Correlating $\delta^{13}$C and $\delta^{18}$O in cellulose of trees

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ABSTRACT

We measured the carbon and oxygen isotopic composition of stem cellulose of Pinus sylvestris, Picea abies, Fagus sylvatica and Fraxinus excelsior. Several sites along a transect of a small valley in Switzerland were selected which differ in soil moisture conditions. At every site, six trees per species were sampled, and a sample representing a mean value for the period from 1940 to 1990 was analysed. For all species, the mean site $\delta^{13}$C and $\delta^{18}$O of stem cellulose are related to the soil moisture availability, whereby higher isotope ratios are found at drier sites. This result is consistent with isotope fractionation models when assuming enhanced stomatal resistance (thus higher $\delta^{13}$C of incorporated carbon) and increased oxygen isotope enrichment in the leaf water (thus higher $\delta^{18}$O) at the dry sites. $\delta^{18}$O-$\delta^{13}$C plots reveal a linear relationship between the carbon and oxygen isotopes in cellulose. To interpret this relationship we developed an equation which combines the above-mentioned fractionation models. An important new parameter is the degree to which the leaf water enrichment is reflected in the stem cellulose. In the combined model the slope of the $\delta^{18}$O-$\delta^{13}$C plot is related to the sensitivity of the $p_{i}/p_{a}$ of a plant to changing relative humidity.

Key-words: $\delta^{13}$C; $\delta^{18}$O; cellulose; intercellular CO₂ concentration; relative humidity; soil moisture; stable isotopes; tree rings.

INTRODUCTION

Carbon and oxygen isotope ratios in plant matter can each be used as a tool to obtain information on environmental conditions during plant growth. The usefulness of this tool depends on understanding of the fundamental processes involved. For $\delta^{13}$C [where $\delta (\%) = (R_{\text{sample}}/R_{\text{Standard}} - 1) \times 1000$ and $R$ is $^{13}$C/$^{12}$C], the theory describing fractionation due to the diffusion of CO₂ into the leaf and subsequent photosynthesis is well developed (Farquhar et al. 1989). Many experiments have been performed to investigate the influence of various environmental factors on $\delta^{13}$C in plants. For instance, soil moisture conditions (Winter 1981), the relative humidity of the atmosphere (Madhavan, Treichel & O’Leary 1991), light (Broadmeadow & Griffiths 1993), nutrition and air pollutants (Saurer et al. 1995a) have been shown to affect the carbon isotopic composition, often in agreement with the above-mentioned model. On the other hand, the processes which determine the oxygen isotope ratio in plant matter are not as well understood (Yakir 1992). Many studies have dealt with $\delta^{18}$O in leaf water, but relatively few with $\delta^{18}$O in organic matter. One point of uncertainty is, for instance, to what degree isotopic leaf water enrichment, which is dependent on evaporative conditions, is reflected in leaf and in stem organic matter (Sternberg & DeNiro 1983). In the near future, a rapid increase in the number of studies using $\delta^{18}$O in organic material can be expected due to the recent development of on-line pyrolysis techniques (Werner et al. 1996).

It might be particularly useful to consider a combination of $\delta^{13}$C and $\delta^{18}$O values for organic matter, in view of the fact that external factors, in particular the moisture conditions, can influence both carbon and oxygen isotope ratios. The relative responses of $\delta^{13}$C and $\delta^{18}$O can then be related to the sensitivity of a plant to the evaporative conditions. This idea will be developed in the section ‘Isotope fractionations’. In this study, we present $\delta^{13}$C and $\delta^{18}$O values for stem cellulose of beech, pine and spruce trees growing at sites which differ in soil moisture conditions. The experimental set-up provides information about the within-site variability, about differences between species and about the dependence on site conditions, data that are lacking for oxygen isotopes in particular. Furthermore, a model is developed for interpretation of $\delta^{13}$C and $\delta^{18}$O data which combines the equations commonly used to describe carbon and oxygen fractionation by plants.

Isotope fractionations

Carbon isotope ratio

In C₃ plants, the carbon isotope composition of organic material, $\delta^{13}$C$_{\text{plant}}$, is approximately related to the ratio of partial pressure of CO₂ inside the leaf, $p_{i}$, and the partial pressure of CO₂ in the atmosphere, $p_{a}$ (Farquhar, O’Leary & Berry 1982):

$$\delta^{13}C_{\text{plant}} = \delta^{13}C_{\text{atm}} - a - (b - a) \frac{p_{i}}{p_{a}},$$

(1a)

where $\delta^{13}C_{\text{atm}}$ is the $\delta^{13}$C of atmospheric CO₂, $a (= 4.4 \text{‰})$ is the fractionation occurring due to the diffusion in air and $b (= 27 \text{‰})$ is the fractionation caused by carboxylation.
(see also Table 1 for a summary of all coefficients used). This equation has been validated convincingly with online techniques (Evans et al. 1986), and has proved useful in numerous studies. Strictly speaking, Eqn 1a is valid only for the first product of photosynthesis and does not include fractionations caused by later biochemical processes. For instance, lipids are known to be isotopically lighter than the bulk material, whereas cellulose is heavier. Therefore, the difference between $\delta^{13}C$ in cellulose and in bulk plant material, $\delta c-p$, has to be considered in inferring $p/p_s$ ratios from $\delta^{13}C$ values of cellulose, $\delta^{13}C_{cell}$ (White et al. 1993):

$$\delta^{13}C_{cell} = \delta^{13}C_{plan} + \delta c-p = \delta^{13}C_{atm} - a - (b - a) \frac{p_i}{p_a} + \delta c-p.$$  \hspace{1cm} (1b)

$\delta c-p$ is $1\% - 2\%$. This value may depend on species and may also vary between different cellulose extraction techniques.

### Oxygen isotope ratio

In summary, the oxygen isotope composition of organic material is determined by (i) the isotopic composition of the source or soil water, $\delta^{18}O_w$, (ii) the enrichment taking place in the leaf water due to transpiration, resulting in an increased $\delta^{18}O_l$ of leaf water compared to $\delta^{18}O_w$, and (iii) biochemical fractionations. The degree of leaf water enrichment is dependent on the ratio of the atmospheric and intercellular vapour pressures ($e_i$ and $e_k$, respectively), the isotopic composition of water vapour in the air, $\delta^{18}O_v$, fractionation due to the change of phase from liquid to vapour, $e_e$ (9.8‰ at 20°C; Majoube 1971), and kinetic fractionation due to the diffusion of vapour into unsaturated air, $e_k$. A formula integrating these processes has been developed by Dongmann et al. (1974):

$$\delta^{18}O_l = \delta^{18}O_w + \epsilon_k + \epsilon_i + (\delta^{18}O_l - \delta^{18}O_w - \epsilon_k) \epsilon_i/\epsilon_k.$$  \hspace{1cm} (2)

$\epsilon_k$ (26-5‰ according to Farquhar et al. 1989) is calculated based on the degree of turbulence in the leaf-boundary layer and on the ratio of the resistance for diffusion through the stomata to the resistance for diffusion through the leaf boundary. This value is usually assumed to be constant but is actually sensitive to the nature of the boundary layer, which is controlled by leaf size and morphology (Buhay, Edwards & Aravena 1996). When water vapour is in isotopic equilibrium with soil water, Eqn 2 can be simplified because $\delta^{18}O_l - \delta^{18}O_w = -\epsilon_k$. This is often the case in European summer conditions (Förstel & Hützen 1983). Equation 2 treats leaf water as one well-mixed pool, which is a crude approximation only. Water in the chloroplasts can be expected to be less enriched than water directly subject to transpiration (Yakir, DeNiro & Gat 1990b; Farquhar & Lloyd 1993). For high transpiration rates especially, such differences should be important. Accordingly, a dependence of $\delta^{18}O_l$ on the transpiration rate has been found (Flanagan, Comstock & Ehleringer 1991), but this effect cannot be easily quantified at present and is therefore neglected in Eqn 2.

The oxygen isotopic composition of organic matter is determined by the oxygen of the water at the site of synthesis. Cellulose is enriched by about 27‰ compared to water (DeNiro & Epstein 1981). This enrichment may be termed biochemical fractionation ($\epsilon_b$) and is probably caused by isotopic exchange of carbonyl oxygen atoms of intermediate products of photosynthesis with water (Sternberg, DeNiro & Savidge 1986). This exchange does not only occur in the leaf, but also in the stem during cellulose formation in numerous studies. Strictly speaking, Eqn 1a is valid only for the first product of photosynthesis and does not include fractionations caused by later biochemical processes. For instance, lipids are known to be isotopically lighter than the bulk material, whereas cellulose is heavier. Therefore, the difference between $\delta^{13}C$ in cellulose and in bulk plant material, $\delta c-p$, has to be considered in inferring $p/p_s$ ratios from $\delta^{13}C$ values of cellulose, $\delta^{13}C_{cell}$ (White et al. 1993):

$$\delta^{13}C_{cell} = \delta^{13}C_{plan} + \delta c-p = \delta^{13}C_{atm} - a - (b - a) \frac{p_i}{p_a} + \delta c-p.$$  \hspace{1cm} (1b)

$\delta c-p$ is $1\% - 2\%$. This value may depend on species and may also vary between different cellulose extraction techniques.

### Table 1. Summary of parameters used in Eqns 1 to 5 (see text for detailed description and references)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
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<tbody>
<tr>
<td>$a$</td>
<td>4.4‰</td>
<td>$^{13}C$ fractionation of CO$_2$ diffusing in air</td>
</tr>
<tr>
<td>$b$</td>
<td>27‰</td>
<td>$^{13}C$ fractionation during carboxylation in C$_1$ plants</td>
</tr>
<tr>
<td>$\epsilon_i$</td>
<td>9.8‰</td>
<td>$^{18}O$ equilibrium fractionation due to the change of phase from liquid water to vapour at 20°C</td>
</tr>
<tr>
<td>$\epsilon_k$</td>
<td>26.5‰</td>
<td>$^{18}O$ kinetic fractionation due to the diffusion of vapour into unsaturated air</td>
</tr>
<tr>
<td>$\delta^{18}O_w$</td>
<td>-8.4‰</td>
<td>$^{18}O$ value of soil water; mean of the summer months April to September in Bern</td>
</tr>
<tr>
<td>$\delta^{18}O_v$</td>
<td>-7.1‰</td>
<td>$^{18}O$ value of atmospheric vapour; $\delta^{18}O_l - \delta^{18}O_w = -\epsilon_i$, assuming isotopic equilibrium with soil water</td>
</tr>
<tr>
<td>$f$</td>
<td>0.3 - 0.5</td>
<td>the degree of dampening of leaf water $^{18}O$ enrichment as reflected in the stem cellulose</td>
</tr>
</tbody>
</table>

Coefficients of the regression lines $\delta^{13}C_{cell} = c_1 + c_2 \delta^{18}O_{cell}$ (as determined in this study; Pinus = *Pinus sylvestris*, Fagus = *Fagus sylvatica*, Picea = *Picea abies*): $c_1^{Pinus} = -119.2\% \pm 36.6\%$, $c_2^{Pinus} = 3.30 \pm 1.25 (r^2 = 0.87)$; $c_1^{Fagus} = -89.0\% \pm 6.3\%$, $c_2^{Fagus} = 2.24 \pm 0.22 (r^2 = 0.98)$; $c_1^{Picea} = -49.6\% \pm 4.8\%$, $c_2^{Picea} = 0.96 \pm 0.17 (r^2 = 0.97)$.

### Difference in $\delta^{13}C$ between stem cellulose and bulk wood (as determined in this study):

$\delta^{13}C_{stem} = 1.33\% \pm 0.20\%$

$\delta^{13}C_{cell} = 1.17\% \pm 0.34\%$

$\delta^{13}C_{air} = 1.36\% \pm 0.10\%$
production (Hill et al. 1995). Therefore, the $\delta^{18}O$ signal of leaf water imprinted on sucrose may be partly lost when sucrose exchanges oxygen atoms with less enriched stem water.

To calculate the $\delta^{18}O$ of cellulose in the stem, we assume a factor $f$ ($0 < f < 1$) which summarizes the dampening effect of (i) leaf water inhomogeneity and (ii) exchange of oxygen atoms of sucrose with stem water. Using the expression for leaf water enrichment (Eqn 2) we propose the following equation:

$$\delta^{18}O_{cell} = \delta^{18}O_i + f(e_k + e_c + (\delta^{18}O_i - \delta^{18}O_k - \epsilon_c) e_i/e_k) + e_c.$$  (3)

### Combining $\delta^{13}C$ and $\delta^{18}O$

Humidity conditions simultaneously influence the carbon isotope and the oxygen isotope ratios: $\delta^{18}O$ is influenced via changing $e_i/e_k$, and $\delta^{13}C$ via stomatal regulation of the plant. Therefore, plants grown under different humidity conditions may show a correlation between the $\delta^{13}C$ and $\delta^{18}O$ values of organic matter. This correlation should be positive because the isotopic shift caused by humidity changes is in the same direction for carbon and oxygen isotopes: drier conditions will increase both $\delta^{13}C$ and $\delta^{18}O$ values. The main restriction of this hypothesis is that other factors which can influence the isotopic ratios should not vary. Important factors altering the isotopic composition are, for $\delta^{18}O$, the light intensity, and for $\delta^{13}C$, the isotopic composition of source water, $\delta^{18}O_v$. A correlation between $\delta^{13}C$ and $\delta^{18}O$ cannot be expected, for instance, in a tree ring study when year-to-year variations are analysed, because the year-to-year variations in $\delta^{18}O$ of source water (i.e. precipitation) are large (Rozanski, Araguas-Araguas & Gonfiantini 1992).

Our data suggest that a correlation exists between the $\delta^{13}C$ and $\delta^{18}O$ values of cellulose of plants growing in different habitats (see ‘Results’). How can this result be interpreted in the light of the equations presented above? For simplicity we assume a linear relationship to exist, that is:

$$\delta^{13}C_{cell} = c_1 + c_2 \delta^{18}O_{cell}.$$  (4)

The coefficients $c_1$ and $c_2$ have to be determined experimentally and are species-dependent. Equations 1b, 3 and 4 form a set of independent equations for the variables $\delta^{13}C_{cell}$, $\delta^{18}O_{cell}$, $p/p_a$ and $e_i/e_k$, when all other parameters are regarded as constants. By simple algebraic operations, two of the variables, $\delta^{13}C_{cell}$ and $\delta^{18}O_{cell}$, are eliminated to yield a relationship for the dependence of $p/p_a$ on changing $e_i/e_k$.

$$p_i/p_a = \frac{1}{b-a} \left[ \delta^{13}C_{cell} + \epsilon_a - a - c_1 - c_2 (\delta^{18}O_{cell} + f(e_k + e_c + \epsilon_c)) \right]$$

$$- \frac{fc_2}{b-a} \left( \delta^{18}O_{cell} - \delta^{18}O_{v} - \epsilon_c \right) \frac{e_a}{e_i}.$$  (5)

We find that $p_i/p_a$ depends linearly on changing $e_i/e_k$, which is due to the presumed linearity of Eqn 4. The slope of Eqn 5, $-\left( f c_2 / (b-a) \right) (\delta^{18}O_{cell} - \delta^{18}O_v - \epsilon_c)$, is the important term: it is the sensitivity of the $p_i/p_a$ of a plant to changing $e_i/e_k$. The parameters which determine the slope are either known from the literature ($b$, $a$, $\epsilon_c$) or can be experimentally determined [$\delta^{18}O_v$, $\delta^{18}O$ and the slope of the $\delta^{18}O_{cell} - \delta^{13}C_{cell}$ plot (i.e. $c_2$)]. Yet considerable uncertainty is involved in the dampening factor $f$; the influence of varying $f$ on Eqn 5 will be evaluated in the sections ‘Results’ and ‘Discussion’. It is important to note that the slope of Eqn 5 is proportional to $c_2$. Accordingly, it should be possible to estimate the influence of changing relative humidity on the $p_i/p_a$ of a plant species from the measurement of $\delta^{18}O$ and $\delta^{13}C$ in organic matter.

### MATERIALS AND METHODS

#### Site description and sampling

The study area is located in the Jura mountain chain which constitutes the northern boundary of the Swiss Central Plateau. The climate is temperate-moist with a mean annual precipitation of c. 1000 mm and a mean annual temperature of c. 9 °C. Five sites were selected in a valley which stretches from east to west near Court (7°22’ E, 47°15’ N). The sites were chosen on an axis perpendicular to the valley (see Fig. 1) in order that site conditions varied within a short distance. Because the sites differed in exposure, the soil moisture conditions amongst the sites differed greatly. An ecologically defined moisture index was determined to characterize the soil conditions. This index is based on the composition and abundance of plant species at a site. In this method (Ellenberg 1974), every plant is assigned a number which is related to its appearance in different habitats in Switzerland, and the entire plant community determines the index of a site. A low soil moisture index indicates dry conditions. Two of the selected sites have a southern exposure and are dry. The site with index 2.05,
which is a Cotoneastro-Amelanchieretum plant community, is very dry (Ellenberg 1986). This site is on a steep slope with shallow soil, and the only tree species growing there is *Pinus sylvestris*. At the site with index 2·8 (Seslerio-Fagetum), *Fagus sylvatica*, *Picea abies* and *Pinus sylvestris* are present. The two sites with northern exposure are more humid (Fagetum silvaticae typicum): at the site with index 2·96 there are *Picea abies* and *Pinus sylvestris*, and the site with index 3·05 has *Fagus sylvatica*. The most humid site is on the valley bottom, near a small river (Aceri-Fraxinetum), where *Picea abies* and *Fraxinus excelsior* are found. Finally, samples from a previous study, from a site at about the same altitude and at a distance of 21 km, were used (Saurer, Borella & Leuenberger 1997). This is a dry site with index 2·6 with *Fagus sylvatica* (Pulmonario-Fagetum melittetosum).

Cores from six trees per species and per site were taken for isotope analysis. Additionally, cores from 16 trees were taken for ring width analysis. The samples were carefully dated by cross-checking and the ring width determined on a semi-automated system (WSL, Birmensdorf, Switzerland). For the isotope analysis we pooled 50 rings of each core representing the period from 1940 to 1989. This composite sample should represent a mean tree value.

**Determination of the isotope ratios**

The samples were milled and cellulose was extracted from the wood (Brenninkmeijer 1983). The $^{18}$O/$^{16}$O ratio of cellulose was measured using a continuous-flow method, similar to the one described by Werner et al. (1996). In the method used here, an elemental analyser (Carlo Erba 1108, Italy) is linked to an isotope ratio mass spectrometer (MS; Delta-S, Finnigan MAT, Germany) via an open split interface (Conflo II, Finnigan MAT). The quartz tube in the elemental analyser is filled to half its length with glassy carbon (Thierhaupten, Germany) and is kept at a temperature of 1080 °C. The samples (c. 1·5 mg) are loaded in tin capsules and dropped into the pyrolysis tube without addition of oxygen. After the pyrolysis, the oxygen evolving from the sample is mainly found in the form of CO. The gases are swept by the helium carrier gas through a water trap and a separation column (Poropak) and are then introduced to the MS. The ratio 30/28 is measured and used to calculate the $\delta^{18}$O value. The calibration versus VSMOW is performed with cellulose standards that have previously been analysed by independent methods. Comparison of the present method with these off-line methods (pyrolysis in nickel tubes, Saurer et al. 1997; pyrolysis with a mercury chloride method, Field, Switsur & Waterhouse 1994) and details of the on-line method will be published elsewhere (manuscript in preparation). The standard deviation for repeated analysis of cellulose is about 0·2‰. The $\delta^{13}$C values were previously determined using an off-line method (Saurer, Siegenthaler & Schweingruber 1995b). The $\delta^{13}$C values (± 0·1‰) are reported versus VPDB.

**RESULTS**

In Fig. 2 the mean tree cellulose isotope values are shown as a function of the soil moisture index of the sites. Clearly, there is a dependence of the isotope values on the soil moisture conditions, both for $\delta^{13}$C and for $\delta^{18}$O. This holds for all species, deciduous as well as coniferous. The slopes of the regression lines correlating the isotope ratios to the moisture index tend to be smaller for *Pinus sylvestris* than for *Fagus sylvatica* and *Picea abies*, and this is clearer for $\delta^{18}$O than for $\delta^{13}$C. However, it could not be determined whether the slopes are significantly different because the error in the moisture index is not precisely known. For $\delta^{13}$C, there are large differences between the species mean values, the conifers having higher values than the deciduous beech (see also Saurer et al. 1995b). These differences are more or less constant as long as trees with similar

moisture conditions are compared. On the other hand, a beech tree from a dry site can have a $\delta^{13}$C value nearly as high as that of a pine tree from a humid site, i.e. the influence of site conditions on the isotope ratio can override the species differences. For $\delta^{18}$O, the differences between the species are less pronounced. This is also reflected in the fact that there is a correlation between $\delta^{18}$O and the moisture index even when the data points from all species are pooled. The $\delta^{18}$O values are in the range from about 27.9 to 29.6‰, and the overall variability is therefore about 3 times smaller than for $\delta^{13}$C ($-26.5$ to $-21.5$‰). Further, the tree-to-tree variability for a given site and species is smaller for $\delta^{18}$O than for $\delta^{13}$C. This can be seen from the standard error bars in Fig. 2 (note the different scales on the y-axis for $\delta^{18}$O and $\delta^{13}$C); the standard errors are much smaller for $\delta^{18}$O, typically 0.15‰, than for $\delta^{13}$C, typically 0.3‰.

Table 2 gives the mean ring widths of the different species at each site (Fig. 1 shows where the trees were growing). High soil moisture content generally resulted in wider rings, i.e. increased growth. It must therefore be concluded that water is a limiting factor for the trees growing in this area. This was particularly true for the conifers, for which at least a doubling in mean ring width was observed when the driest and the most humid sites were compared. On the other hand, the increase in ring width due to enhanced water availability was not large for beech.

Since both the carbon and the oxygen isotope ratios are related to the soil moisture index, it was to be expected that the carbon and oxygen isotope ratios would be related to each other. To elucidate this point we plotted $\delta^{13}$C against $\delta^{18}$O using the mean site values as presented above. Figures 3a, b and c show results for Picea abies, Pinus sylvestris and Fagus sylvatica, respectively. A correlation is apparent for each species, and the coefficients of the respective regression lines $c_1$ and $c_2$ (where $\delta^{13}$C$_{Cell} = c_1 + c_2 \cdot \delta^{18}$O$_{Cell}$) are given in Table 1. There are significant differences between the species; in particular, the slopes (= $c_2$) vary by a factor of 3. The correlation coefficients $r$ are very high (see Table 1), although some caution is necessary in the interpretation of these results as only three or four data points are involved.

The model developed in the section ‘Isotope fractionations’ will now be applied to the results of this study, and the parameters necessary to evaluate Eqn 5 are discussed (see also Table 1). For the isotopic composition of atmospheric CO$_2$, $\delta^{13}$C$_{atm}$, a mean value representing the period from 1940 to 1990, was calculated, because the cellulose data also represent this period. This value was calculated to be $-7.1$‰ ($\pm 0.36$‰), using data from Friedli et al. (1986) and Keeling et al. (1989). The relatively large standard deviation is due to the fact that $\delta^{13}$C$_{atm}$ was not constant but steadily decreasing. The $\delta^{18}$O value of soil water, $\delta^{18}$O$_{soil}$ was calculated using the monthly $\delta^{18}$O data of precipitation from the period 1971–1990 at Bern (IAEA 1992). Bern is about 40 km from the study area. Data before 1971 are not available, but this period of 20 years should be long enough to represent adequately the period from 1940 to 1990. Taking the mean of the summer months April to September, we calculated a mean value of $\delta^{18}$O$_{soil} = -8.4$‰. The $\delta^{18}$O value of atmospheric vapour, $\delta^{18}$O$_{vap}$, was estimated by assuming isotopic equilibrium with soil water, that is $\delta^{18}$O$_{vap} = \delta^{18}$O$_{soil} = -\epsilon$. The difference between cellulose and bulk plant material was measured for each species (see Table 1). The difference was around 1.3‰, and no significant differences between the species were found. Most uncertainty is involved for $f$, i.e. the degree of dampening of leaf water $\delta^{18}$O variations as reflected in the stem cellulose. To study the effect of varying $f$, we evaluated Eqn 5 for $f = 0.3$, $f = 0.5$ and $f = 1.0$. Figure 4 shows the results obtained after solving in the whole range of possible values (0–1) for $e_j/e_i$ and $p/p_z$. The slopes depend on the value of $f$, whereas the ratio of the slopes between the species remains the same (provided that $f$ does not vary between species). Pinus sylvestris and Fagus sylvatica exhibit a steeper slope than Picea abies, that is, a stronger dependence on $e_j/e_i$. The curves shown in Fig. 4 correspond to an extrapolation of the isotope data beyond the range where data are actually present. To determine the relevant range, Eqn 3 has to be solved for $e_j/e_i$:

$$e_j/e_i = \frac{1}{f} \frac{(\delta^{18}O_{cell} - \delta^{18}O_{v} - \epsilon_k - \epsilon_e)}{\delta^{18}O_{v} - \delta^{18}O_{soil} - \epsilon_k}.$$

The range of $e_j/e_i$ was then calculated using the maximum and the minimum measured isotope values $\delta^{18}$O$_{cell}$. For

<table>
<thead>
<tr>
<th>Soil moisture index</th>
<th>Ring width (mm)</th>
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<tr>
<td></td>
<td>Picea abies</td>
</tr>
<tr>
<td>2-05</td>
<td>1.29 ± 0.20</td>
</tr>
<tr>
<td>2.8</td>
<td>2.04 ± 0.27*</td>
</tr>
<tr>
<td>2.96</td>
<td>1.59 ± 0.19</td>
</tr>
<tr>
<td>3-05</td>
<td>2.68 ± 0.35</td>
</tr>
<tr>
<td>3.15</td>
<td>2.68 ± 0.35</td>
</tr>
</tbody>
</table>

Table 2. Mean ring width at the investigated sites (± standard deviation; n=16). Each value is the mean of 16 trees (per species) whereby the mean ring width of an individual tree was calculated by pooling all rings from the period 1900 to 1989. The asterisks indicate that the ring width of a species at a site is significantly different ($P < 0.01$, t-test) from the ring width of the next-driest site, i.e. from the value of the next line. For instance, the mean ring width of Picea abies at the site with soil moisture index 2.96 (2.04 mm) is significantly different from the ring width at the site with soil moisture index 3.15 (2.68 mm)
does not vary between sites, this effect must be attributed to evaporative enrichment occurring in the leaf due to lower $e_a/e_i$ at the dry sites. The results of our study therefore indicate that leaf water enrichment is passed on to the $\delta^{18}O$ of leaf sucrose and finally reflected in the cellulose of

$e_a/e_i$ covers the range 0.4–0.5 (for all species), which is reasonable for the relative humidity in this climatic area, whereas the range is too low for $f=0.3$ and too high for $f=1.0$.

**DISCUSSION**

The sensitivity of $\delta^{13}C$ in plant material to humidity conditions (soil moisture and/or relative humidity of the atmosphere) has been demonstrated in widely differing experimental set-ups, ranging from tree-ring studies (Leavitt & Long 1988; Saurer et al. 1995b) to measurements on container-grown plants in the greenhouse (Farquhar & Richards 1984). Periods of low precipitation and of water stress treatment, respectively, result in increased $\delta^{13}C$ values. Our data show that this effect is reflected in the long term in the cellulose of stem material when trees from sites with differing soil moisture are compared. The $\delta^{18}O$ measured on these same samples is also dependent on the moisture conditions. Provided that the $\delta^{18}O$ of the soil water

![Figure 3. $\delta^{18}O$–$\delta^{13}C$ plots using the data of Fig. 2. (a) Picea abies, (b) Pinus sylvestris, (c) Fagus sylvatica. Regression lines are indicated.](image)

![Figure 4. Results of model evaluation showing $p_i/p_a$ as a function of $e_a/e_i$. The different line types show the responses of the different species: Picea abies, solid line; Pinus sylvestris, long-dashed line; Fagus sylvatica, short-dashed line. In (a) Eqn 5 was evaluated using $f=0.3$, in (b) $f=0.5$ and in (c) $f=1.0$.](image)
the stem. However, this straightforward interpretation may be too simple. Whereas the leaf water response to varying relative humidity has been extensively investigated, it is not known to what degree this enrichment can be found in leaf organic material. Yakir, DeNiro & Ephrat (1990a) found higher leaf water $d^{18}O$ in non-irrigated cotton plants compared to irrigated plants, which was reflected in the leaf organic material of one species only (of two investigated). The authors suggest that the metabolic water compartment of the species which did not respond may be clearly separated from the water at the site of evaporation where the isotopic enrichment takes place. Ferhi, Bariac & Letolle (1983) found a linear relationship between mesophyll water $d^{18}O$ and cellulose $d^{18}O$ of bean plants grown under differing relative humidity, although the slope of the linear fit was significantly below unity (0.39). From these results we conclude that there is a transfer of the $d^{18}O$ signal in leaf water to organic material, but (i) the signal may be damped and (ii) the signal transfer may depend on species. Regarding the transfer to cellulose in the stem, a possible exchange of oxygen between stem water and sucrose during cellulose production could remove the $d^{18}O$ signal from the leaf water. Sternberg et al. (1986) estimated an exchange of 45% for cellulose-producing bacteria, a value which in principle could also hold for trees. This view is supported by Hill et al. (1995), who found early-wood $d^{18}O$ of oak to be determined by the $d^{18}O$ of current-year water, although the material is built up from carbohydrates from the previous year. Nevertheless, several studies have found an influence of varying relative humidity on stem cellulose. Lipp et al. (1996) found a dependence of $d^{18}O$ in stem cellulose of *Tamarix jordanis* on relative humidity similar to that for the $d^{18}O$ of leaf water (though not as strong as predicted by the enrichment model, Eqn 2) and concluded that complete re-equilibration between cellulose and trunk water does not occur. Further, in tree-ring studies an influence of relative humidity was found (Edwards & Fritz 1986; Saurer et al. 1997). In the latter study it was estimated from correlation analysis on meteorological data, and from evaluation of Eqn 2, that the variation of $d^{18}O$ in leaf water might be damped by a factor of 3 in stem cellulose. From all this evidence we conclude that the assumption of the dampening factor $f$ as presented in the theoretical section is justified and that its value probably is in the range from 0.3 to 0.5.

So far we have assumed that the differing soil moisture conditions, along with the resulting differences in the relative humidity of the atmosphere, are the main cause of the isotope variations. Can we be sure that other factors are negligible? In particular, differing exposure to light may influence $d^{13}C$, and differing altitude could violate the assumption of equal $d^{18}O$ of precipitation at all sites (Siegenthaler & Oeschger 1980). However, there is no simple relation between moisture index and light or altitude (as can be seen from Fig. 1), so these factors cannot explain the correlation with the moisture index that we find for both isotopes. The good correlation may also be due to the use of 50 year averages, which avoids consideration of short-term variations of source water $d^{18}O$ (in response to seasonal and year-to-year variations of the $d^{18}O$ of precipitation).

Combining the present fractionation models, the linear relationship between the $d^{13}C$ and $d^{18}O$ of cellulose means that $p_i/p_o$ depends linearly on $e_i/e_o$. This outcome of Eqn 5 is a physiologically meaningful result, although plants are thought to be sensitive to vapour pressure deficit more directly than to relative humidity (Aphalo & Jarvis 1991). Our findings agree well with results obtained by Zhang & Nobel (1996), who found $p_i/p_o$ to decrease linearly as vapour pressure deficit increased for a range of C$_3$ species. We suggest using the dampening factor $f$ in Eqn 3, which reduces the influence of $d^{18}O$ leaf water enrichment. Both the transfer from leaf water to leaf sucrose and the transfer from sucrose to stem cellulose may contribute to $f$. Obviously, the use of a single factor is a very simple concept. However, its simplicity may be an advantage in the absence of a better understanding of the relevant processes. Evaluation of the proposed model (Eqn 5) allows estimation of $f$, and such calculated values can be compared to experimental data as $f$ is directly proportional to the slope in the $d^{18}O$–$d^{13}C$ plot. We find that the model and data are consistent for $f = 0.5$, i.e. a value which is within the range of values reported above. Therefore, we conclude that a humidity signal is indeed stored in the $d^{18}O$ of stem cellulose, although it is strongly damped. In future controlled experiments, Eqn 5 can be more thoroughly tested by growing plants in varying relative humidity or water supply while ensuring that the $d^{18}O$ of soil water, light and other interfering factors are kept constant. Besides improving understanding of fractionation processes, Eqn 5 may ultimately be useful as a tool in photosynthesis research. For instance, for modelling photosynthesis in response to varying environmental conditions it is important to know the dependence of the $p_i/p_o$ of a plant on changing $e_i/e_o$. This species-dependent function may be experimentally determined by the combined analysis of carbon and oxygen isotope ratios in organic material.

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REFERENCES


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