01) \) \( \Delta^{18}O_{\text{NW}} \), however, was not significantly
related to values for the other tree tissues and most importantly not to \( \delta^{18}O_{\text{tree-ring}} \) (Table 1). These relationships were weaker at the tree line and the only significant correlation was found between \( \delta^{18}O_{\text{tree-ring}} \) and \( \delta^{18}O_{\text{NW}} \) (\( \tau = 0.63; P < 0.05 \)). This correlation, however, also disappeared when \( \Delta^{18}O_{\text{NW}} \) was compared with \( \delta^{18}O_{\text{tree-ring}} \) (Table 1). A comparison between the two sites indicated high common variability (\( P < 0.01 \)) in particular for \( \delta^{18}O_{\text{tree-ring}} \) (0.83) and \( \delta^{18}O_{\text{xylem}} \) (0.78). Values for the other tree tissues were also significantly correlated but the absolute \( \tau \) values were much lower (Table 1).

### Calibration with environmental variables

\( \delta^{18}O_{\text{xylem}} \) closely followed the seasonal patterns of \( \delta^{18}O_{\text{soil}} \) (\( \delta^{18}O_{\text{soil}_{-16}}: \tau = 0.63; P < 0.05 \); \( \delta^{18}O_{\text{soil}_{-60}}: \tau = 0.83; P < 0.01 \)) (Fig. 4a; Table 2) at the tree line, where \( \delta^{18}O \) of soil water from 10 cm (\( \delta^{18}O_{\text{soil}_{-10}} \)) and 60 cm (\( \delta^{18}O_{\text{soil}_{-60}} \)) depth was available at biweekly resolution during almost the whole growing season. This clear dependence of xylem water on soil water variations can only be assumed to hold also at the valley site; here, however, only ten data points were available for \( \delta^{18}O_{\text{soil}_{-10}} \) and only two for \( \delta^{18}O_{\text{soil}_{-60}} \).
Especially at the valley bottom, the seasonal patterns of $\delta^{18}O_{\text{xylem}}$ were significantly fingerprinted in the $\delta^{18}O_{\text{NW}}$ variations ($r = 0.69$; $P < 0.01$). If, however, only the evaporative enrichment of needle water above source water was considered ($\Delta^{18}O_{\text{NW}}$), it strongly reflected the weekly and seasonal variations of meteorological variables at both sites, particularly relative humidity (valley: $r = -0.74$; tree line: $r = -0.83$; $P < 0.01$) and VPD (valley: $r = 0.76$; tree line: $r = 0.70$; $P < 0.01$) (Fig. 4b; Table 2).

$\delta^{18}O_{\text{tree-ring}}$ at the tree line most strongly mirrored variations in $\delta^{18}O_{\text{soil}}$ ($\delta^{18}O_{\text{soil,}10}$: $r = 0.85$; $P < 0.01$; $\delta^{18}O_{\text{soil,}60}$: $r = 0.71$; $P < 0.05$) (Fig 4c). In particular, the increasing trends in $\delta^{18}O_{\text{tree-ring}}$ and $\delta^{18}O_{\text{soil}}$ in the first half of the growing season were strongly correlated. This relationship also seemed to hold for the valley site, where, however, comparison with $\delta^{18}O_{\text{soil}}$ was limited because of the small number of data. Correlations between $\delta^{18}O_{\text{tree-ring}}$ and $\delta^{18}O_{\text{ppt}}$ were also significant at both sites, although weaker compared with soil water (valley: $r = 0.58$; tree line, $r = 0.59$; $P < 0.05$). The relationships between $\delta^{18}O_{\text{tree-ring}}$ and external variables such as relative humidity and VPD, which strongly drove $\Delta^{18}O_{\text{NW}}$ at both sites, were significantly weaker than for the source water signal (Table 2).

Seasonal variations of $\delta^{18}O_{\text{phloem}}$ contained less clear signals than xylem and needle water variations. At the valley site, there was a significant relationship with $\delta^{18}O$ in precipitation ($\delta^{18}O_{\text{ppt}}$) ($r = 0.74$; $P < 0.01$) (Table 2), whereas this relationship disappeared at the tree line. Furthermore, $\delta^{18}O_{\text{phloem}}$ was correlated to maximum and average temperatures of the sampling day or the preceding week ($T_{\text{max}}$: $r = 0.49$; $P < 0.05$; $T_{\text{mean}}$: $r = 0.48$; $P < 0.05$; $T_{\text{max}}$: $r = 0.54$; $P < 0.05$) only in the valley.

No other environmental fingerprints such as those from relative humidity, VPD and/or $T$, which may have been expected to propagate via needle water enrichment, were present.

### Mechanistic modelling

Considering the fact that our measured needle water data only represent a temporal snapshot of the day which may not fully correspond to the signal finally fingerprinted in the exported assimilates, we tried to create a more temporally integrated picture by comparing (1) the modelled needle water data (Eqn 1) using input data representative of the sampling time with (2) the results from the SWR/VPD-weighted model.

Both modelled $\delta^{18}O_{\text{NW,}e}$ based on (1) and (2) were close to measured $\delta^{18}O_{\text{NW}}$ at the tree line, where sampling took place at noon (Fig. 5a). At the valley site, however, where sampling took place in the afternoon, modelled $\delta^{18}O_{\text{NW,}e}$ at sampling time overestimated the measured data by $1.56^{\circ}_{\text{soo}}$ (Fig. 5a). The modelled $\delta^{18}O_{\text{NW,}e}$ weighted by SWR/VPD underestimated the measured data by $4.04^{\circ}_{\text{soo}}$.

We estimated the influence of evaporative needle water enrichment on the oxygen isotope composition of the tree ring independently from statistical correlations by modelling $\delta^{18}O_{\text{tree-ring}}$ variations using Eqn 3. The best fit (valley, $r = 0.64$; $P < 0.01$; tree line, not significant) of the mean absolute values was obtained with the highest damping factors ($f = 1$; Fig. 5b).
Fig. 4 Environmental influences on δ\(^{18}\)O in xylem water (δ\(^{18}\)O\textsubscript{xylem}), the enrichment of needle water above source water (\(\Delta^{18}\)O\textsubscript{sw}) and δ\(^{18}\)O in tree rings (δ\(^{18}\)O\textsubscript{tree-ring}) of Larix decidua. (a) Seasonal variations of δ\(^{18}\)O\textsubscript{xylem}, δ\(^{18}\)O in precipitation (δ\(^{18}\)O\textsubscript{p}) and δ\(^{18}\)O in soil water. δ\(^{18}\)O from 10 cm soil depth (δ\(^{18}\)O\textsubscript{sw10}) and δ\(^{18}\)O from 60 cm soil depth (δ\(^{18}\)O\textsubscript{sw60}) were partly pooled among the five suction elements and partly measured as single samples (number of data points consisting of single measurements, 7; mean number of available replications, 3.6), resulting in a mean standard deviation of 0.9‰. All values represent a mixed sample of the previous week. (b) Seasonal variations of needle water enrichment above xylem water (δ\(^{18}\)O\textsubscript{sw}), relative air humidity (RH, for better comparison RH was multiplied by –1) and vapour pressure difference (VPD) (c) Seasonal variations of δ\(^{18}\)O\textsubscript{tree-ring}, δ\(^{18}\)O\textsubscript{p} and δ\(^{18}\)O\textsubscript{sw10}. Normalized data were adapted to a common mean and standard deviation to enable visual comparison of more than two data sets with different units.

However, seasonal variations were represented best when a strong damping of the needle water signal was considered with \(f=0.2\) at the valley site (\(r=0.61;\ P<0.01\)) and \(f=0.0\) at the tree line (not significant). Generally, the model worked better at the valley site than at the tree line, where stronger time lags in the signal transfer are to be expected as a result of lower metabolic rates.

Finally, we also considered the fact that the quasi-instantaneous δ\(^{18}\)O\textsubscript{NW} measured or modelled at 1 d per week might not be directly comparable with our wood data, where signals were integrated over days/weeks. Therefore, we calculated weeklong runs of the SWR/VPD-weighted needle water model and compared these data with the δ\(^{18}\)O\textsubscript{tree-ring} data for both high- and low-frequency variations. At the valley site, the relationship between the modelled weeklong δ\(^{18}\)O\textsubscript{NW} and δ\(^{18}\)O\textsubscript{tree-ring} did not markedly improve (\(r=0.56;\ P<0.05\)) compared with correlations with measured δ\(^{18}\)O\textsubscript{NW} (\(r=0.52;\ P<0.05\)). As with the measured values, any significant correlations disappeared when the xylem water trend was eliminated (\(\Delta^{18}\)O\textsubscript{NW}). At the tree line, no significant correlation was found any more between δ\(^{18}\)O\textsubscript{tree-ring} and modelled week-long δ\(^{18}\)O\textsubscript{NW} (\(r=-0.08\)) or δ\(^{18}\)O\textsubscript{NW} (\(r=-0.28\)), but only the soil water trend was mirrored in the wood.

Discussion

Coherency among individual trees

The strong inter-tree relationships for δ\(^{18}\)O\textsubscript{xylem}, δ\(^{18}\)O\textsubscript{NW} and δ\(^{18}\)O\textsubscript{phloem} respectively, suggest that all trees at a site use the same water source and are exposed to similar atmospheric conditions at the canopy. The slightly reduced, although still strong coherency of δ\(^{18}\)O\textsubscript{tree-ring} suggests that individual tree features (age, crown density etc.) do not influence oxygen isotope fractionation until assimilate production, but become important during xylem cell formation.

Differences in the signal transfer between the valley bottom and the tree line

Generally, the significant, although not very high, common signal between all tree δ\(^{18}\)O parameters at the valley site indicates a faster and more continuous transfer of the isotope signal from water to organic matter than at the tree line. The low temperatures slowing down tree metabolism at the tree line obviously lead to greater but not uniform time lags.

At the valley site, where needle water samples were taken in the afternoon, the mechanistic needle water model overestimated values at sampling time by 1.56‰. This suggests an important role of the Péclet effect. As the Péclet effect is scaling with transpiration,
we expect its influence to be strongest in the afternoon during highest transpiration (e.g. Barnard et al., 2007). It is probably of minor relevance at the tree line, with generally lower transpiration rates as a result of lower temperatures (King et al., 2013), and sampling times at noon, when peak transpiration has not yet been reached.

The inclusion of diurnal variations of needle water enrichment and the photosynthetic rate (represented by the SWR/VPD index) in the model further emphasized the effect of sampling time on δ18O. Particularly at the valley site, with afternoon conditions of high needle water enrichment and already decreasing photosynthetic activity, the measured values of δ18ONW seemed to lead to a systematic overestimation of the impact of needle water 18O enrichment on the isotopic composition of the assimilates during a diurnal course. We calculated the overestimation (as the difference between SWR/VPD-weighted δ18O and measured δ18ONW) to be c. 4‰. Thus, the use of measured absolute raw values of δ18ONW, as a 'snapshot' during the day, could introduce a bias in the interpretation of the δ18O signal transfer into photosynthetic assimilates. At the tree line, however, the values of δ18ONW, measured at noon, seemed to more closely represent the average photosynthesis-weighted diurnal values.

Temporal decoupling of xylem water δ18O from source water δ18O

At both sites, the exceptionally high δ18Oxylem values at the beginning of the growing season followed by a marked decrease are not consistent with the trend expected to result from the influence of isotopically depleted snowmelt water (Robertson et al., 2001; Trebyite et al., 2006). As upward water flow through deciduous larch trees probably stops in winter (King et al., 2013), we hypothesize a coupled effect of water storage from the previous autumn (Waring et al., 1979; Brandes et al., 2007) and additional enrichment as a result of evaporation effects in the twigs and stems (Dawson & Ehleringer, 1993). This is supported by the observation that full stomatal transpiration probably started to occur in needles as soon as source water and xylem sap flow were fully coupled again. Although not found in our study, the possibility generally cannot be excluded that this early season enrichment could cause a potential bias in the isotopic signature of the very first early wood cells, at least during the phase of cell enlargement and primary cell wall development.

The fact that δ18Oxylem consistently lay between the values of the two soil layers during the growing season suggests integrated water uptake across these relatively shallow soil depths. A certain residence time of xylem water in the tracheids (Brandes et al., 2007) and considerable time lags for water at the trunk base to reach the crown in coniferous species (2.5–21 d; Meinzer et al., 2006) could, however, also temporally decouple twig δ18Oxylem patterns from δ18Osoil during the growing season.

Decoupling of the phloem isotopic signal from the other tree tissues

The mean difference between δ18ONW and δ18Ophloem of 17.9‰ (valley) and 19.7‰ (tree line) was lower than the expected 27‰, which is given in the literature as the average value for the equilibrium fractionation factor between carbonyl oxygen and water (DeNiro & Epstein, 1979, DeNiro & Epstein, 1981; Sternberg et al., 1986; Yakir & Deniro, 1990; Cernusak et al., 2003). This mismatch was also found with modelled SWR/VPD-weighted diurnal means of δ18ONW, accounting for the fact that the transfer of the needle water isotope signal into assimilates is highest before noon when δ18ONW is not yet at its diurnal maximum (Cernusak et al., 2005). Moreover, δ18Ophloem was between 0.7 and 0.8‰ lower than δ18Otree-ring and consequently more than 5‰ below the estimated δ18Otree-ring values taking into account the ε18O values given by Grieder (2013). According to current understanding (e.g. Cernusak et al., 2005), δ18Otree-ring should, however, be lower than δ18Ophloem, because, during cellulose formation from phloem-derived sucrose, partial exchange of oxygen atoms with unenriched xylem water occurs (Rodén et al., 2000).

This lower than expected δ18O enrichment of phloem organic matter indicates a partial decoupling of the phloem isotopic signal from the leaf-level processes. In the past it was mainly assumed that phloem sugars convey the δ18O signal from the leaf water via the assimilates to the trunk without any change, and that only during cellulose production is the leaf-level isotopic signal modified as a result of the exchange of carbonyl oxygen with xylem water. Only recently, Gessler et al. (2013) reported a species-specific decoupling between the leaf water and the phloem organic matter δ18O signal over the short term (i.e. during a diel course) in five tree species representing several life forms. By tracking the transfer of the leaf water δ18O signal to leaf sugars and phloem-transported organic matter, they found that, while leaf sugars showed the expected δ18O enrichment of 27‰ above leaf water in all species, phloem sugars were up to 10‰ less enriched than expected in Scots pine, European larch and Alpine ash (Eucalyptus delegatensis). Here, we can show that such an effect also holds for European larch over the whole growing season. Gessler et al. (2013) explored several mechanisms that might be responsible for their observation as well as for the consistent findings obtained here. The first mechanism is related to the fact that phloem organic matter in trees consists of sugars with various origins and residence times and thus integrates the canopy isotope signal between hours and days (Keitel et al., 2003; Brandes et al., 2006). Phloem is partly loaded with sugars during the day, but assimilates is accumulated in the chloroplasts as transitory starch and loaded into the phloem during the night (Smith et al., 2003; Weise et al., 2004; Gessler et al., 2008). In this case, not only is the needle water signal time-shifted and damped, but phloem exported sugars are also partially labelled with lower night values of δ18ONW, as assimilates are influenced by medium water during starch breakdown (Gessler et al., 2007).

A second mechanism discussed by Gessler et al. (2013) is related to phloem loading in the central cylinder of the needles in conifers. The authors assume that the water in this compartment is clearly less enriched than the water in the leaf lamina and any exchange of organic oxygen with the surrounding water either directly before or during phloem loading would result in a decrease in 18O enrichment of phloem sugars.
Moreover, \(^{18}\text{O}\) enrichment of organic matter (re)fixed in the bark, where reaction water is not or only slightly enriched, should be approximately 27\(^{\circ}\)\text{o} higher than source water (Cernusak et al., 2005), being well below the enrichment for sugars fixed in leaves. Such bark photosynthesis could also contribute to the twig phloem organic matter of our larch trees with a photosynthetically active (green) layer within the twig bark during the whole growing season. Finally, the rather low \(^{18}\text{O}\)phloem compared with \(^{18}\text{O}\)tree-ring or \(^{18}\text{O}\)cellulose might be partially explained by our phloem sampling strategy. \(^{18}\text{O}\) in phloem organic matter sampled from twigs, unlike in trunk phloem organic matter, might not be fully representative for the whole crown. Studies with Scots pine (Barnard et al., 2007; Brandes et al., 2007) showed that trunk phloem organic matter was in fact more enriched than twig phloem. Despite this uncertainty, our results, however, do not indicate a further strong oxygen atom exchange between organic matter and reaction water during cellulose synthesis.

Variations in source water \(^{18}\text{O}\) dominate the \(^{18}\text{O}\)tree-ring pattern on a seasonal scale

We found strong evidence that seasonal variations in source water may have a stronger impact on \(^{18}\text{O}\)tree-ring variations than seasonal variations in needle water enrichment. First, this conclusion is supported by the finding that variations in \(^{18}\text{O}\)soil,10 explained 72\% of the variance of \(^{18}\text{O}\)tree-ring at the tree line and by the low contribution of variations in \(^{18}\text{O}\)NW at both sites. This low contribution also holds when \(^{18}\text{O}\)tree-ring is compared with modelled \(^{18}\text{O}\)NW integrated over a whole week.

Secondly, strong relationships between the sites with respect to \(^{18}\text{O}\)xylem and \(^{18}\text{O}\)tree-ring suggest a dominating influence of source water as a common external driver at both sites. By contrast, lower \(r\) values for \(^{18}\text{O}\)NW and \(^{18}\text{O}\)NW, respectively, suggest a dominating influence of more site-specific environmental conditions such as VPD. Also, associations of \(^{18}\text{O}\)tree-ring with external variables such as relative humidity and VPD, which strongly drove \(^{18}\text{O}\)NW at both sites, were significantly weaker than for the source water signal or even absent (Table 2).

Thirdly, our observations on decoupling of the phloem signal suggest that a large part of the leaf water enrichment is already lost in the phloem organic matter and that the chemical reactions during cellulose synthesis play a minor role in incorporating the source water signal into the tree ring tissue, at least in European larch. Finally, mechanistic modelling supports our findings. At the valley site, where the tree-ring model worked well, seasonal variations were captured best when the damping factor \(f\) (Eqn 2) was small, that is, the original needle water isotope signal was strongly damped. The agreement of absolute values are better for a higher \(f\) value, but this caused the amplitude of \(^{18}\text{O}\)tree-ring variations to be greatly overestimated.

Remaining uncertainties in the tree-ring model could result from the fact that, after the cell enlargement phase used for the dating of our tree-ring sections in this study, carbon allocation still continues for approximately 2 months during subsequent cell wall thickening and lignification. In addition, the application of the damping factor \(f\), integrating several physiological processes at the leaf level (Péclet effect) and in the stem (oxygen atom exchange), might not be adequate for modelling intra-annual courses. If the Péclet effect and/or oxygen atom exchange during wood/cellulose synthesis is not constant over the year, then \(f\) is not constant either.

One could also argue that the \(\varepsilon_{\text{cp}}\) value of \(-4.55\%\) could potentially vary during the season as a result of varying amounts of primary (e.g., cellulose) and secondary (e.g., lignin and lipids) compounds in the xylem cells with distinctly different isotope values (Wilson & Grinsted, 1977). An ultrastructural perspective on cell wall lignification indicates, however, little difference in lignin distribution between early and latewood tracheids in gymnosperms (Donaldson, 2001 and references therein).

In conclusion, our findings based on both statistical and mechanistic models indicate that seasonal variations in \(^{18}\text{O}\)tree-ring predominantly mirror seasonal variations in the source water. The distinct week-to-week variations in \(^{18}\text{O}\)NW and their clear dependence on weather conditions were scarcely propagated into the tree rings. The source water signal relies partly on \(^{18}\text{O}\)xylem preserved in the needles and partly on post-photosynthetic oxygen atom exchange with medium water. It was postulated that such an exchange might occur not only during cellulose synthesis (e.g., Sternberg et al., 1986; Cernusak et al., 2005) but also during phloem loading and phloem transport and can lead to a strong uncoupling between the leaf water and \(^{18}\text{O}\)tree-ring signals (Offermann et al., 2011; Gessler et al., 2013).

The \(^{18}\text{O}\) of the source water includes the isotopic signal of recent precipitation and water pools in the soil that damp the isotopic variation. Consequently, the strength of the precipitation signal recorded in the source water is most crucial for the application of \(^{18}\text{O}\)tree-ring for climate reconstruction. Based on the mechanistic insights provided here, we suggest that the strongest climate (or at least source water) signal should be recorded at sites where soils are most frequently supplied with precipitation water during the growing period, namely in temperate regions with high summer precipitation. This finding is supported by results from a European tree-ring isotope network containing the strongest climate signal at temperate sites in the UK and northern France and weak signals at dry Mediterranean sites (Treydte et al., 2007). Furthermore, we recommend that future reconstruction efforts should focus on the reconstruction of atmospheric phenomena that are directly linked to the determination of the isotope ratio in source water. We note that the latter probably varies as a function of geographical location and therefore requires spatially widespread networks of annually resolved tree-ring-based \(^{18}\text{O}\) measurements.

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