

# **Seasonal pathway of oxygen isotopes from precipitation and soil to the tree ring: Source water versus needle water enrichment**

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## Summary

- ❖ For an accurate interpretation of tree-ring  $\delta^{18}\text{O}$  is disentangling the mechanisms underlying the variations in the tree's internal water cycle and understanding the transfer of source versus leaf water  $\delta^{18}\text{O}$  to the phloem and stem wood.
- ❖ We studied the seasonal pathway 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  $\delta^{18}\text{O}$  records of precipitation, soil water, xylem and needle water, phloem organic matter and tree rings were developed.
- ❖ Week-to-week variations in needle-water  $^{18}\text{O}$  enrichment were strongly controlled by weather conditions during the growing season. These seasonal variations were, however, not significantly fingerprinted in tree-ring  $\delta^{18}\text{O}$ . Instead, seasonal trends in tree-ring  $\delta^{18}\text{O}$  predominantly mirror trends in the source water, including recent precipitation and soil water pools. Modelling results support these findings: Seasonal tree-ring  $\delta^{18}\text{O}$  variations are captured best when the week-to-week variations of the leaf water signal are suppressed.
- ❖ Our results suggest that best climate signals in tree-ring  $\delta^{18}\text{O}$  variations should be recorded at temperate sites with humid conditions and precipitation maxima during the growing season.

*Key words:* Dendroecology; *Larix decidua*; leaf water enrichment; oxygen isotopes; phloem; tree line; tree physiology; xylem water

## INTRODUCTION

Oxygen isotopes in tree rings ( $\delta^{18}\text{O}_{\text{tree-ring}}$ ) 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-Henne *et al.*, 2007; Saurer *et al.*, 2008), relative air humidity (Ramesh *et al.*, 1986; Saurer *et al.*, 1997b; Robertson *et al.*, 2001; Edwards *et al.*, 2008), or 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  $\delta^{18}\text{O}$  seems to be resistant against air pollution (Rinne *et al.*, 2010), the strength of the climate signal can strongly vary. Several environmental parameters may be represented with near equal fidelity (Kress *et al.*, 2010) or may show temporally unstable relations with  $\delta^{18}\text{O}_{\text{tree-ring}}$  (Treydte *et al.*, 2007; Reynolds-Henne *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  $\delta^{18}\text{O}$  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). Up to now it is assumed that no measureable 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  $\delta^{18}\text{O}$  are reasonably well

described with mechanistic models. These processes are evaporative enrichment (Dongmann *et al.*, 1974) and the modification of the leaf water enrichment by the convection of unenriched source water to the leaf evaporative sites. Lamina leaf water  $\delta^{18}\text{O}$  is on the one hand determined by the evaporative enrichment at the sites of evaporation (Dongmann *et al.* 1974) and the diffusion of this  $^{18}\text{O}$  enriched water back to the leaf lamina. This diffusion process is on the other hand opposed by the convective transport of unenriched xylem water through the leaf lamina to the evaporation sites via the transpiration stream. The net effect of these convective vs. diffusive transport processes is called 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  $\delta^{18}\text{O}$  of the leaf water (i.e. the water in which the chemical reactions occur) is imprinted on the newly assimilated organic matter. The carbonyl group is enriched in  $^{18}\text{O}$  by 27‰ compared to 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 in downstream metabolic processes remains still fragmentary, in particular how these processes are influenced by environmental versus plant internal factors (Farquhar *et al.*, 1998; Brandes *et al.*, 2006; Sternberg 2008).

Many studies have focused on the mechanistic processes behind these fractionation processes and their implications for  $\delta^{18}\text{O}_{\text{tree-ring}}$  (Farquhar *et al.*, 1998; Barbour & Farquhar, 2000; Roden *et al.*, 2000; Barbour *et al.*, 2002; Roden *et al.*, 2005; Ogée *et al.*, 2009). Only 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 do not allow general conclusions but are often species specific although taking into account (i) transport time lags and the (ii) generally acknowledged exchange of 42% of the sucrose oxygen with non-enriched stem water during cellulose synthesis (Gessler *et al.*, 2009): Whereas the  $\delta^{18}\text{O}$  signal could be traced from leaf water to the tree ring in Scots pine, the tree-ring oxygen isotopic signal in European beech 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 follow the complete pathway of  $\delta^{18}\text{O}$  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  $\delta^{18}\text{O}$  of *Larix decidua* Mill. growing at a cool/moist and a warm/dry site in the Lötschental/Swiss Alps. These high-resolution tree measurements are related to precipitation and soil water  $\delta^{18}\text{O}$ , and to external environmental variables such as air temperature, relative air humidity and vapor pressure deficit via both statistical and mechanistic modelling approaches. We systematically address the system across (i) 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, (ii) time, by studying the temporal variability in the fixation of the  $\delta^{18}\text{O}$  signal in the tree ring across an entire growing season (2008) and (iii) climate conditions, by investigating trees both at the upper treeline and at the valley bottom.

In particular, we tested the following assumptions: 1) the strength of the signal transfer differs between both sites due to different temperature and soil moisture conditions. 2) Twig xylem water  $\delta^{18}\text{O}$  consistently represents source water  $\delta^{18}\text{O}$ . 3) Phloem  $\delta^{18}\text{O}$  is the transmitter of the leaf water signal on the seasonal scale. And most importantly 4) both seasonal variations in source water  $\delta^{18}\text{O}$  and needle water  $^{18}\text{O}$ -enrichment propagate into the tree ring at similar strength.

## **MATERIAL AND METHODS**

### *Study sites and trees*

Our study region is the Lötschental, an inner-alpine dry valley in the Swiss Alps. Sites are located at two altitudes: At the upper tree line (2100m asl, 46°23'58N, 7°44'34E, SSE) of a south-facing slope and at the valley bottom on a rocky hill (1350m asl, 46°23'29N, 7°45'38E, NNW). 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, soil moisture) are collected since 2007 (Moser *et al.*, 2010; King *et al.*, 2013). During the 2008 growing season, the mean temperature was 11.6°C at the valley bottom and 8.3°C at the treeline. The valley site is

generally drier than the tree line site ( $0.16\text{m}^3/\text{m}^3$  volumetric water content (VWC) versus  $0.51\text{m}^3/\text{m}^3$  VWC, measured in 10cm depth; week 29-43) due to less precipitation (292mm versus 410mm; week 25-42) and greater evaporative demand. Soils at both sites are ~60cm deep podzolic cambisols.

At both sites we selected four *Larix decidua* trees with an average age of ~150-200 years, average height of ~20 meters and average breast height diameter of 35cm (valley) and 48cm (treeline) respectively. The stands were quite open with all individuals exposed to direct sunlight. During the vegetation period the development of phenological stages was determined by recording the times of bud break, needle maturing, yellowing and fall. We defined the beginning of the growing season by bud break (valley: week 16, treeline: week 20) and the end by needle fall (valley: week 46, treeline: 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 15cm long twigs of the sun exposed crown at heights between 2 and 5 meters and separating them into needles, bark and wood. This was done around noon at the treeline and around 2-4 pm at the valley bottom. Needles and bark-free twigs were put into 18x180mm airtight closed glass tubes. Approximately  $\sim 1\text{cm}^2$  of bark removed from the twigs was placed into 1.8ml deionized water in 2ml exudation vials. Phloem exudation was conducted for 5 hours until an equilibrium between phloem sap and water was reached (Gessler *et al.*, 2004; Gessler *et al.*, 2013). All samples (water and twigs) were packed in ice in the field and during transport and later kept in the freezer at  $-18^\circ\text{C}$ .

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  $80^\circ\text{C}$  and evaporating water was trapped in U-tubes, submerged in liquid nitrogen. Extraction was performed under a vacuum of 0.03hPa for at least 2 hours.

Phloem organic matter was obtained from the exudation solutions by successively pipetting and drying the solution at  $65^\circ\text{C}$  in silver capsules. To avoid any hygroscopic effects such as adsorption of water vapor to the sugar surfaces of the phloem samples

(Cernusak *et al.*, 2003) the crimped capsules were dried for 24 hours in a desiccator and then kept under dry argon in the autosampler of the Elemental Analyser.

### *Sample preparation for tree-ring $\delta^{18}O$*

At the end of the growing season, we took tree cores of 10mm diameter from all sampled trees at breast height. Due to 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 3 trees per site were cut into sequences of 20 $\mu$ m 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 treeline and no significant differences in the climate response of both wood materials during the study period 1982-2011 (Grieder 2013).

For each 20 $\mu$ m tangential section, the timing of cell formation was estimated by a model derived from the growth rate of four other representative trees at the same site, for which the amount of enlarging, wall thickening and mature cells was determined by taking micro cores on a weekly basis (Rossi *et al.*, 2003; Moser *et al.*, 2010). A Gompertz function, relating cell position in the tree ring with its time of formation, was calculated for the enlarging phase of cell development. The week of cell enlargement of each section was estimated as the centre 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 an 314cm<sup>2</sup> opening connected by tubing to a two-liter storage bottle. These bottles were buried into 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 50ml aliquot was sampled in a PE 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 10cm depth and ceramic suction cups at 60cm depth with five replicates per depth. The lysimeters were connected to 250ml glass bottles, stored in an insulated and light-protected box. A vacuum of about 400hPa was generated and renewed every five hours to obtain a sample representative for conditions between the weekly samplings. Because of lower extracted volume during the summer, soil water was first flushed in a sealed 10ml glass-vial to minimize the vapor loss, which would alter the isotopic composition of the remaining water. The overflow was collected in the 250ml bottle. Both water samples (aliquots and overflow) were collected weekly; dry conditions at the valley site allowed only for the collection of mainly spring water.

We tested potential biases of the isotopic composition of rainwater and soil water resulting from evaporation effects during the sampling period by putting samples of Evian water (commercial mineral water) of known  $\delta^{18}\text{O}$  values (Spangenberg & Vennemann, 2008) into the rain collector and in the lysimeter box for one week. The test was performed twice in August during a warm period (16°C- 28°C). We found no significant difference between the  $\delta^{18}\text{O}$  values of the Evian water from the original bottle ( $10.21\text{‰} \pm 0.11\text{‰}$ ) and the Evian samples from the rain collector ( $10.14\text{‰} \pm 0.12\text{‰}$ ) and the lysimeter box respectively ( $10.30\text{‰} \pm 0.04\text{‰}$ ). The soil and rainwater samples were stored at -18°C as well, until oxygen isotope measurements.

### *Isotope measurements*

Isotope measurements were distributed between the WSL, the PSI and the University of Trier. The liquid samples were either equilibrated and analyzed for  $\delta^{18}\text{O}$  via a gas bench - Delta V Advantage mass spectrometer (MS) configuration or with a TC/EA High Temperature-Conversion Elemental analyzer, linked to a Delta Plus XP MS via a Conflo III interface. The latter instrumentation was also used to determine the  $\delta^{18}\text{O}$  values of phloem organic matter ( $\delta^{18}\text{O}_{\text{phloem}}$ ). Bulk wood samples were analyzed with an Elemental analyzer (EA-1102, Carlo Erba, Milano Italy), linked to a Delta Plus XL MS via a Conflo III interface (all MS with periphery are from Thermo Fisher Scientific, Bremen, Germany). To ensure comparability of the measurements between the three labs, working standards, which were calibrated against the internationally accepted standard, were routinely cross-calibrated. The overall analytical precision was  $\pm 0.2\text{‰}$  for water and  $\pm 0.3\text{‰}$  for wood samples. For phloem organic matter



measurements with two replicates of the same sample the standard deviation was  $\pm 0.6\text{‰}$  probably due to inhomogeneity of phloem exudates. All data are referenced to VSMOW and given in  $\text{‰}$  as deviation from this standard.

#### *Meteorological data*

At both sites air temperature and relative humidity were measured in 15-minute intervals with HOBO pro v2 sensors (U23-002) in the stands at 2m height. The vapor pressure difference (VPD) was calculated as the difference between the actual vapor pressure ( $e_a$ ) and the saturation vapor pressure ( $e_s$ ).

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

#### *Mechanistic modelling*

Mechanistic models were applied to test our findings from statistical relations and to derive a better understanding of the main factors and processes regulating needle water evaporative  $^{18}\text{O}$  enrichment and tree-ring  $\delta^{18}\text{O}$  fixation at our sites. Needle water  $\delta^{18}\text{O}$  at the sites of evaporation ( $\delta^{18}\text{O}_{\text{NWE}}$ ) was modelled according to Dongman *et al.* (1974) based on assumptions of Craig & Gordon (1965) and Farquhar & Lloyd (1993):

$$\delta^{18}\text{O}_{\text{NWE}} = \delta^{18}\text{O}_{\text{source}} + \varepsilon^+ + \varepsilon_k + (\delta^{18}\text{O}_{\text{vapor}} - \delta^{18}\text{O}_{\text{source}} - \varepsilon_k) e_a/e_i \quad (\text{Eqn 1})$$

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

$$\varepsilon^+ (\text{‰}) = 2.664 - 3.206 (10^3 / T_1) + 1.534 (10^6 / T_1^2) \quad (\text{Eqn 2})$$

where  $T_1$  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  $T_{\text{leaf}}=T_{\text{air}}$  as previously done for the same stand by Gessler *et al.* (2013).  $\varepsilon_k$  represents the kinetic fractionation during water diffusion through stomata and 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 vapor in the air ( $\delta^{18}\text{O}_{\text{vapor}}$ ) can be estimated as the difference of  $\delta^{18}\text{O}_{\text{source}}$  and  $\varepsilon^+$ , assuming that water vapor is in isotopic equilibrium with soil water (Förstel & Hütten, 1983). The parameter, which controls  $\delta^{18}\text{O}_{\text{NW}}$  the most is RH, given in Eqn 1 as the ratio of the atmospheric ( $e_a$ ) and intercellular ( $e_i$ ) vapor pressure, assuming the needles' intercellular spaces are water vapor saturated and therefore  $e_i = e_s$ .

In a second needle water modelling approach, we calculated photosynthesis-weighted average daily  $\delta^{18}\text{O}_{\text{NW}_e}$  values. This approach considers the fact that photosynthesis and the organic matter production is usually highest in the morning when the leaf water enrichment is relatively low, and in the afternoon the assimilation is often lowest, when leaf water is most enriched (Cernusak *et al.*, 2005). For every sampling date we calculated diurnal courses of  $\delta^{18}\text{O}_{\text{NW}_e}$  as well as 15min-resolved diurnal courses of the ratio between short wave radiation and VPD (SWR/VPD). From our perspective 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. The  $\delta^{18}\text{O}_{\text{NW}_e}$  data were multiplied with the SWR/VPD index data, 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 changed after Saurer *et al.* (1997a):

$$\delta^{18}\text{O}_{\text{tree-ring}} = \delta^{18}\text{O}_{\text{source}} + f[\varepsilon^+ + \varepsilon_k + (\delta^{18}\text{O}_{\text{vapor}} - \delta^{18}\text{O}_{\text{source}} - \varepsilon_k) e_a/e_i] + \varepsilon_{\text{wc}} + \varepsilon_{\text{cp}} \quad (\text{Eqn 3})$$

with  $\delta^{18}\text{O}_{\text{tree-ring}}$  being  $\delta^{18}\text{O}$  of whole wood in the tree ring. The biochemical fractionation ( $\varepsilon_{\text{wc}}$ ) 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.  $\varepsilon_{\text{cp}}$  accounts for the difference between cellulose and bulk wood. As value for  $\varepsilon_{\text{cp}}$  we use here a constant of -4.59 (valley

bottom) and -4.55‰ (treeline), derived from  $\delta^{18}\text{O}$  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  $\delta^{18}\text{O}$  and that of the source water  $\delta^{18}\text{O}$  signal.

## RESULTS

### *Comparison of individual trees*

The  $\delta^{18}\text{O}$  values of xylem water ( $\delta^{18}\text{O}_{\text{xylem}}$ ), needle water ( $\delta^{18}\text{O}_{\text{NW}}$ ) and phloem organic matter ( $\delta^{18}\text{O}_{\text{phloem}}$ ) respectively are remarkably coherent among all trees at a site (Fig. 2a-c) with mean inter-series correlations always at  $p < 0.01$ . The  $\delta^{18}\text{O}_{\text{tree-ring}}$  patterns show common variability particularly in the long-term trends, with notably lowest but increasing  $\delta^{18}\text{O}$  values at the beginning of the growing season at both sites (Fig. 2d). The short-term variations are generally quite modest and not particularly coherent among trees. Besides, the  $\delta^{18}\text{O}_{\text{tree-ring}}$  data tend to display offsets between the trees.

### *Distribution of mean values*

The mean values for all tree tissues at a site averaged over the growing season indicate that  $\delta^{18}\text{O}_{\text{phloem}}$  is by 0.7‰ (valley) and 0.8‰ (treeline) lower than  $\delta^{18}\text{O}_{\text{tree-ring}}$ . The offset between  $\delta^{18}\text{O}_{\text{phloem}}$  and  $\delta^{18}\text{O}_{\text{NW}}$  is 17.9‰ in the valley and 19.7‰ at the treeline, compared to an offset between  $\delta^{18}\text{O}_{\text{phloem}}$  and  $\delta^{18}\text{O}_{\text{xylem}}$  of 32.6‰ and 30.7‰ respectively (Fig. 3b, c).

Mean  $\delta^{18}\text{O}_{\text{tree-ring}}$  values are similar at both sites (25.3‰ valley and 25.2‰ treeline) (Fig. 3b). There exists only a slight difference of 1.5‰ between mean  $\delta^{18}\text{O}_{\text{xylem}}$  (-8.1‰ valley and -9.6‰ treeline) and also  $\delta^{18}\text{O}_{\text{phloem}}$  values are with 1.2‰ quite low (24.6‰ valley and 23.4‰ treeline). Mean  $\delta^{18}\text{O}_{\text{NW}}$ , however, shows a more distinct offset of

3.6‰ (8.1‰ valley and 4.5‰ treeline) and this value holds also for mean  $\Delta^{18}\text{O}_{\text{NW}}$  (16.4‰ and 12.8‰ respectively).

### *Seasonal patterns at both sites*

The  $\delta^{18}\text{O}_{\text{NW}}$  data and also the data of evaporative enrichment of needle water ( $\Delta^{18}\text{O}_{\text{NW}}$ ; calculated by subtracting  $\delta^{18}\text{O}_{\text{xylem}}$  from  $\delta^{18}\text{O}_{\text{NW}}$ ) show high week-to-week variability, while the  $\delta^{18}\text{O}$  data of the other tree tissues contain damped short-term variations and more pronounced long-term seasonal trends (Fig. 3a).

In particular,  $\delta^{18}\text{O}_{\text{xylem}}$  at both sites starts at high values in early spring and decreases markedly at the beginning of the growing season (Fig. 3a). The minimum was reached shortly before needle maturation middle of May/beginning of June (valley: week 22; treeline: week 27). This initial trend is obviously decoupled from the trend in soil water at the same time with strongly depleted  $^{18}\text{O}$  isotope values. We regard it here as a delayed physiological signal from winter evaporative enrichment and disregarded all early values before the minimum from the comparison with external factors.

At the valley bottom correlations between all the tree internal parameters are significant, although not very high (mean of all r-values = 0.58,  $p < 0.05$ ) with maximum correlation between  $\delta^{18}\text{O}_{\text{xylem}}$  and  $\delta^{18}\text{O}_{\text{NW}}$  ( $r = 0.67$ ,  $p < 0.01$ ). Particularly  $\Delta^{18}\text{O}_{\text{NW}}$ , however, is not significantly related to the other tree tissues and most importantly not to  $\delta^{18}\text{O}_{\text{tree-ring}}$  (Table 1). These relations are weaker at the treeline and the only significant correlation is found between  $\delta^{18}\text{O}_{\text{tree-ring}}$  and  $\delta^{18}\text{O}_{\text{NW}}$  ( $r = 0.63$ ,  $p < 0.05$ ). This correlation, however, also breaks down if the xylem water trend is eliminated and instead  $\Delta^{18}\text{O}_{\text{NW}}$  is compared with  $\delta^{18}\text{O}_{\text{tree-ring}}$  (Table 1). Comparison between both sites indicates high common variability ( $p < 0.01$ ) in particular for  $\delta^{18}\text{O}_{\text{tree-ring}}$  (0.83) and  $\delta^{18}\text{O}_{\text{xylem}}$  (0.78) respectively. Also the other tree tissues are significantly related but the absolute r-values are much lower (Table 1) ( $\delta^{18}\text{O}_{\text{phloem}}$  0.59,  $\delta^{18}\text{O}_{\text{NW}}$  0.57 and  $\Delta^{18}\text{O}_{\text{NW}}$  0.58).

### *Calibration with environmental variables*

$\delta^{18}\text{O}_{\text{xylem}}$  nicely follows the seasonal patterns in  $\delta^{18}\text{O}_{\text{soil}}$  ( $\delta^{18}\text{O}_{\text{soil}_{10}}$ :  $r = 0.63$ ,  $p < 0.05$ ;  $\delta^{18}\text{O}_{\text{soil}_{60}}$ :  $r = 0.83$ ,  $p < 0.01$ ) (Fig. 4a; Table 2) at the treeline, where  $\delta^{18}\text{O}$  of soil water from 10cm ( $\delta^{18}\text{O}_{\text{soil}_{10}}$ ) and 60cm ( $\delta^{18}\text{O}_{\text{soil}_{60}}$ ) depth are mostly weekly available during

the growing season. This clear dependency of xylem water on soil water variations can just be assumed to hold also at the valley site due to the fact that only ten data points were available for  $\delta^{18}\text{O}_{\text{soil}_{10}}$  and even only two for  $\delta^{18}\text{O}_{\text{soil}_{60}}$ .

Especially at the valley bottom the seasonal patterns of  $\delta^{18}\text{O}_{\text{xylem}}$  are significantly fingerprinted in the  $\delta^{18}\text{O}_{\text{NW}}$  variations ( $r=0.69$ ,  $p<0.01$ ). If, however, only the evaporative enrichment of needle water above source water is considered by  $\Delta^{18}\text{O}_{\text{NW}}$ , it strongly reflects the short- and long-term variations of meteorological variables at both sites (Fig. 5a), particularly RH (valley:  $r=-0.74$ , treeline:  $r=-0.83$ ;  $p<0.01$ ) and VPD (valley:  $r=0.76$ , treeline:  $r=0.70$ ;  $p<0.01$ ) (Fig. 4b; Table 2).

$\delta^{18}\text{O}_{\text{tree-ring}}$  at the treeline most strongly mirror variations in  $\delta^{18}\text{O}_{\text{soil}}$  ( $\delta^{18}\text{O}_{\text{soil}_{10}}$ :  $r=0.85$ ,  $p<0.01$ ;  $\delta^{18}\text{O}_{\text{soil}_{60}}$ :  $r=0.71$ ,  $p<0.05$ ) (Fig 4c). Particularly the increasing trend in the first half of the growing season is well matched. This relation seems to hold also for the valley site, where, however, comparison with  $\delta^{18}\text{O}_{\text{soil}}$  is limited due to the small number of data. Correlations between  $\delta^{18}\text{O}_{\text{tree-ring}}$  and  $\delta^{18}\text{O}_{\text{ppt}}$  are also significant at both sites, although lower compared to the soil water (valley:  $r=0.58$ , treeline:  $r=0.59$ ;  $p<0.05$ ). Any relations of  $\delta^{18}\text{O}_{\text{tree-ring}}$  to external variables such as RH and VPD, which strongly drive  $\Delta^{18}\text{O}_{\text{NW}}$  at both sites, are significantly weaker than the source water signal (Table 2).

Seasonal variations of  $\delta^{18}\text{O}_{\text{phloem}}$  contain unclear signals compared to xylem and needle water variations. At the valley site there is a significant relation to  $\delta^{18}\text{O}$  in precipitation ( $\delta^{18}\text{O}_{\text{ppt}}$ ) ( $r=0.74$ ,  $p<0.01$ ) (Table 2) whereas this relation disappears at the treeline. Furthermore  $\delta^{18}\text{O}_{\text{phloem}}$  is correlated to maximum and average temperatures of the sampling day or the preceding week ( $T_{\text{max}}$ :  $r=0.49$ ,  $p<0.05$ ;  $T_{\text{mean week}}$ :  $r=0.48$ ,  $p<0.05$ ;  $T_{\text{max week}}$ :  $r=0.54$ ,  $p<0.05$ ) only at the valley. Any other environmental fingerprints such as those from RH, VPD and/or T, which may have been expected to propagate downstream from the needles, are not present anymore.

### *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 temporarily integrated picture by

comparing i) the modelled needle-water data using input data representative for the sampling time with ii) the results from the SWR/VPD-weighted model (Eqn. 1).

Both modelled  $\delta^{18}\text{O}_{\text{NWe}}$  based on i) and ii) are close to measured  $\delta^{18}\text{O}_{\text{NW}}$  at the treeline where sampling took place at noon. At the valley site, however, where sampling took place in the afternoon, modelled  $\delta^{18}\text{O}_{\text{NWe}}$  at sampling time overestimate the measured data by 1.56‰ (treeline: measured=5.21‰, modelled=5.75‰; valley: measured=8.20‰, modelled=9.76‰) (Fig. 5a). In contrast, the SWR/VPD-weighted modelled  $\delta^{18}\text{O}_{\text{NWe}}$  are on average 4.04‰ lower than measured  $\delta^{18}\text{O}_{\text{NW}}$ .

We estimated the influence of evaporative needle water enrichment on the oxygen isotope composition of the tree ring independently from statistical correlations by modelling  $\delta^{18}\text{O}_{\text{tree-ring}}$  variations with Eqn 3. The best fit (valley:  $r=0.64$ ,  $p<0.01$ ; treeline not significant) of the mean absolute values appears with the highest damping factors ( $f=1$ ; Fig. 5b). However, seasonal variations are represented best when a strong damping of the needle water signal is considered with  $f=0.2$  at the valley site ( $r=0.61$ ,  $p<0.01$ ) and even  $f=0.0$  at the treeline (not significant). Generally, the model works better at the valley site than at the treeline, where stronger time lags in the signal transfer are to be expected due to lower metabolism rates.

Finally, we also considered the fact that the quasi-instantaneous  $\delta^{18}\text{O}_{\text{NW}}$  measured or modelled at one day per week might not be directly comparable with our wood data where signals are integrated over days/weeks. Therefore, we calculated weeklong runs of the SWR/VPD-weighted needle water model and compared these data with the  $\delta^{18}\text{O}_{\text{tree-ring}}$  data for both high and low frequency variations. At the valley site the relation between the modelled weeklong  $\delta^{18}\text{O}_{\text{NWe}}$  and  $\delta^{18}\text{O}_{\text{tree-ring}}$  does not markedly improve ( $r=0.56$ ,  $p<0.05$ ) compared to correlations with measured  $\delta^{18}\text{O}_{\text{NW}}$  ( $r=0.52$ ,  $p<0.05$ ). As with the measured values any significant correlations disappear when the xylem water trend is eliminated ( $\Delta^{18}\text{O}_{\text{NWe}}$ ). At the treeline, no significant correlation is found anymore between  $\delta^{18}\text{O}_{\text{tree-ring}}$  and modelled weeklong  $\delta^{18}\text{O}_{\text{NWe}}$  ( $r=-0.08$ ) or  $\Delta^{18}\text{O}_{\text{NWe}}$  ( $r=-0.28$ ), but only the soil water trend is mirrored in the wood.

## DISCUSSION

### *Coherency among individual trees*

The strong inter-tree relations for  $\delta^{18}\text{O}_{\text{xylem}}$ ,  $\delta^{18}\text{O}_{\text{NW}}$  and  $\delta^{18}\text{O}_{\text{phloem}}$  respectively suggest that all trees at a site use the same water source and are exposed to similar atmospheric conditions at the canopy. Slightly lowered, although still strong coherency of  $\delta^{18}\text{O}_{\text{tree-ring}}$  suggests that individual tree features (e.g 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 treeline*

Generally, the significant although not very high common signal between all tree parameters at the valley site indicates a faster and continuous transfer of the isotope signal from water to organic matter than at the treeline. There low temperatures slowing down the tree metabolism 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 overestimates 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 most likely of minor relevance at the treeline with generally lower transpiration rates due to lower temperatures (King *et al.*, 2013), and sampling times at noon, when the peak transpiration has not yet been reached.

The inclusion of diurnal variations of needle water enrichment and the photosynthetic rate (represented by a SWR/VPD index) in the model further emphasises the effect of the sampling time on  $\delta^{18}\text{O}_{\text{NW}}$ . Particularly at the valley site, with afternoon conditions of high needle water enrichment and low photosynthetic activity, the measured values of  $\delta^{18}\text{O}_{\text{NW}}$  seem to lead to a systematic overestimation of the impact of the needle water  $^{18}\text{O}$  enrichment on the isotopic composition of the assimilates during a diurnal course. We calculated the overestimation (as the difference between SWR/VPD weighted  $\delta^{18}\text{O}_e$  and measured  $\delta^{18}\text{O}_{\text{NW}}$ ) to be about 4%. Thus, the use of measured absolute raw values of  $\delta^{18}\text{O}_{\text{NW}}$ , as “snapshot” during the day, could introduce a bias in

the interpretation of the  $\delta^{18}\text{O}$  signal transfer into photosynthetic assimilates. At the treeline, however, the values of  $\delta^{18}\text{O}_{\text{NW}}$ , measured at noon, seem to closer represent the average photosynthesis-weighted values of the day.

#### *Temporal decoupling of xylem water $^{18}\text{O}$ -enrichment from source water $\delta^{18}\text{O}$*

At both sites the exceptionally high  $\delta^{18}\text{O}_{\text{xylem}}$  values at the beginning of the growing season followed by a remarkable decrease contradict the trend expected by the influence of isotopically depleted snowmelt water (Robertson *et al.*, 2001; Treydte *et al.*, 2006). Since the upward water flow through the deciduous larch trees is most likely stopped in winter (King *et al.*, 2013), we hypothesize a coupled effect of water storage from previous autumn (Waring *et al.*, 1979; Brandes *et al.*, 2007) and additional enrichment due to evaporation effects in the twigs and stems (Dawson & Ehleringer, 1993). This is supported by the observation that needles most likely start with full stomatal transpiration as soon as source water and xylem sap flow are fully coupled again. Although not found in our study, it can, however, generally not 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  $\delta^{18}\text{O}_{\text{xylem}}$  consistently lies between the values of both 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 days; Meinzer *et al.*, 2006) could, however, also temporarily decouple twig  $\delta^{18}\text{O}_{\text{xylem}}$  patterns from  $\delta^{18}\text{O}_{\text{soil}}$  during the growing season.

#### *Decoupling of the phloem isotopic signal from the other tree tissues*

The mean difference between  $\delta^{18}\text{O}_{\text{NW}}$  and  $\delta^{18}\text{O}_{\text{phloem}}$  of 17.9‰ (valley) and 19.7‰ (treeline) is lower than the expected value of 27‰ from literature as the equilibrium fractionation factor between carbonyl oxygen and water (DeNiro and Epstein, 1979;1981; Sternberg *et al.*, 1986; Yakir and DeNiro, 1990; Cernusak *et al.*, 2003). This mismatch also persists with modelled SWR/VPD-weighted diurnal means of  $\delta^{18}\text{O}_{\text{NWe}}$ , accounting for the fact that the transfer of the needle water isotope signal into



assimilates is highest before noon when  $\delta^{18}\text{O}_{\text{NW}}$  is not yet at its diurnal maximum (Cernusak *et al.*, 2005). Moreover,  $\delta^{18}\text{O}_{\text{phloem}}$  is between 0.7 and 0.8‰ lower than  $\delta^{18}\text{O}_{\text{tree-ring}}$  and consequently more than 5‰ below the estimated  $\delta^{18}\text{O}_{\text{tree-ring}}$  values taking into account the  $\varepsilon_{\text{cp}}$  values given by Grieder (2013). According to current understanding (e.g. Cernusak *et al.*, 2005),  $\delta^{18}\text{O}_{\text{tree-ring}}$  should, however, be lower than  $\delta^{18}\text{O}_{\text{phloem}}$  since during cellulose formation from phloem-derived sucrose, partial exchange of oxygen atoms with un-enriched xylem water occurs (Roden *et al.*, 2000). This lower than expected  $^{18}\text{O}$  enrichment of phloem organic matter points to 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  $\delta^{18}\text{O}$  signal from the leaf water via the assimilates to the trunk without any change and only during cellulose production the leaf level isotopic signal gets modified due the exchange of carbonyl oxygen with xylem water. Only recently, Gessler *et al* (2013) reported a species-specific uncoupling between the leaf water and the phloem organic matter  $\delta^{18}\text{O}$  signal over the short term (i.e. during a diel course) in five tree species covering several life forms. By tracking the transfer of the leaf water  $\delta^{18}\text{O}$  signal to leaf sugars and phloem-transported organic matter they found that while leaf sugars showed the expected  $^{18}\text{O}$  enrichment of 27‰ above leaf water in all species, phloem sugars were up to 10‰ lower than expected in Scots pine, European larch and Alpine ash. 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, which might be responsible for their observation as well as for the consistent findings made 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 another part of assimilates is accumulated in the chloroplasts as transitory starch and loaded into the phloem during the night (Gessler *et al.*, 2008; Weise *et al.*, 2004; Smith *et al.*, 2003). In this case, the needle water signal is not only time shifted and damped, but phloem exported sugars are also partially labelled with lower night values of  $\delta^{18}\text{O}_{\text{NW}}$ , since 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 in conifers. The authors assume that the water in

this compartment is clearly less enriched than 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 of  $^{18}\text{O}$  enrichment of phloem sugars.

Moreover organic matter (re)fixed in the bark, where reaction water is not or only slightly enriched, should be approximately 27‰ 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  $\delta^{18}\text{O}_{\text{phloem}}$  compared to  $\delta^{18}\text{O}_{\text{tree-ring}}$  or  $\delta^{18}\text{O}_{\text{cellulose}}$  might be partially explained by our phloem sampling strategy.  $\delta^{18}\text{O}$  in phloem organic matter sampled from twigs might not be fully representative for the whole crown as would be the trunk phloem organic matter. 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. Given this uncertainty, our results, however, do not point to a further strong oxygen atom exchange between organic matter and reaction water during cellulose synthesis.

#### *Variations in source water $\delta^{18}\text{O}$ dominate the $\delta^{18}\text{O}_{\text{tree-ring}}$ pattern on a seasonal scale*

We see strong evidence that seasonal variations in the source water may have a stronger impact on  $\delta^{18}\text{O}_{\text{tree-ring}}$  variations than seasonal variations in the needle water enrichment. First this is supported by the fact that variations in  $\delta^{18}\text{O}_{\text{soil}_10}$  explain 72% of the variance of  $\delta^{18}\text{O}_{\text{tree-ring}}$  at the treeline and by the low contribution of the variations in  $\Delta^{18}\text{O}_{\text{NW}}$  to seasonal cvariation in  $\delta^{18}\text{O}_{\text{tree-ring}}$  at both sites. This also holds when  $\delta^{18}\text{O}_{\text{tree-ring}}$  is compared with modelled  $\delta^{18}\text{O}_{\text{NW}}$  integrated over a whole week.

Second strong relations between the sites with respect to  $\delta^{18}\text{O}_{\text{xylem}}$  and  $\delta^{18}\text{O}_{\text{tree-ring}}$  suggest a dominating influence of source water as common external driver at both sites. In contrast, lower r-values of  $\delta^{18}\text{O}_{\text{NW}}$  and  $\Delta^{18}\text{O}_{\text{NW}}$  respectively suggest a dominating influence of more site-specific environmental conditions such as VPD. Besides, any relations of  $\delta^{18}\text{O}_{\text{tree-ring}}$  to external variables such as RH and VPD, which strongly drive

$\Delta^{18}\text{O}_{\text{NW}}$  at both sites are significantly weaker than the source water signal or are even absent (Table 2).

Third 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 works well, seasonal variations are captured best when the damping factor  $f$  (Eqn 2) is small, i.e. the original needle water isotope signal is strongly damped. The agreement of absolute values are better for a higher  $f$ -value, but causing the amplitude of  $\delta^{18}\text{O}_{\text{tree-ring}}$  variations to be much overestimated.

Remaining uncertainties in the tree-ring model could result from the fact that after the cell enlargement phase used for dating of our tree-ring sections in this study, carbon allocation still continues for a couple of more months during subsequent cell wall thickening and lignification. Therefore this phase might not be the best estimate for the timing of the average carbohydrate content in the wood. 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 fully adequate for modelling intra-annual courses. If the Péclet effect and/or the oxygen atom exchange during wood/cellulose synthesis are not constant over the year, then  $f$  is not constant either.

One could also argue that the  $\varepsilon_{\text{cp}}$  value of  $-4.55/-4.59\text{‰}$  could potentially vary during the season due to varying amounts of primary (e.g. cellulose) and secondary (e.g. lignin, 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 herein).

In conclusion our findings based on both statistical and mechanistic models indicate that seasonal variations in  $\delta^{18}\text{O}_{\text{tree-ring}}$  predominantly mirror seasonal variations in the source water. The distinct week-to-week variations in  $\Delta^{18}\text{O}_{\text{NW}}$  and their clear

dependency on weather conditions scarcely propagated into the tree rings. The source water signal partly relies on  $\delta^{18}\text{O}_{\text{xylem}}$  preserved in the needles and partly on a post-photosynthetic oxygen atom exchange with medium water. It was postulated that such an exchange might not only occur 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 the  $\delta^{18}\text{O}_{\text{tree-ring}}$  signals (Offermann *et al.*, 2011; Gessler *et al.*, 2013).

The  $\delta^{18}\text{O}$  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  $\delta^{18}\text{O}_{\text{tree-ring}}$  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 United Kingdom 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 ratios in source water. We note that this likely varies strongly as a function of the geographic location and thereby require spatially wide-spread networks of annually-resolved tree ring based  $\delta^{18}\text{O}$  measurements.

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## TABLES

**Table 1: Correlations between tree internal isotope parameters within and between sites**

r = pearson's correlation coefficient, N = number of valid cases; stars indicate significance levels at  $p < 0.05$  and  $p < 0.01$  respectively. Correlation coefficients are calculated over the whole growing season, for  $\delta^{18}\text{O}_{\text{xylem}}$ , however, excluding the early period of strongest enrichment but starting with minimum values in spring (see text). Bold framed fields indicate correlations between the valley and the treeline.

		VALLEY				TREELINE				
		Xylem water $\delta^{18}\text{O}$	Needle water $\delta^{18}\text{O}$	Needle water $\Delta^{18}\text{O}$	Phloem $\delta^{18}\text{O}$	Xylem water $\delta^{18}\text{O}$	Needle water $\delta^{18}\text{O}$	Needle water $\Delta^{18}\text{O}$	Phloem $\delta^{18}\text{O}$	Tree ring $\delta^{18}\text{O}$
Xylem water $\delta^{18}\text{O}$	r					<b>0.78**</b>				
	N					17				
Needle water $\delta^{18}\text{O}$	r	<b>0.69**</b>				0.47	<b>0.57**</b>			
	N	20				14	20			
Needle water $\Delta^{18}\text{O}$	r	0.24	—			0.25	—	<b>0.58**</b>		
	N	20				14		20		
Phloem $\delta^{18}\text{O}$	r	<b>0.73**</b>	<b>0.55*</b>	0.49		0.15	0.15	0.32	<b>0.59**</b>	
	N	16	16	16		14	13	16	20	
Tree ring $\delta^{18}\text{O}$	r	<b>0.58*</b>	<b>0.52*</b>	0.36	<b>0.59*</b>	0.28	<b>0.63*</b>	0.52	-0.15	<b>0.83**</b>
	N	18	16	16	14	11	11	11	11	14

**Table 2: Correlations between isotope and climate parameters**

r = pearson's correlation coefficient, N = number of valid cases; stars indicate significance levels at  $p < 0.05$  and  $p < 0.01$  respectively. Correlation coefficients are calculated for the whole growing period, for  $\delta^{18}\text{O}$  xylem, however, excluding the early period of strongest enrichment but starting with minimum values in spring (see text). Tmean week, Tmax week and RH week are average data of daily mean and maximum temperature and relative humidity respectively of the 7 days before and the day of sampling.

		VALLEY						TREELINE					
		Xylem water $\delta^{18}\text{O}$	Needle water $\delta^{18}\text{O}$	Needle water $\Delta^{18}\text{O}$	Phloem $\delta^{18}\text{O}$	Tree-ring $\delta^{18}\text{O}$	Precipitation $\delta^{18}\text{O}$	Xylem water $\delta^{18}\text{O}$	Needle water $\delta^{18}\text{O}$	Needle water $\Delta^{18}\text{O}$	Phloem $\delta^{18}\text{O}$	Tree-ring $\delta^{18}\text{O}$	Precipitation $\delta^{18}\text{O}$
Precipitation $\delta^{18}\text{O}$	r	0.26	0.40	0.37	<b>0.74**</b>	<b>0.58*</b>		-0.16	0.24	0.22	0.37	<b>0.59*</b>	
	N	17	15	15	11	15		12	12	12	13	12	
Soil water 10 $\delta^{18}\text{O}$	r	0.46	0.07	-0.07	0.39	0.38	0.51	<b>0.63*</b>	0.46	0.37	0.15	<b>0.85**</b>	<b>0.58*</b>
	N	8	7	7	7	7	7	12	12	12	11	12	15
Soil water 60 $\delta^{18}\text{O}$	r							<b>0.83**</b>	0.41	0.30	0.29	<b>0.71*</b>	0.38
	N	0	0	0	1	0	0	12	12	12	11	12	15
Tmax sampling time	r	0.17	<b>0.59**</b>	<b>0.65**</b>	<b>0.49*</b>	0.21	0.41	0.20	0.33	0.38	0.17	0.22	0.38
	N	21	20	20	17	18	17	17	17	17	19	14	17
Tmean week	r	-0.18	0.25	0.40	<b>0.48*</b>	0.19	<b>0.69**</b>	-0.44	0.14	0.24	0.06	0.25	<b>0.63**</b>
	N	22	21	21	17	18	17	17	17	17	19	14	17
Tmax week	r	-0.08	0.29	0.42	<b>0.54*</b>	0.33	<b>0.76**</b>	-0.41	0.15	0.25	0.08	0.29	<b>0.68**</b>
	N	22	21	21	17	18	17	17	17	17	19	14	17
RH sampling time	r	-0.20	<b>-0.64**</b>	<b>-0.74**</b>	-0.14	-0.28	-0.36	-0.43	<b>-0.80**</b>	<b>-0.83**</b>	-0.07	<b>-0.54*</b>	0.11
	N	22	21	21	17	18	17	17	17	17	19	14	17
RH week	r	0.25	0.04	-0.18	-0.12	-0.21	<b>-0.57*</b>	0.40	-0.24	-0.33	-0.14	-0.18	0.01
	N	22	21	21	17	18	17	17	17	17	19	14	17
VPD	r	0.16	<b>0.66**</b>	<b>0.76**</b>	0.32	0.22	0.33	0.33	<b>0.63**</b>	<b>0.70**</b>	0.11	0.28	-0.02
	N	21	20	20	17	18	17	16	16	16	17	13	16
Precipitation amount	r	-0.40	-0.26	-0.09	0.01	-0.33	<b>0.57*</b>	-0.29	0.16	0.19	-0.36	0.15	0.45
	N	21	19	19	14	18	17	16	14	14	14	13	14



## FIGURE CAPTIONS

**Figure 1: Daily means of temperature (T), relative humidity (RH) and vapour pressure deficit (VPD) at the valley site and the tree line in 2008.** WOY is week of the year.

**Figure 2: Seasonal variations of all measured tree  $\delta^{18}\text{O}$  parameters for the individual trees per site (a) xylem water, (b) needle water, (c) phloem organic matter, (d) tree-ring  $\delta^{18}\text{O}$  measured on whole wood.** Weekly data for (a-c) consist either of 12 (3 twigs from 4 trees) or 4 (3 twigs pooled from 4 trees) data points. Deviations from these numbers rely on difficulties during sample preparation. Grey arrows: Biased values due to contamination (e.g. rainwater) during sampling or sample preparation (excluded from further analyses).  $r$  is the mean inter-series correlation (Pearson's correlation coefficient) calculated as average of all the correlations between the individual trees per site and parameter.

**Figure 3: Seasonal  $\delta^{18}\text{O}$  variations and ranges per site and parameter (a)** Weekly records of  $\delta^{18}\text{O}_{\text{xylem}}$ ,  $\delta^{18}\text{O}_{\text{NW}}$ , the oxygen isotope enrichment of needle water above source (xylem) water ( $\Delta^{18}\text{O}_{\text{NW}}$ ) and  $\delta^{18}\text{O}_{\text{tree-ring}}$  (whole wood). Dotted lines represent the transition between early- and latewood. bb=bud break, nm=needle maturing, ny=needle yellowing, nf=needle fall. **(b)**  $\delta^{18}\text{O}$  boxplots of all tree tissues, precipitation (ppt) and soil water at 10cm (sw10) and 60cm (sw60) soil depth. Note, that xylem water calculations were done starting with minimum values at the beginning of the growing season (see text). **(c)** Mean values of source water and all tree tissues at the treeline.

## **Figure 4: Environmental influences on $\delta^{18}\text{O}_{\text{xylem}}$ , $\Delta^{18}\text{O}_{\text{NW}}$ and $\delta^{18}\text{O}_{\text{tree-ring}}$**

**(a)** Seasonal variations of  $\delta^{18}\text{O}_{\text{xylem}}$ ,  $\delta^{18}\text{O}_{\text{ppt}}$  and  $\delta^{18}\text{O}_{\text{soil}}$ .  $\delta^{18}\text{O}_{\text{SW10}}$  (from 10 cm soil depth) and  $\delta^{18}\text{O}_{\text{SW60}}$  (from 60 cm soil depth) were partly pooled between the 5 suction elements and partly measured as single samples (amount of data points consisting of single measurements: 7; mean amount 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  $\Delta^{18}\text{O}_{\text{NW}}$  (needle water enrichment above xylem water), relative air humidity (RH, plotted inverse) and vapor pressure difference (VPD) **(c)** Seasonal variations of  $\delta^{18}\text{O}_{\text{tree-ring}}$ ,  $\delta^{18}\text{O}_{\text{ppt}}$ , and  $\delta^{18}\text{O}_{\text{SW10}}$ . Normalized data were adapted

to a common mean and standard deviation to enable visual comparison of more than 2 data sets with different units.

**Figure 5: Measured and modelled  $\delta^{18}\text{O}_{\text{NW}}$  and  $\delta^{18}\text{O}_{\text{tree-ring}}$  of *Larix decidua***

(a) Measured and modelled  $\delta^{18}\text{O}_{\text{NW}}$ ; modelled data at sampling time are based on meteorological data 30 minutes before sampling time. For the calculation of the SWR/VPD-weighted diurnal means see text. (b) Measured versus modelled tree-ring  $\delta^{18}\text{O}$  based on Eqn 3.

**Figure 6: Long-term trends of  $\delta^{18}\text{O}_{\text{xylem}}$ ,  $\delta^{18}\text{O}_{\text{NW}}$ ,  $\Delta^{18}\text{O}_{\text{NW}}$  and  $\delta^{18}\text{O}_{\text{tree-ring}}$  at both sites calculated as 10-week splines with a 50% frequency cutoff; data of  $\delta^{18}\text{O}_{\text{NWe}}$  and  $\Delta^{18}\text{O}_{\text{NWe}}$  have been calculated from weeklong runs of the SWR/VPD-weighted needle water model (see text). Normalized data were adapted to a common mean and standard deviation to enable visual comparison of data sets with more than 2 different units.**

**Figure 1**

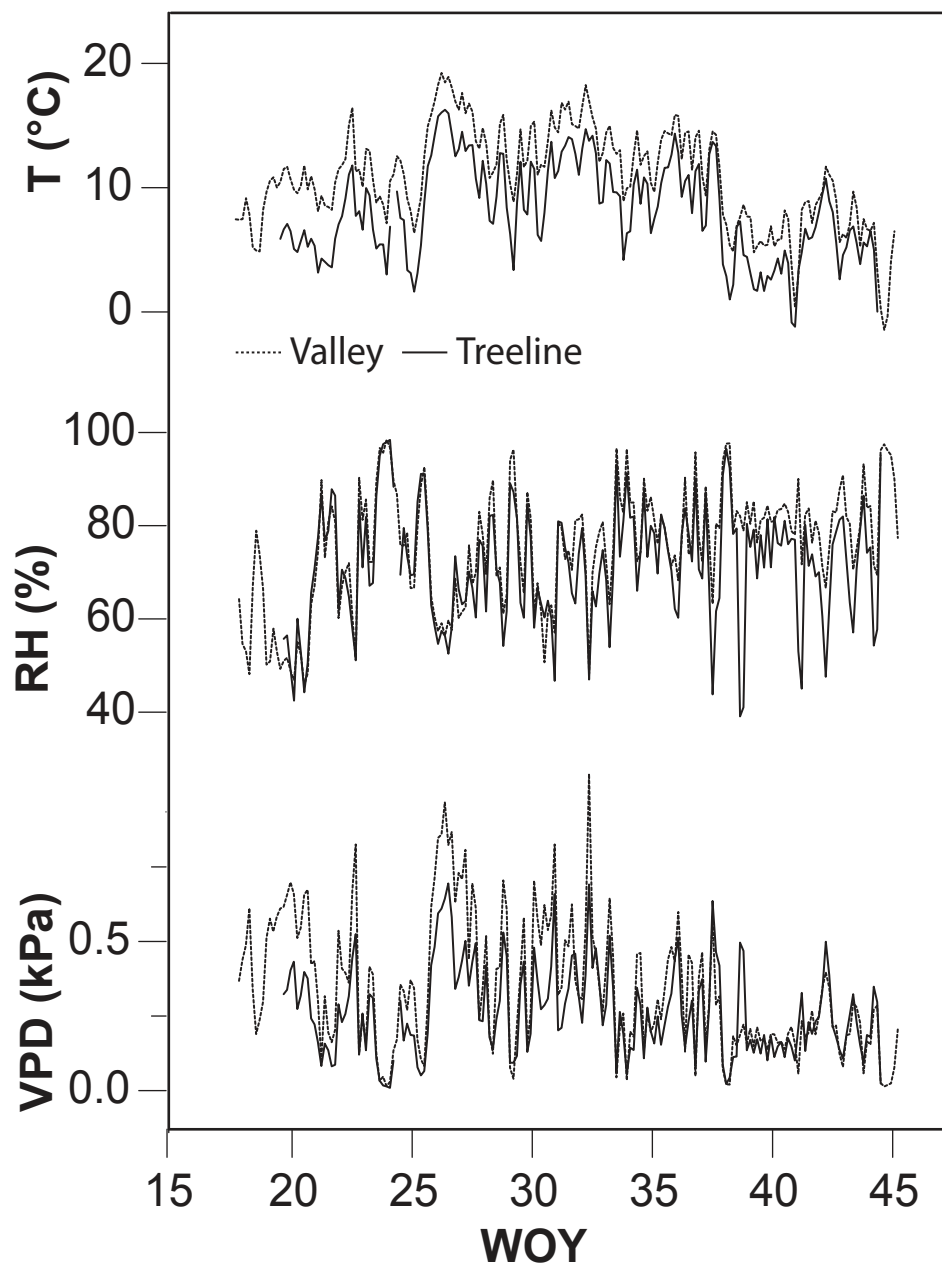


Figure 2

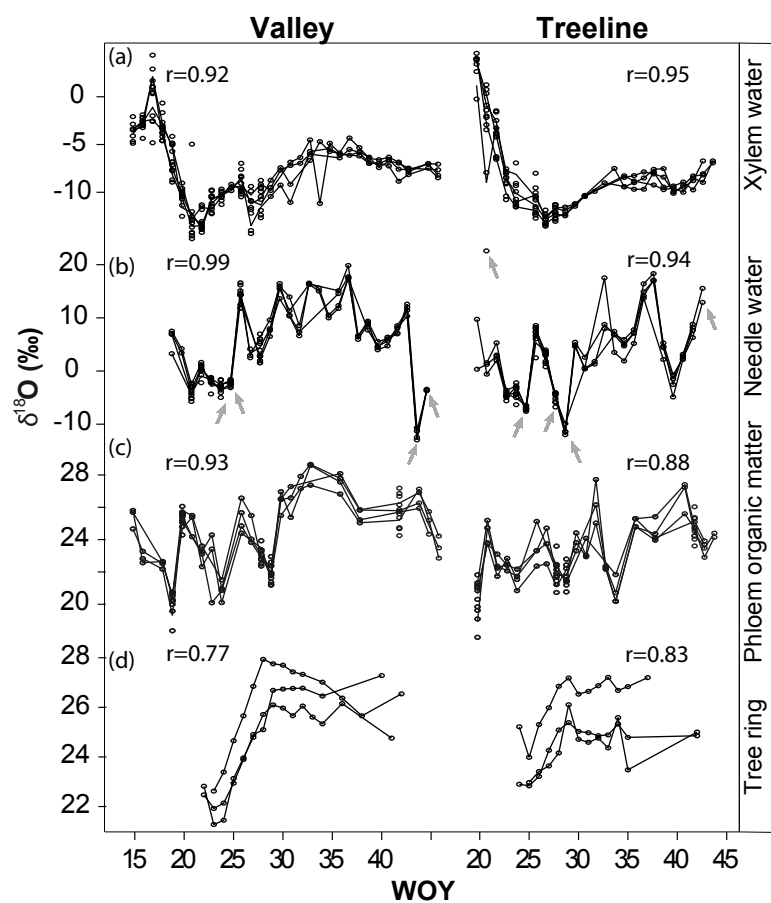
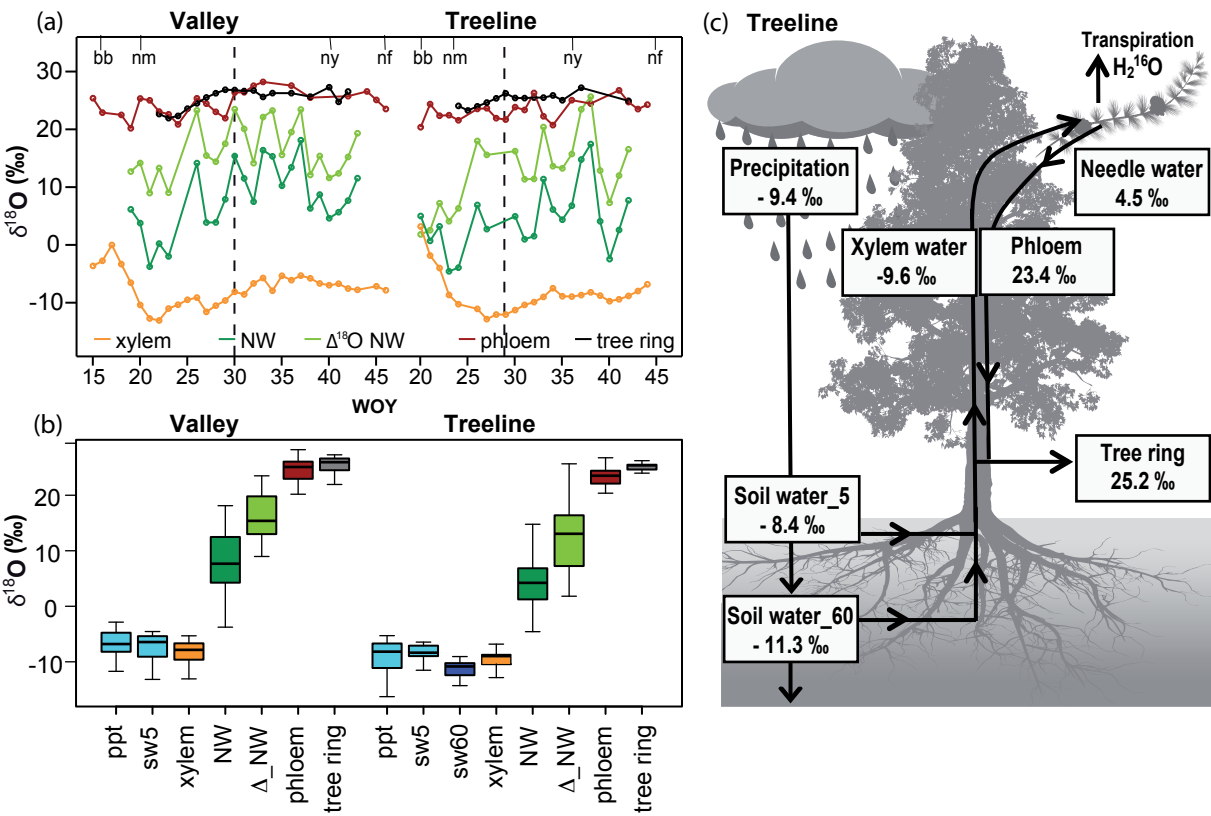
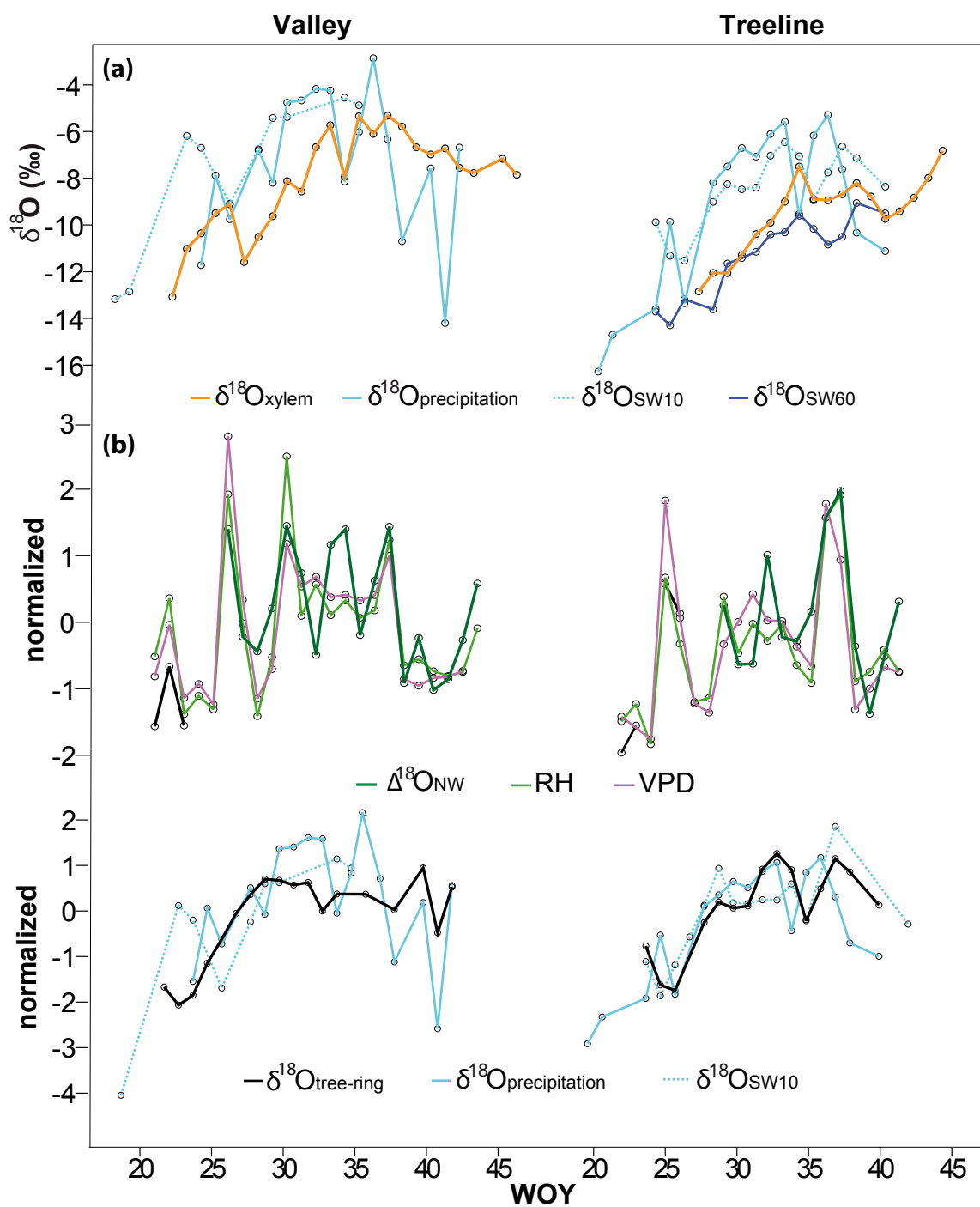


Figure 3



**Figure 4**



**Figure 5**

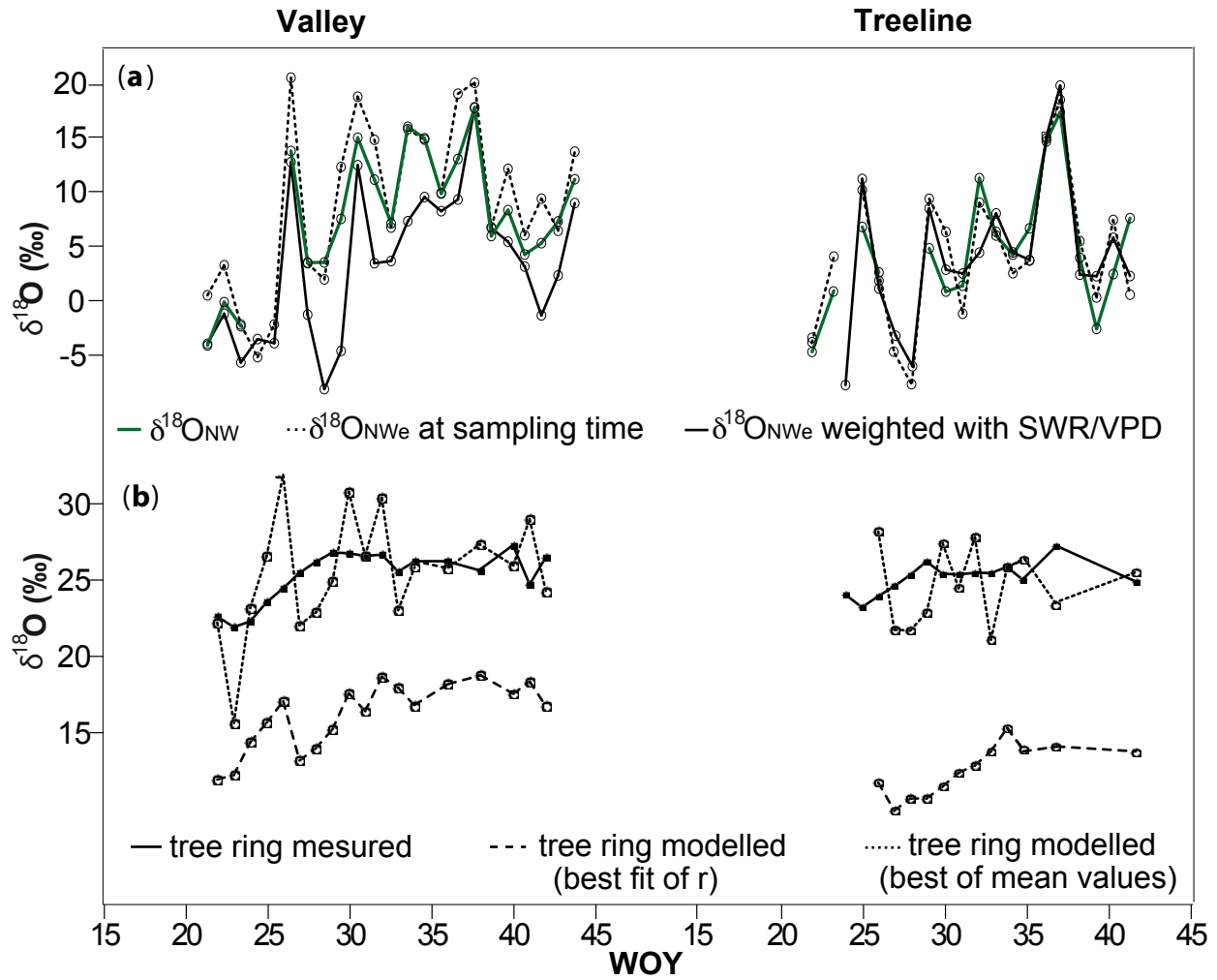


Figure 6

