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Temperature versus species-specific influences on the stable oxygen isotope ratio of tree rings

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Abstract Stable isotopic ratios integrate ecosystem variability while reflecting change in both environmental and biological processes. At sites, where climate does not strongly limit tree growth, co-occurring trees may display large discrepancies in stable oxygen isotopic ratios ($\delta^{18}O$) due to the interplay between biological processes (competition for light and nutrients, individual tree physiology, etc.) and climate. For a better quantification of the isotope variability within and among trees, the climatic and/or individual tree effects on seasonal $\delta^{18}O$ variations in precipitation, soil water, leaf water and leaf organic material (whole leaf, cellulose and starch) and annual $\delta^{18}O$ variations in tree-ring cellulose for *Fagus sylvatica* (Fs), *Quercus robur* (Qr), *Carpinus betulus* (Cb) and *Pinus sylvestris* (Ps) were studied in a mature temperate forest in Switzerland, using a mixed linear regression model technique. Furthermore, the influence of environmental factors on $\delta^{18}O$ was assessed by means of three common isotope fractionation models. Our statistical analysis showed that except for Ps, a greater portion of $\delta^{18}O$ variance in leaf compounds can be explained by individual tree effects, compared to temperature. Concerning tree-ring cellulose, only Fs and Ps show a significant temperature signal (maximum 12% of the variance explained), while the individual tree effect significantly explains $\delta^{18}O$ for all species for a period of 38 years. Large species differences resulted in a limited ability of the isotope fractionation models to predict measured values. Overall, we conclude that in a diverse mixed forest stand, individual tree responses reduce the potential extraction of a temperature signal from $\delta^{18}O$.

Keywords Oxygen isotope · Temperature · Tree ring

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Introduction

Tree rings consist of annually resolved layers of cells that integrate the biological and climatic influences on growth in a given year. Their ability to store information derives from processes of exchange between trees and their environment, such as uptake and loss of water. As such, the natural abundance of stable oxygen isotope ratios ($^{18}O/^{16}O$) in trees reveals stomatally controlled evaporative demands (Yakir et al. 1990; Barbour et al. 2004) and climatic conditions during growth (McCarroll and Loader 2004; Treydte et al. 2006). Correlations have been reported for stable oxygen isotopic composition ($\delta^{18}O$) of tree-ring cellulose and inter-annual temperature (DeNiro and Epstein 1979; Burk and Stuiver 1981; Saurer et al. 1997a; Rebetez et al. 2003), precipitation (Burk and Stuiver 1981; Anderson et al. 1998; Saurer et al. 2000; Robertson et al. 2001; Rebetez et al. 2003) and air relative humidity (Edwards and Fritz 1986; Ramesh et al. 1986; Yakir et al. 1993; Berkelhammer and Stott 2008). However, due to the complexity of the biological response to environmental conditions, physiological processes that are important on a seasonal scale such as growth rates, carbohydrate storage and senescence can also influence the isotopic composition

of plant compounds (Helle and Schleser 2002), as well as species-specific water use such as differing rooting patterns (Marshall and Monserud 2006).

Overall, the $\delta^{18}O$ signal in trees is governed by the water cycle (Yakir et al. 1993). The systematic relationship between $\delta^{18}O$ of precipitation and temperature produces decreasing isotopic composition from low to high latitudes, ocean to continental regions and low to high elevations, as a result of evaporation and condensation (Dansgaard 1964; Jouzel et al. 1997; Rozanski et al. 1993, 1997; Bowen and Wilkinson 2002). The source water of co-occurring trees contains the same large-scale temperature signal, and during water uptake by trees no isotope fractionation occurs (White et al. 1985; Dawson and Ehleringer 1991). Preferential loss of the lighter isotope ^{16}O as a result of evapo-transpiration leads to isotopic enrichment amounting to up to +20‰ in leaf water (Dongmann et al. 1974; Flanagan et al. 1991). Steady-state leaf water $\delta^{18}O$ enrichment (Craig and Gordon (1965), Dongmann et al. (1974)) is described mathematically as follows:

$$\delta^{18}O_{LW} = \delta^{18}O_s + \varepsilon_k + \varepsilon_e + (\delta^{18}O_v - \delta^{18}O_s - \varepsilon_k)e_a/e_i \quad (1)$$

where leaf water ($\delta^{18}O_{LW}$) is a function of source water ($\delta^{18}O_s$), kinetic and equilibrium fractionation factors (ε_k and ε_e, respectively), atmospheric water vapour ($\delta^{18}O_v$) and the ratio of atmospheric and intercellular vapour pressures (e_a/e_i). The main free parameter in this equation is e_a/e_i, corresponding to the relative humidity of the atmosphere, which is therefore an important driver of variations in leaf water enrichment. Saurer et al. (1997b) expanded this formula for leaf organic material as:

$$\delta^{18}O_{organic} = f[\delta^{18}O_s + \varepsilon_k + \varepsilon_e + (\delta^{18}O_v - \delta^{18}O_s - \varepsilon_k)e_a/e_i] + \varepsilon_c \quad (2)$$

where ε_c represents the biochemical fractionation between carbonyl oxygen and water and f the degree of dampening of $\delta^{18}O$ in organic material compared to leaf water ($0 < f < 1$, full dampening when $= 0$). This dampening is an empirical factor that reflects the observation of generally much smaller $\delta^{18}O$ variability in leaf organic matter compared to leaf water. It is on the one hand caused by lower enrichment in the chloroplast water compared to water at the sites of evaporation (where the Craig–Gordon model applies), and on the other hand is due to integration of different environmental conditions over the buildup period of the organic matter, which is in the order of weeks to months. Furthermore, Roden et al. (2000) defined $\delta^{18}O$ of tree-ring cellulose as:

$$\delta^{18}O_{cellulose} = f_o(\delta^{18}O_x + \varepsilon_c) + (1 - f_o)(\delta^{18}O_{LW} + \varepsilon_c). \quad (3)$$

This model for cellulose considers two points of exchange of carbon-bound oxygen: (1) at the site of sucrose synthesis in the leaf, and (2) during biochemical exchange reactions in the trunk with xylem water ($\delta^{18}O_x$). Therefore, f_o represents the fraction of carbon-bound O atoms undergoing exchange with xylem water, and $(1 - f_o)$, the isotopic exchange during cellulose synthesis.

The three models (Eqs. 1–3) illustrate the increasing complexity of $\delta^{18}O$ signals within trees as molecules containing oxygen become transformed, as well as the potentially confounding influences on $\delta^{18}O$ in trees (environmental versus biological effects). Climate reconstructions, however, rely on a clearly detectable climate signal in $\delta^{18}O$. Whether or not this assumption holds for sites where climate does not strongly limit tree growth is questionable.

In this paper, we present a quantification of the effect of temperature on $\delta^{18}O$, as compared to biological factors. $\delta^{18}O$ was measured across different time scales in leaf whole material, leaf starch, leaf cellulose and tree-ring cellulose in *Quercus robur* L. (Qr), *Fagus sylvatica* L. (Fs), *Carpinus betulus* L. (Cb) and *Pinus sylvestris* L. (Ps) from a mixed temperate forest in Switzerland. A mixed linear regression model (MLR-model) and three isotope fractionation models were used to compare leaf compound $\delta^{18}O$ and tree-ring $\delta^{18}O$ with climate, with an emphasis on temperature. A better understanding of the isotope fractionation processes in various compounds in trees and enhanced knowledge of the factors determining the seasonal course of $\delta^{18}O$ is important to improve interpretation of climatic signals in high-resolution studies. Therefore, the question of the reliability of climate reconstructions from $\delta^{18}O$ values of trees located at non-ecologically and non-climatically limited sites was explored.

Materials and methods

Sampling took place at the Swiss Canopy Crane site (Pépin and Körner 2002) located within a mixed forest stand ~10 km south of the city of Basel, Switzerland (7°30′E, 47°28′N, 550 m asl). This 80–120 year-old highly diverse mature temperate forest includes a 45 m construction crane as a research tool and is partly enriched in free-air CO_2. The mean canopy height is 35 m, tree density (diameter ≥0.1 m) 415 trees ha^{-1} and leaf area index ~5.0 m^2 m^{-2} ground area. The forest is characterized by a dominance of *Fagus sylvatica* L., and *Quercus robur* L., with a strong presence of *Tilia platyphyllos* Scop., *Pinus sylvestris* L.,

Picea abies L. and *Abies alba* Mill.. The climate is a typical humid temperate zone climate with 990 mm of annual precipitation and mean January and July air temperatures of 2.1 and 19.1°C, respectively. The soil is a loamy rendzina on calcareous bedrock with an average pH of 5.8. Trees were selected based on crane accessibility and replication ($N = 19$).

Sampling

Soil water

Soil water was collected at monthly intervals from 2002 to 2004, except for the months with frozen or completely dry soil. Water was extracted from 5 and 15 cm depths at 20–30 positions in the forest (depending on sampling date), using suction cups (high-flow porous ceramic cups, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) connected to a vacuum pump.

Leaves

A group of 19 trees (five Qr, six Fs, five Cb and three Ps) were sampled monthly from May to September 2003 on cloud-free days between 12:00 and 13:00 hours. Three leaves per sampling date were collected from sun-exposed branches. Leaves were frozen at −21°C and stored in air-tight vials until water extraction was possible.

Tree rings

In 1997, two trees from each of Fs, Ps and Qr were cored in a control area adjacent to the study site (restrictions at the study site prevented coring of trees directly sampled for leaves). Trees ranged in age from 70 to 80 years. Latewood $\delta^{18}O$ was analysed, apart from 10% of Qr where the rings were too narrow to separate latewood. In the latter case, the entire ring was analysed. In order to avoid the juvenile period (Francey and Farquhar 1982), where growth is strongly variable and influenced by factors other than climate, only rings after AD 1960 were included in this study.

Sample preparation

Leaf water

Leaf water (including needle water) was extracted by vacuum distillation by quantitatively collecting leaf water in glass U-traps submersed in liquid N_2 (−196°C), while leaves were heated in a water bath to 80°C. The dry relic of whole leaf material was milled in a steel ball mill (Mixer Mill, Retsch MM2000, Haan, Germany) for analysis by mass spectrometry.

Organic material

Whole leaf organic material was ground in a steel ball mill (as above). Similarly, wood samples were homogenized in a centrifugal mill (Retsch ZM1000, Haan, Germany) for the extraction of starch and cellulose.

Starch

Starch was extracted from milled whole leaf material according to Wilson et al. (1995) with modifications according to Jäggi et al. (2002). In summary, polar substances were extracted with ethanol and non-polar substances with a mixture of 12 parts methanol, 5 parts chloroform and 3 parts water, followed by centrifugation. The acid-soluble starch fraction was extracted with 20% HCl, then precipitated by the addition of ethanol.

Cellulose

Cellulose was extracted from milled tree rings and whole leaf material by treatment with 5% NaOH (removal of fats, oils, resins and hemicelluloses) and 7% $NaClO_2$ (removal of lignin), according to Loader et al. (1997).

Isotope analysis

For solid samples, $\delta^{18}O$ was determined by pyrolysis to CO at 1,080°C (Saurer et al. 1998) with an elemental analyser (EA-1108, Carlo Erba, Milano, Italy) connected to a continuous flow isotope ratio mass spectrometer (Delta-S, Finnigan MAT, Bremen, Germany) via a variable open split interface (CONFLO II, Finnigan MAT, Bremen, Germany). For water samples, $\delta^{18}O$ was determined in a high-temperature pyrolysis unit at 1,450°C (TC/EA, Thermo Finnigan, Bremen, Germany), connected to a continuous flow isotope ratio mass spectrometer (Delta Plus XP), via a variable open split interface (CONFLO III, both Thermo Finnigan, Bremen, Germany). For all samples, the isotope signature is expressed as:

$$\delta^{18}O = \left(R_{sample}/R_{standard} - 1\right) \times 1000 \quad (4)$$

relative to the international standard Vienna Standard Mean Ocean Water (VSMOW), where R represents the ratio $^{18}O/^{16}O$ of a sample and standard, respectively. While each sample was only measured once, the $\delta^{18}O$ was determined with a reproducibility of 0.3‰ for organic material and 0.2‰ for water as determined from the repeated analysis of in-house standards and IAEA reference materials.

Climatic data

$\delta^{18}O$ in precipitation (available from 1972 onwards) was obtained from a Global Network of Isotopes in Precipitation (GNIP) station in Bern, Switzerland (7°26′E, 46°57′N, 541 m asl, ~60 km from the site), where a unique long record is available (data provided by the Federal Office of Water and Geology (FOWG) and measured by the Climate and Environmental Physics Department of the Physics Institute of the University of Bern, Switzerland). Monthly values of $\delta^{18}O$ of precipitation are highly correlated among different stations on the Swiss Plateau (e.g. Bern and Suhr, 70 km apart, correlate with $r^2 = 0.91$ for the period AD 1994–2004); therefore, the data from Bern can be considered as representative for our site.

The temperature was recorded at 10-min intervals during the growing season at a weather station at the sampling site. Temperature measured at the Basel meteorological station (Swiss meteorological station network MeteoSwiss; 7°35′E/47°32′N, 317 m asl, ~10 km from the site) was available for the period AD 1960–1997 for comparison with tree rings. A comparison of the summer temperature for 2000–2004 for the weather station at the sampling site with that at Basel indicated a highly significant correlation coefficient of $r^2 = 0.93$.

CO_2 effect

Of the 19 trees studied, 6 were within the CO_2-enriched area and therefore exposed to an average 520 ppm CO_2 during the growing season (Pépin and Körner 2002). This involved two *Quercus*, two *Fagus* and two *Carpinus* trees (for leaf samples only, not for tree rings). Prior to other analyses, we evaluated the CO_2 effect on the oxygen isotopic composition of the different materials (whole leaf, cellulose, starch), but did not find systematic differences between control and CO_2-treated trees. Therefore, the samples from all trees were combined for further analysis and CO_2 treatment was not considered in this study.

Isotope fractionation models

We compared measured $\delta^{18}O$ of leaf water, whole leaf material, and tree-ring cellulose with values predicted by the Craig–Gordon (Eq. 1), Saurer (Eq. 2) and Roden (Eq. 3) models, respectively (Table 1). Due to shallow soil, the source water was assumed to be equivalent to soil water and was also used to estimate xylem water (Eq. 3). $\delta^{18}O_v$ of atmospheric vapour was considered in equilibrium with soil water and calculated as ($\delta^{18}O_s + \varepsilon_e$). The equilibrium fractionation factor ε_e varied from −9.5 to −10.3‰, according to temperature (Majoube 1971). The kinetic fractionation factor, ε_k, was assigned the value of 30.2 for moderately turbulent conditions characteristic of the upper tree canopy on fair-weather days (Cappa et al. 2003). For the modified Saurer model, the "least-squares best fit" principle between expected and measured values was used to determine the dampening factor, f, of 0.4. A value 0.35 was assigned for f_o (Roden et al. 2000), and the value was

Table 1 Input parameters for models calculating $\delta^{18}O$ of leaf water, leaf organic matter or tree-ring cellulose (Eqs. 1–3)

Model	Craig–Gordon	Saurer	Roden
Modelled parameter	$\delta^{18}O$ leaf water	$\delta^{18}O$ leaf organic	$\delta^{18}O$ cellulose
Time scale	Daily, 2003	Monthly, 2003	Yearly, 1972–1999
Model terms			
$\delta^{18}O_s$	Monthly average1-month lag	Monthly average1-month lag	–
$\delta^{18}O_v$	$\delta^{18}O_s + \varepsilon_e$	$\delta^{18}O_s + \varepsilon_e$	$\delta^{18}O_s + \varepsilon_e$
e_i/e_a	Relative humidity 12:00-13:00 h, leaf sampling day	Relative humidity monthly average	Relative humidity growing season average
ε_k	+30.2‰	+30.2‰	+30.2‰
ε_e	Based on average temperature, leaf sampling day	Based on average temperature, monthly	Based on average temperature, growing season
ε_c	–	+27‰	+27‰
f	–	0.35	–
f_o	–	–	0.4
$\delta^{18}O_x$	–	–	Growing season average $\delta^{18}O_s$
$\delta^{18}O_{LW}$	–	–	Craig–Gordon calculated with growing season relative humidity

Model terms are: $\delta^{18}O_s$ of source water, $\delta^{18}O_v$ of atmospheric water vapour, e_a/e_i the ratio of atmospheric and intercellular vapour pressures, ε_k the kinetic fractionation factor, ε_e the equilibrium fractionation factor, ε_c the biochemical fractionation factor between carbonyl oxygen and water, f the degree of dampening of $\delta^{18}O$ in organic material compared to leaf water, f_o the fraction of carbon-bound O atoms undergoing exchange with xylem water, $\delta^{18}O_x$ of xylem water and $\delta^{18}O_{LW}$ of leaf water

+27‰ for the biochemical fractionation factor, ε_c (Sternberg et al. 1986).

Mixed linear regression model

We used a mixed linear regression (MLR-model, Dalgaard 2002) to describe the influence of individual tree effects and of temperature on $\delta^{18}O$ of different tree compartments. The model is as follows:

$$\delta^{18}O = a + b \times \text{temperature} + \beta_{s1} + \beta_{s2} + \beta_{s3} + \beta_{s4} \quad (5)$$

with a being the intercept, b the coefficient of the continuous variable, temperature, and β_{s1}–β_{s4}, the treatment effects of the discrete variable, tree, for each of the four species (s1–s4). For leaf compartments (leaf water, whole material, starch and cellulose), we used the temperature of the sampling day. Even for whole leaf organic material, the effect of substituting the average monthly temperature was very small, perhaps because sampling occurred under clear-sky conditions. For tree-ring cellulose, we compared the average growing season temperature (May–August) and the average temperature of individual months. Other climate parameters were also tested (precipitation amount, relative humidity), but were found to be less important than temperature. The MLR-model is used here to distinguish between the climatic (temperature) and the biological (individual tree effect) influences on $\delta^{18}O$ when looking at the data from species means. In addition, the simple linear regression between $\delta^{18}O$ of a single species at time versus temperature was calculated for each species. This information is needed if one is only interested in the question of how temperature alone affects $\delta^{18}O$. Statistical analysis was carried out using R version 2.3.

Results

Monthly precipitation and soil water $\delta^{18}O$

Monthly precipitation and soil water showed a seasonal course, with $\delta^{18}O$ maxima in summer and minima in winter. No significant isotopic difference was observed between the two sampling depths (5 and 15 cm) except for the transitional months (October and May; data not shown). Compared to precipitation, soil water $\delta^{18}O$ was dampened by about 40% (maximum variations over the course of a year are 6.6‰ for precipitation and 3.8‰ for soil water; Fig. 1). Dry conditions at leaf sampling dates (summer 2003) meant that there was insufficient soil water for extraction. For the model calculations using Eqs. 1–2, soil water $\delta^{18}O$ for summer 2003 was therefore estimated according to the relationship between precipitation $\delta^{18}O$ and soil water $\delta^{18}O$ for the period 2002–2004.

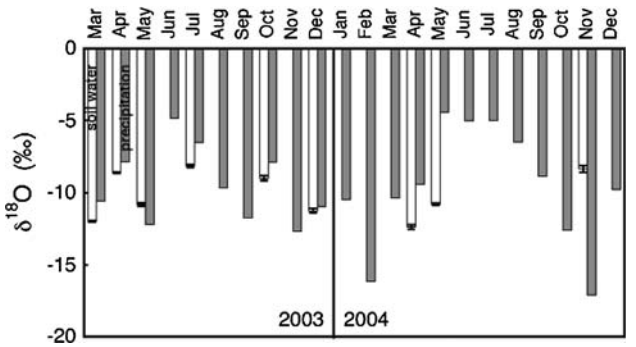

Fig. 1 Seasonal course of soil water $\delta^{18}O$ at −15 cm (*white bars*) and $\delta^{18}O$ of precipitation (*grey bars*) for the period March 2003–December 2004. Soil water values are averages of 20–30 sampling positions for a given month, ±standard error

Measured leaf $\delta^{18}O$

$\delta^{18}O$ of leaf water and whole leaf material followed a similar seasonal course for all species, except for Ps, where whole leaf organic material and leaf water were the least enriched in May (Fig. 2). The $\delta^{18}O$ values of whole leaf material were dampened by 38% compared to the leaf water values. Regarding differences among species, whole leaf material values were highest for Cb (up to +2‰ higher than other species) and most variable for Ps (about 4‰ difference between May and September). While leaf water and whole leaf organic show similarities in the seasonal course, the highest values in June and August coinciding with extremely high temperatures during these 2 months in 2003 seasonal trends were different for cellulose and starch: cellulose $\delta^{18}O$ was most enriched in August for all species, whereas starch $\delta^{18}O$ tended to decrease over the course of the growing season, except for Fs. $\delta^{18}O$ signal amplitudes, corresponding to maximum $\delta^{18}O$ variation from 1 month to the next (averages across all species), are ranked as: leaf water (6.3‰) > leaf cellulose (3.9‰) > whole leaf organic material and leaf starch (both 1.7‰).

Measured tree-ring $\delta^{18}O$

Tree-ring cellulose $\delta^{18}O$ varied considerably among years and species (Fig. 3). Only in a few years did all three species show the same isotopic response. $\delta^{18}O$ similarities between individuals of the same species, based on common percentage of year-to-year intervals with similar positive or negative trends, amounted to 70% (Fs), 43% (Qr) and 51% (Ps) (significant at $P < 0.05$ for Fs only). Correlations with average monthly temperature and precipitation were significant ($P < 0.001$) for temperature and marginally significant ($0.01 < P < 0.05$) for precipitation, whereby months showing strongest correlations for either temperature or precipitation were species-dependent: Fs (April,

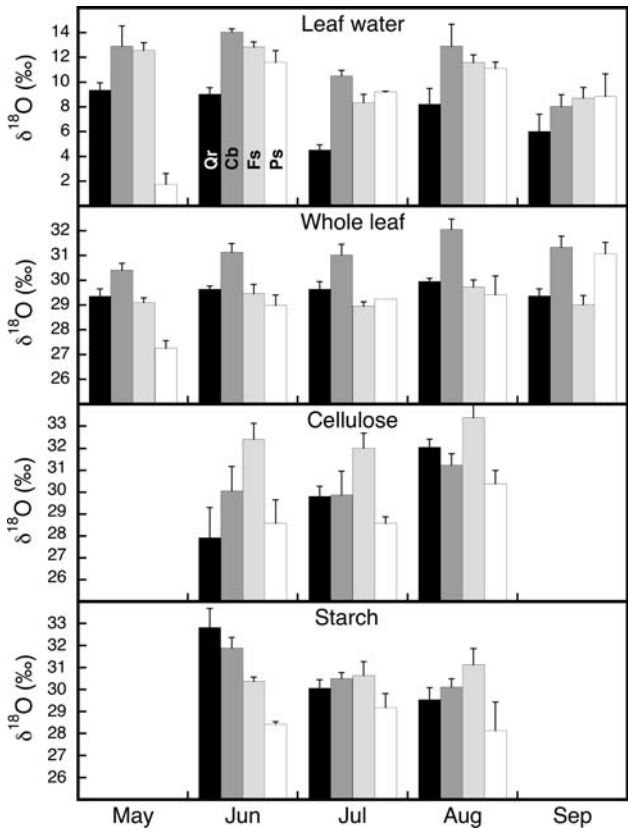

Fig. 2 $\delta^{18}O$ of leaf water, whole leaf organic material, leaf cellulose and leaf starch for *Quercus rbur* (Qr), *Carpinus betulus* (Cb), *Fagus sylvatic* (Fs) and *Pinus sylvestris* (Ps). Values represent averages of 2–6 trees sampled three times on one given day per month, ±standard error

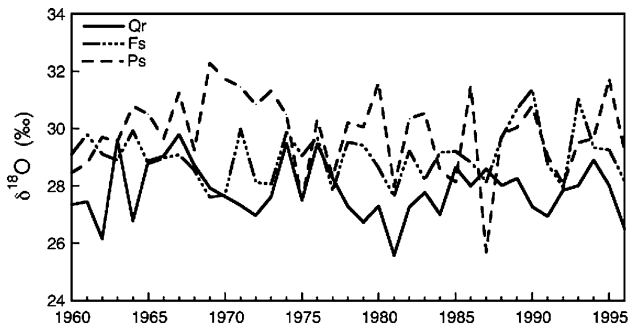

Fig. 3 Tree-ring cellulose $\delta^{18}O$ for *Quercus robur* (Qr), *Fagus sylvatica* (Fs) and *Pinus sylvestris* (Ps) for AD 1960–1996. Values are averages of two trees per species

May, June, July), Qr (July) and Ps (May, June, July, August). First differences of the data (given year minus previous year) corresponding to the year-to-year variation in $\delta^{18}O$ were more strongly correlated with climate than the raw data series. The average signal amplitude (maximum year-to-year variation in $\delta^{18}O$) was 4.8‰ for the investigated period.

Model comparisons

$\delta^{18}O$-values for leaf water, leaf organic material and cellulose were calculated according to Eqs. 1–3 (Table 1). For leaf water comparison, measured daily (24-h average) temperature and midday relative humidity values were used (Craig–Gordon model, Eq. 1). For whole leaf organic material $\delta^{18}O$, average monthly relative humidity values were used (Saurer model, Eq. 2), reflecting the longer integration time of the $\delta^{18}O$ signal in organic material, compared to leaf water. For yearly resolved tree-ring cellulose (Roden model, Eq. 3), $\delta^{18}O$ was determined according to the average growing season conditions (May–August), justified by the use of latewood cellulose for analysis.

The non-species-specificity of all three models provided a measure of environmental effects on $\delta^{18}O$, based on the agreement between modelled and measured values. Overall, correlations for leaf water were as follows: (r values): Qr (0.93) > Cb (0.72) > Fs (0.57) > Ps (0.56) (Table 2). The physically defined model therefore explained leaf water $\delta^{18}O$ somewhat better for the three deciduous species studied than for the conifer Ps. A convincing test of the applicability of the model was provided by the results for meteorological conditions (relative humidity) for different hours of the day as input variable, where for all species, the highest correlation coefficient r was achieved during actual sampling time. For Qr, for instance, r was 0.53 at 10:00–11:00 hours, increased to $r = 0.75$ at 11:00–12:00 hours, reached the highest value at 12:00–13:00 h ($r = 0.93$) and decreased to $r = 0.69$, 1 h later. Neither the Craig–Gordon nor Saurer model completely explained $\delta^{18}O$, with slightly significant results for Qr (leaf water) and Cb (whole leaf organic) only (Table 2). In addition, the Roden model predictions for tree-ring cellulose were significant for Fs only ($r = 0.63$, Table 2). Regarding these models, it should be noted that several of the parameters could be species-dependent, like f, ε_c and ε_k, but were kept constant for simplicity and because species-dependency could not be quantified. However, using different values for different species would not affect the correlation coefficients shown in Table 2, because these values reflect the correlation between the temporal course either modelled or measured. If, for instance, f would be decreased, this would result in lower variability of the resulting modelled isotope time course, but the correlation to the measured isotope variations would remain the same.

Influence of biology and temperature for leaf components and tree rings

As calculated by the MLR-model, the portion of variance in leaf component $\delta^{18}O$ explained by temperature (Fig. 4,

Table 2 Correlation coefficients (r) for relationship between modelled and measured $\delta^{18}O$ in different tree compartments for *Quercus robur* (Qr), *Fagus sylvatica* (Fs), *Carpinus betulus* (Cb) and *Pinus sylvestris* (Ps)

Model Tree compartment	Craig–Gordon Leaf water	Saurer Whole leaf organic	Roden Tree-ring cellulose
n	5	5	37
Qr	*0.93*	0.75	0.32
Fs	0.57	0.47	**0.63**
Cb	0.73	*0.96*	–
Ps	0.56	0.70	0.35

In bold, $P < 0.05$; in italics, $0.05 < P < 0.1$. n = number of months (leaf water, whole leaf organic) or years (tree-ring cellulose)

"Temperature") showed more significant relationships than that explained by individual tree effects (Fig. 4, "Tree"). Significant relationships were found for Ps leaf water, Ps cellulose, Cb whole leaf material ($P < 0.05$), Cb leaf starch ($P < 0.10$; "Temperature"), and Fs and Cb whole leaf material ("Tree", $P < 0.05$ and $P < 0.10$, respectively). Taken alone, the effect of temperature (Fig. 4, "Temperature only") significantly explained variance in Ps leaf water ($P < 0.05$). While not significant, "Temperature only" explained large portions of the variance in whole leaf material for Qr and Cb, in leaf cellulose for Ps and Cb, and in leaf starch for Fs, Ps and Cb (Fig. 4). A principal problem should also be noted in that it is difficult to distinguish between the effect of temperature on $\delta^{18}O$ of tree components via directly influencing $\delta^{18}O$ of source water or via influencing biological fractionation mechanisms. Finally, a correlation analysis with the influence of relative humidity did not further explain $\delta^{18}O$ variance for leaf water, whole leaf material, starch or cellulose, supporting the selection of temperature as the single climate variable studied.

For tree rings, the effect of temperature on $\delta^{18}O$ was only significant for Fs (average growing season temperature and May temperature, $P < 0.05$) and for Ps (May temperature, $P < 0.10$; Fig. 5). Removing the effect of individual trees in correlations calculated for average species $\delta^{18}O$ ("Temperature only" bars) improved the extracted temperature signal for Ps (May, $P < 0.10$) and Fs (May, $P < 0.05$). For Qr, explained variance by temperature increased later in the growing season (July and August). For all species, the calculated individual tree effect was identical across all months, since there is a single $\delta^{18}O$ value per year in tree rings and a fixed number of individual trees. Overall, the individual tree effect explains the greatest portion of variance in tree-ring $\delta^{18}O$ compared with temperature: 34% (Ps), 15% (Qr) and 12% (Fs).

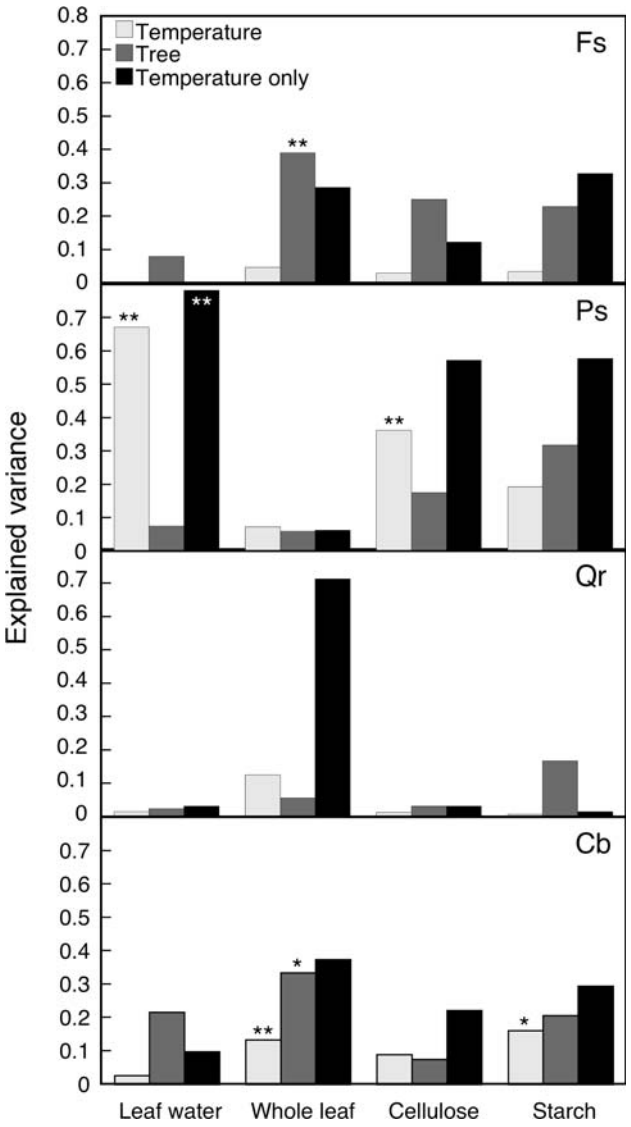

Fig. 4 Explained variance in $\delta^{18}O$ for leaf components. The *grey columns* were derived from a mixed model (Eq. 5), whereas the *black columns* correspond to results from a separate test. "*Temperature*" represents the influence of temperature on day of sampling and "*Tree*" the influence of the individual tree, on $\delta^{18}O$ in a given compartment. "*Temperature only*" represents the influence of temperature of the sampling day, taken as the single predictor of the average species $\delta^{18}O$. *Double asterisk* significant influences ($P < 0.05$), *single asterisk* ($P < 0.10$)

Discussion

We observed that variance in tree-ring $\delta^{18}O$ is best explained by individual tree effects rather than by the influence of temperature. This statement summarizes the effect of the primary factor (temperature) on the final output (tree-ring $\delta^{18}O$), but is a reflection of all the intermediary fractionation processes involved in the soil, leaf and stem, which are dependent on the ecological setting and physiological and biochemical properties of the trees.

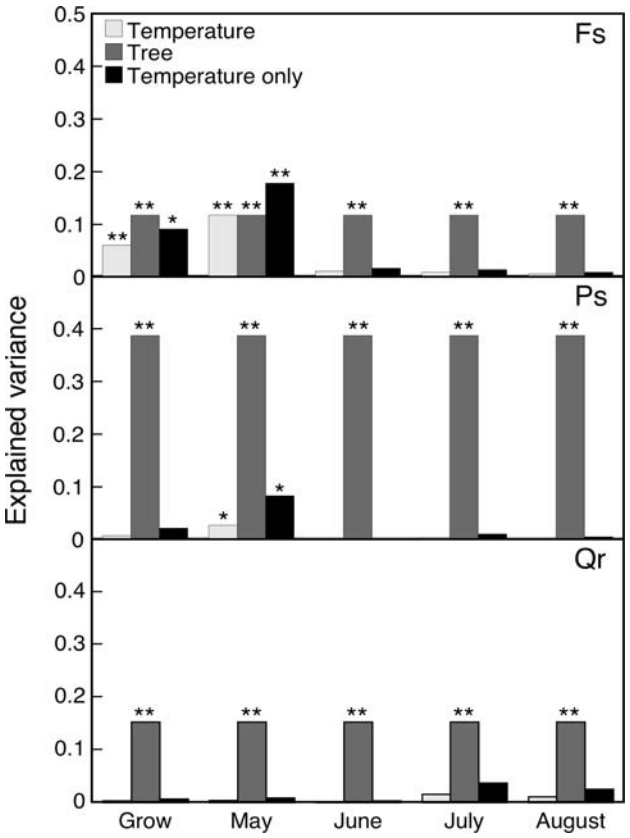

Fig. 5 Explained variance in tree-ring $\delta^{18}O$ with average monthly temperature (May, June, July and August), and average growing season ("Grow"; average May–August) temperature. The *grey columns* were derived from a mixed model (Eq. 5), whereas the *black columns* correspond to results from a separate test. "*Temperature*" represents the variance explained by temperature and "*Tree*" the variance explained by the individual tree, on tree-ring $\delta^{18}O$. '*Temperature only*' represents the influence of temperature only, as a single predictor of the average species $\delta^{18}O$. *Double asterisk* significant influences ($P < 0.05$), *single asterisk* ($P < 0.10$)

Strictly speaking, fractions of explained variance may not be compared among models (Tree, Temperature or Temperature only). Their function is to contrast significance estimates of $\delta^{18}O$ qualitatively among models.

Accordingly, understanding how $\delta^{18}O$ is laid down in trees requires knowledge of the variety of processes involving oxygen in an ecosystem. As shown for the Swiss Jura, atmospheric temperature is related to the seasonal course of precipitation $\delta^{18}O$ ($r^2 = 0.67$; Rebetez et al. 2003). At our site, the precipitation signal is reflected in soil water with a 1–3 month time lag (Fig. 1). For example, for May 2004, high values of precipitation $\delta^{18}O$ are observed (average −4‰), whereas low soil water $\delta^{18}O$ (average −11‰) reflects the precipitation signal of March 2004. Similarly, Tang and Feng (2001) and Jäggi et al. (2003) found that early spring soil water $\delta^{18}O$ reflected winter precipitation. Time lags have been shown to increase for deeper soils (i.e. 6 months at −80 cm, Hesterberg and Siegenthaler 1991), while the extent of signal dampening (reduction of the annual amplitude) is dependent on total annual precipitation and soil properties (Hsieh et al. 1998). We observe ~60% of the $\delta^{18}O$ range of precipitation in soil water at −15 cm, which varies annually by an average +3.8‰. Both dampening and variation in the $\delta^{18}O$ soil signal can be explained by soil characteristics such as porosity that influence the residence time and mixing of water in the soil. Our results show how a $\delta^{18}O$ temperature signal already becomes dampened in the soil, even for soils that are considered shallow (−30 cm), and soil processes therefore constitute a first major disturbance for the relationship between temperature and $\delta^{18}O$-signals in trees.

The influence of temperature on leaf water $\delta^{18}O$ is significant only for Ps (67% variance explained, Fig. 4). However, the overall seasonal $\delta^{18}O$ signal amplitudes for leaf water of all species are nearly as high as for precipitation (6.3 vs. 6.6‰), suggesting leaf water $\delta^{18}O$ sensitivity to external variables other than temperature, e.g. relative humidity as a driving variable in Eq. 1. The observed temperature signal in Ps leaf water may reflect shallow rooting depth, where the bulk of the pine root system consists of horizontal roots close to the soil surface, which stores the most recent precipitation (Laitakarai 1927). Pine tree rings have been shown to be produced mostly from current photosynthates and not reserves (Dickmann and Kozlowski 1970; Glerum 1980). From the onset of the growing season, the strong photosynthetic demand may be accompanied by high rates of respiration and in particular high water uptake by roots, providing rapid turnover of the source $\delta^{18}O$ signal in leaves. In contrast, deciduous trees depend more strongly on the breakdown of reserves from previous growing seasons for initial growth (Pilcher 1995), which may affect initial water uptake and transpiration rates. In addition, poor Craig–Gordon model predictions suggest that species-dependent physiological parameters should be considered in leaf water $\delta^{18}O$ predictions.

Whole leaf material $\delta^{18}O$ signal amplitude is very strongly dampened by 75% compared to leaf water (Fig. 2). Similarly, Jäggi et al. (2003) observed a signal reduction of 80% between modelled leaf water and whole leaf organic material $\delta^{18}O$ for the conifer *Picea abies*. Unlike leaf water, which is continuously in flux in a sun-exposed deciduous leaf, heterogeneous whole leaf material, consisting of a mixture of solid organic compounds (e.g. cellulose, hemicellulose and lignin), pigments and lipids accumulated over a period of weeks to months, may conceal part of the climatic signal initially present in leaf water $\delta^{18}O$. High $\delta^{18}O$ values in cellulose (e.g. Fig. 2, Fs) and starch (e.g. Qr, June) may be masked in whole leaf material by isotopically light compounds, such as lignin (Barbour et al. 2001) and lipids. Overall, a maximum 13% of the

variance in whole leaf material $\delta^{18}O$ is explained by temperature (for Cb, Fig. 4), which is less than the portion that can be explained in leaf water. A refinement of isotope fractionation models that incorporate intra-molecular isotope variations could help in distinguishing between source water and leaf water influences, but also better explain dampening effects. Sternberg et al. (2007) observed that the $\delta^{18}O$ content of the oxygen attached to the second carbon of the cellulose molecule may most closely reflect the $\delta^{18}O$ of the source water. In this study, a further curvilinear relationship between $\delta^{18}O$ of source water and cellulose was observed, which could result in the relative insensitivity of $\delta^{18}O$ to temperature above a certain threshold value and thus a dampening effect similar to what we observed, although this phenomenon is not fully understood.

$\delta^{18}O$ of leaf structural and storage components is significantly explained by temperature in Ps (cellulose) and Cb (starch). In Ps, this is in spite of the fact that no significant explanations of variance can be made for whole leaf material (compared to large portions of explained variance for other species). Pine stores substantial quantities of lipids in woody tissues (Hoch et al. 2003) compared to the starch storage of many deciduous species. If excess photosynthates in Ps needles become largely sequestered to lipid pools in stems, the remaining average whole leaf material $\delta^{18}O$ may not reflect current temperature as strongly as climatic conditions earlier in the growing season when the leaf skeleton or other fundamental structures were formed. When temperature only is considered, it explains a similar proportion of the $\delta^{18}O$ signal of starch and cellulose as for leaf water (approximately 60% variance in $\delta^{18}O$ explained). In Qr, the known use of reserves early in the growing season may explain why leaf cellulose, a structural material, is poorly correlated with current temperature. In this species, temperature explains 70% of whole leaf material $\delta^{18}O$ in the absence of individual tree effects ("Temperature only"), while in all other leaf compartments neither temperature nor individual tree effects significantly explain $\delta^{18}O$. The strong climatic constraint produced by drought and heat stress during the record-breaking heat wave of summer of 2003 had no effect in unifying the $\delta^{18}O$ signals in leaves of these co-occurring trees. Weak correlations with the Saurer model for leaf organic material further supports species-specific influences on $\delta^{18}O$, such as the timing and rate of formation of structural or storage material and other physiological processes.

Overall, we observe a reduced temperature signal in tree rings of all species, compared to other tree and soil compartments. Despite common trends in tree-ring $\delta^{18}O$ being considered a result of climate (Saurer et al. 1997a, 2000), our data show low correlations between $\delta^{18}O$ series of different trees growing at a single location, suggesting the influence of other local or tree-specific factors in determining $\delta^{18}O$ (e.g. soil nutrition, inter-specific competition, etc.). In Ps, individual tree effects significantly explain 40% of the variance in tree-ring $\delta^{18}O$. In contrast, temperature significantly explains at most 12% of the year-to-year variance in tree-ring $\delta^{18}O$, as observed for Fs, the only species for which the Roden model significantly predicts tree-ring $\delta^{18}O$ and which shows significant similar year-to-year trends between individuals of the same species. While the Roden model was used successfully to explain tree-ring $\delta^{18}O$ in hydroponically greenhouse-grown *Betula* and *Populus* spp. (Roden and Ehleringer 1999), it appears that for mature, natural forest stand trees, the information stored in the $\delta^{18}O$ signal cannot be applied across a variety of species by a single model, especially one developed largely with data acquired from deciduous saplings (Roden et al. 2000). Correlations between tree-ring cellulose and temperature are highest for the month of May in Fs and Ps, and July in Qr, showing the importance of origin and timing of earlywood and latewood formation among species. Seasonal differences in $\delta^{18}O$ reflect processes related to fructification and carry-over effects from stored carbohydrates. At a heterogeneous site where climatic conditions do not especially limit tree growth, tree-ring $\delta^{18}O$ may be influenced by leaf and root differences and function, varying with species, size or age, in addition to environmental factors.

Conclusions

We show significant biological effects on $\delta^{18}O$ contained in different tree compartments despite the traditional view of trees as climate proxies. Limited model predictions for leaf water, leaf organic matter and tree-ring cellulose $\delta^{18}O$ of different species challenge the purely physical nature of $\delta^{18}O$ signals in a temperate forest. Average species $\delta^{18}O$ generated by pooling ("Temperature only") reveals the effect of temperature on $\delta^{18}O$ more strongly. At this site, tree-ring $\delta^{18}O$ of Fs is the best proxy for average growing season temperature in a given year, while intra-annual temperature is recorded best in Ps leaf water and in whole leaf organic material for Cb, Qr and Fs. We show both seasonally and annually that species-specific effects compete with climate in determining $\delta^{18}O$ in trees of mixed temperate forests, reducing the climatic potential of $\delta^{18}O$ and necessitating the development of isotope models that more accurately incorporate physiological constraints. While this information offers a note of caution with respect to climatic reconstructions, it supports using $\delta^{18}O$ signals to determine the role of individual trees in overall forest response to a changing climate, a reminder that stable

isotopic ratios in trees may serve as both ecological and climatic indicators.

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