Reducing uncertainties in $\delta^{13}C$ analysis of tree rings: Pooling, milling, and cellulose extraction

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Abstract. Recent developments of on-line methods have provided another boost to the determination of stable isotope ratios in organic material. Along with a significant increase in sample throughput, the sample sizes decrease, both of which are necessary conditions to acquire long time series from limited wood amounts. In view of this new technique we reconsidered the most important factors influencing the measured isotopic signature which are (1) pooling, (2) homogeneity, and (3) cellulose extraction. In most cases, pooling (i.e., mixing wood of the same year from different trees) can be made in a simple way by mixing the whole wood available because mass-weighted and unweighted isotope measurements were the same within the error. More attention must be paid in homogenizing the sample. Theoretical considerations underpinned by experimental results suggest a fineness of 0.15 mm (115 mesh) if cellulose is extracted and 0.1 mm (165 mesh) for direct wood analysis. Many of previous studies did not achieve this fineness. We find that wood is as good a climate proxy as cellulose. This is shown by comparing correlations of wood and corresponding cellulose isotope values with meteorological data, which are identical within the uncertainty.

1. Introduction

The recent development of on-line methods for the stable isotope determination in organic material based on gas chromatograph mass spectrometer techniques opens new research opportunities [*Barrie*, 1991]. This technique leads to a significant increase in sample throughput and a potential to decrease the sample size, which are both necessary conditions to acquire long time series from limited wood amounts in tree-ring studies. As a consequence of this technical improvement, sample preparation (e.g. cellulose extraction) becomes the time-limiting step, and one has to take greater care of the sample homogeneity.

Some comparisons between $\delta^{13}C$ of cellulose and bulk wood or lignin have already been published. *Francey* [1986], *Leavitt and Long* [1991], *Schleser* [1990], *Yildiz* [1995], and *Livingston and Spittlehouse* [1996] found that interannual or intra-annual signal patterns of $\delta^{13}C$ values measured on cellulose and wood are highly correlated. *Wilson and Grinsted* [1977] and *Mazany et al.* [1980] found similar results for cellulose and lignin. However, despite these results, the postulated necessity of extracting cellulose for isotope measurements [*Wilson and Grinsted*, 1977; *Grinsted et al.*, 1979; *Livingston and Spittlehouse*, 1996] has not yet been questioned.

Concerning sample homogeneity, we note that very few researchers mention the fineness of their samples in their publications. *Leavitt* [1993b] and *Leavitt and Lara* [1994] report that their samples were ground to 20 mesh ($\approx$0.78 mm), *Yildiz*

[1995] used a "Ultrazentrifugalmühle" with sieve wideness of 0.75 mm, and *Livingston and Spittlehouse* [1996] used a cyclone mill (<0.2 mm). In some cases the samples are cut into very fine shaves (20–40 μm) and are therefore not milled [*Switsur et al.*, 1995].

Besides good homogeneity, pooling or averaging material from different radii and trees is needed. *Leavitt and Long* [1984] noted that pooling material from at least four trees is required to have a site-representative sample, for *Pinus edulis*. Some other researchers [e.g., *Ramesh et al.*, 1985; *Leavitt and Lara*, 1994; *Saurer et al.*, 1997] compared the $\delta^{13}C$ curves of different trees from the same site, but to our knowledge, only *Leavitt and Long* [1984] compared mass-weighted and unweighted means of $\delta^{13}C$ from different trees, and they concluded that a difference cannot be detected.

The aim of this paper is to present theoretical considerations on (1) pooling, (2) homogeneity, and (3) cellulose extraction and to compare them with new experimental $\delta^{13}C$ results on beech and oak wood. We will address the following questions: How do we have to pool material from different trees? How fine do we have to mill the wood? Is it necessary to extract the cellulose for $\delta^{13}C$ measurements?

2. Theory

2.1. Pooling

When reconstructing the local or regional environmental conditions (e.g., climate), the results from different trees have to be averaged in order to eliminate genetic or site-specific influences. Pooling of the material from different trees accounts for this and additionally saves time, compared to individual

measurements. However, we note that pooling previous to the analysis will give a different mean compared to averaging the individual δ^{13}C values. Pooling all material of a given year from different trees will produce a mean mass-weighted δ^{13}C value, which can be approximated by the ring-width weighted mean. Let us calculate the possible shift with realistic growth variations and δ^{13}C differences. As has been shown before, trees from the same site can differ in their absolute δ^{13}C values by up to 2‰ [*Saurer et al.*, 1997; *Leavitt*, 1993a; *Leavitt and Long*, 1984] (see also section 4.1). If one of the trees shows a rapid growth change, a weighted mean would create an unreal shift in the resulting record. As an illustration, we took four trees from one of our sites and calculated the ring width of one tree relative to the sum of all four trees, i.e., the contribution of this tree to the total material. Over the ~200 years long record this contribution varied between 15 and 65% with shifts of as much as 25% between two adjacent rings (from 65 to 40%). If, in a similar manner, the δ^{13}C values of this tree would deviate by 2‰ from the mean of the other three trees, a weighted mean would artificially yield variations of up to 1‰ over the whole period and up to 0.5‰ in one year. Although this is an extreme case, this calculation shows that one must pay attention to this problem.

The same reflection can be made for the pooling of material from different radii of one tree. However, we think that it is a priori acceptable to mix all the material of the same year, because the genetical variability does not exist, and the growth rate differences most likely come from an unequal apportionment of material produced in the leaves.

2.2. Homogeneity

The second important step in the preparation of the samples is the milling, especially for measurements on very small subsamples. A very coarsely milled sample could lead to an overrepresentation of some parts of the ring. To estimate the fineness, which is required to assure that subsamples do represent the whole sample, we model the sample as consisting of two parts with distinct isotopic values $\delta+\Delta$ and $\delta-\Delta$. (This is of course extremely simplified, since a large part of the wood will have intermediate δ-values. However, it permits simple calculations and gives an upper limit for homogeneity needs.) We set Δ to 1‰ or 1.5‰, respectively, in accordance with intra-annual differences for different tree species (e.g., *Pinus radiata, Pinus strobus, Acer saccharinum, Juniperus, Pseudotsuga, Quercus robur*)[*Wilson and Grinsted*, 1977; *Leavitt and Long*, 1991; *Leavitt*, 1993a, b; *Switsur et al.*, 1995]. If the measured subsample is made of n pieces of cellulose (or wood) with the same weight, a binomial distribution gives the following results: k pieces with $\delta+\Delta$ and n-k pieces with $\delta-\Delta$ gives a mean value of $\delta_{measured}$ = $\delta + (2k-n)/n\Delta$ instead of δ. The probability that one measures a value $(2k-n)/n\Delta$ higher than expected is

$$P\left(\delta_m = \delta + 2\frac{k}{n}\Delta - \Delta\right) = P(k) = \left(\frac{1}{2}\right)^n \frac{n!}{k!(n-k)!} \quad (1)$$

Summing equation (1) for $\delta_m = \delta-\varepsilon$ to $\delta+\varepsilon$ gives the probability to measure a δ_m with a maximum deviation of ε from the correct value δ; it is given by

$$P\left(\left|\delta_m - \delta\right| < \varepsilon\right) = \dots\dots\dots$$

$$= 2 \sum_{k>\frac{n}{2}}^{k<\frac{n}{2}\left(1+\frac{\varepsilon}{\Delta}\right)} \left(\left(\frac{1}{2}\right)^n \frac{n!}{k!(n-k)!}\right) + P\left(k = \frac{n}{2}\right) \quad (2)$$

For $n > 36$, this formula can be approximated by a Gaussian distribution $(\mu = n/2, \sigma = \sqrt{n}/2)$ (see any mathematical table).

As the reproducibility of today's on-line methods is in the range of 0.1 to 0.2‰, we calculate the number of pieces of a subsample necessary to restrict the potential deviation to less than 0.1‰ (ε<0.1‰, 0.15‰, 0.2‰, respectively) with a probability of better than 68% ($1\sigma = \varepsilon$) or 95% ($2\sigma = \varepsilon$), respectively. (The approximation with a Gaussian distribution mentioned above leads to $n \geq (\alpha\Delta/\varepsilon)^2$, where $\alpha = \varepsilon/\sigma$.)

Table 1 shows that ~250 pieces are required to restrict the deviation to less than 0.1‰ with a 68.3% (1σ) probability for $\Delta = 1.5$‰. This is probably an extreme case because a real sample is not constituted of two pools but covers a continuous isotopic spectrum. These 250 pieces of a sample of 1.5 mg correspond to pieces of ≤ 6 µg. With a cellulose content of 40% and a wood density of about 600 kg/m^3 the fineness of the milled wood has to be better than ~0.025 mm^3, i.e. cubes of less than 0.29 mm side length (Wood is milled prior to cellulose extraction, because (1) the extraction is not well achieved on very large pieces and (2) cellulose is extremely difficult to ground). Using a sieve, it has to be taken into account that longer pieces with the same cross section could pass, so we would suggest using a sieve of 0.15-0.2 mm to be sure that all pieces are small enough. Measuring wood instead of cellulose, the sieve must be $\sqrt[3]{2.5}$ times finer, i.e. ~0.10 mm. An alternative to a sieve to control the fineness of a sample is to weigh the largest pieces after milling.

These theoretical results show that one must pay attention to the milling. For example, we calculated the scatter caused by the inhomogeneity of samples "ground to 20 mesh" as described by *Leavitt and Lara* [1994]. We find a 1σ error of $\varepsilon \approx 0.2$‰, which is not negligible in comparison with their "usual overall precision of 0.15-0.20‰."

All these calculations rest on the assumption that the subsample is taken from an infinite sample. This assumption is approximately valid in our case, as we measure subsamples of 1-1.5 mg out of samples of 10-50 mg (in most cases). In the extreme case that all of the sample material is used for the measurement homogeneity is unimportant, of course. As an intermediate case, we calculated the probabilities for subsamples making up one half of the sample. Our results show that one needs about half as much pieces in a subsample than in the "infinite case" to obtain the same precision.

Table 1. Homogeneity Requirements

Probability	0.1‰	0.15‰	0.2‰
68.3%	110/239	53/110	29/68
95.4%	410/914	187/410	104/232

Number of pieces in a subsample as required in order to measure a value which deviates less than ε (0.1, 0.15, 0.2‰, respectively) from the correct value with a probability of 68.3% or 95.4%, respectively. The first value applies to Δ=1‰, the second to Δ=1.5‰; see text.

2.3. Cellulose Extraction

Until now most authors have made their stable isotope measurements in tree rings on pure α cellulose or on holocellulose. This procedure has been generally accepted for two principal reasons: (1) to avoid influence of variable relative abundance of the different wood constituents on the stable isotope results, within a single tree [Narayanamurti and Das, 1955], and (2) to minimize the influence of possible material exchange between adjacent rings (resins are not bound to a given ring [Tans et al., 1978]) or atom exchange after wood synthesis (cellulose is known to be rather stable).

We now look at these two arguments more in detail. (1) We cannot a priori say that the environmental signal is better recorded in cellulose than in wood. What we can do is to evaluate what influence variations in the wood composition could have on the isotope ratio of whole wood. For this, let us consider a two-box model of wood composed of α cellulose and lignin:

$$\delta_W = \delta_C p_C + \delta_L p_L \qquad (3)$$

where δ is the relative isotope ratio ($\delta = (R_{sample}-R_{standard})/R_{standard}$, where R = isotope ratio $^{13}C/^{12}C$, respectively, $^{18}O/^{16}O$, or $^{2}H/^{1}H$) ($\delta^{13}C$, $\delta^{18}O$, or $\delta^{2}H$), p is the relative part of the constituents in the wood, and the subscripts W, C, and L refer to wood, cellulose, and lignin.

If we assume a constant difference between δ_C and δ_L ($\Delta_{CL}:=\delta_C-\delta_L$) for material produced under the same conditions, equation (3) can be simplified:

$$\delta_W = \delta_C - \Delta_{CL} p_L \qquad (4)$$

or $\qquad \Delta_{CW}:= \delta_C - \delta_W = \Delta_{CL} p_L \qquad (4')$

For Δ_{CL} we find a value of 3.25‰ (± 0.45‰), from measurements of birch, beech, oak, and spruce wood after different extraction steps (see section 4.3 and Figure 1), which agrees with the value from Wilson and Grinsted [1977] of 3.5‰ for Pinus radiata. We used the values given by Narayanamurti and Das [1955] for cellulose and lignin content of wood of different tree species (Cedrus deodara, Cupressus torulosa, Pinus longifolia, Shorea robusta, Tectona grandis, and Dalbergia sissoo) to calculate Δ_{CW}. Assuming Δ_{CL} = 3.25‰, we

find Δ_{CW} to be in the range of 1.00 and 1.37‰ for different tree species, and the difference between extremes for one species should not exceed 0.18‰. (2) Up to now we have only considered two components of wood (cellulose and lignin). One other group of constituents which is important for our considerations are the extractives, i.e., materials that can be exchanged between adjacent annual rings, like resins. Tans et al. [1978] showed with Δ^{14}C measurements that C-atom exchange between the annual rings 1962 and 1963 A.D. of an oak grown in the Netherlands amounts to 4-5% of the total C-content of the wood. Typical year-to-year $\delta^{13}C$ variations are ≤ 2-3‰; hence for oak trees we can expect a $\delta^{13}C$ variation dampening of ≤ 0.15‰ caused by material exchange. However, trees with possibly high resin content, like pines, could be much more affected by material exchange [Darkwa, 1996]. Even long-term changes could be strongly distorted because the resin content can vary considerably between sapwood and heartwood. For example, a 0.5 to 6.5% change in resin content (as measured for Dalbergia sissoo [Narayanamurti and Das, 1955]) with a $\delta^{13}C$ difference of about 3‰ between α cellulose and extractives [Deines, 1980] would lead to a shift in $\delta^{13}C$ of the whole wood of about 0.3‰.

On the basis of these theoretical considerations about cellulose extraction we conclude that measuring $\delta^{13}C$ on whole wood should yield reliable values in most cases. Problems could be associated with heartwood and sapwood, if the extractives are not removed. Other problems could arise for $\delta^{18}O$ and $\delta^{2}H$ measurements associated with O or H atoms exchange or selective bacterial or fungal degradation of old wood. It should also be mentioned that an incomplete or varying degree of cellulose extraction could be as detrimental as no extraction. A 5% lignin residual in the cellulose would diminish the apparent $\delta^{13}C$ of the cellulose as much as 0.15‰ with $\Delta_{CL} \approx 3$‰ as mentioned before.

3. Experiment

3.1. Material

The first sampling site is an oak plantation (Quercus sp) located on a hill (584 masl) in the Swiss "Mittelland", near
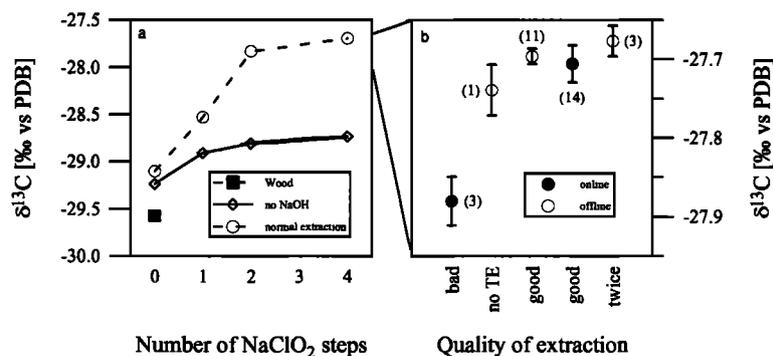

Figure 1. (a) The δ^{13}C increase during α cellulose extraction, i.e., during lignin removal. Symbol size characterizes the errors. (b) Measurements on different complete extraction-series from our internal standard shows that a bad extraction can lower the δ^{13}C value by almost 0.2‰. However, we can consider our extraction technique as suitable for δ^{13}C measurements, as there is no significant shift after a second extraction. The errors of the means and the numbers of measurements (in brackets) are given in Figure 1b. The lines connecting the two figures should emphasize that the variations between different complete extractions are much smaller than the difference between wood and cellulose.

Salvenach (sample notation "Sa") (7°10'20"E/46°54'50"N). The slightly elevated position ensures that our >250-year-old sampled trees have no access to groundwater. The second site is located also on a hill (380 masl), in northern Germany, near Limlingerode ("Li") (10°30'00"E/51°33'41"N). More information on site and tree characteristics are given by *Artmeyer* [1996].

Most isotope analyses were undertaken on α cellulose (C). However, to gain a supplementary information, we also measured the whole wood (W) of some samples and samples extracted with toluene ethanol (TE) only.

Additionally, we took subsamples of wood from birch, beech, as well as from heartwood and sapwood of oak and spruce during the different steps of our cellulose-extraction procedure (see later) and analyzed their $^{13}C/^{12}C$ ratios, in order to extract information about isotopic signatures of each wood component.

3.2. Methods

3.2.1. Tree rings separation. After having measured the ring widths of all our wood cores and sectors, we cross-dated them. When the dating was made, we separated 1-year samples. For one oak we even separated earlywood from latewood. First, we cut through earlywood and then shaved small earlywood slices until latewood is reached.

3.2.2. Milling. Wood samples were milled with a modified commercial coffee mill (Moulinex 980, 60 g). The milling device consists of two rotating cutting blades. We only added a deflection plate to direct the wood pieces toward the cutter. Our first samples were not milled fine enough as the cellulose pieces amounted up to 0.1mg, which was much too coarse as documented by the high standard deviation of our first $\delta^{13}C$ measurements. Afterward, we milled longer, and prior to the milling, we dried the wood for > 4 hours in an oven at 100°C. The resulting cellulose pieces were all less than 10 μg.

3.2.3. Cellulose extraction. We usually extract α cellulose according to a method described by *Brenninkmeijer* [1983]: (1) dissolution of the extractives with a 1:1 mixture of toluene and ethanol for 5-7 hours in a Soxhlet apparatus; (2) extraction of the lignin with a 0.5% $NaClO_2$ + 0.15% acetic acid solution for 3-6 x 12 hours at 70°C; this step is repeated until the sample is "white"; (3) extraction of the hemicellulose with a 4% NaOH solution for 24 hours at 70°C; (4) cleaning the α cellulose in deionized water for 24 hours at 70°C; between the different steps the samples are rinsed with deionized water.

To find out how important each extraction step is, we measured the $\delta^{13}C$ at different stages of the extraction. Figure 1 shows the results of this experiment. At each $NaClO_2$ step, more lignin is removed, which is documented by the increasing $\delta^{13}C$. The $\delta^{13}C$ plateau after three $NaClO_2$ steps, as well as the nonsignificantly different $\delta^{13}C$ of the "twice-extracted" cellulose, shows clearly that our method is suitable for $\delta^{13}C$ measurements. However, Figure 1b also shows that an incomplete extraction (i.e., stopped before the material is white) can produce samples with a $\delta^{13}C$ of up to 0.2‰ lower than α cellulose.

3.2.4. Isotope ratio measurement. The first group of our samples has been analyzed with an off-line method: 2.8-3.5 mg of material together with cupric oxide is placed in quartz tubes, sealed under vacuum, and then heated to 950°C for 2 hours. The produced CO_2 is purified by removing H_2O at -80°C and by pumping off noncondensable gases at the tem-

perature of liquid N_2. The isotopic composition of CO_2 is determined with a 250 Finnigan MAT mass spectrometer. The reproducibility of the method, including cellulose extraction, is 0.02‰, which is of the same magnitude as the reproducibility of the instrument itself.

However, most samples were analyzed with an on-line method at the Paul Scherrer Institute (PSI), in Villigen, Switzerland. This method uses an elemental analyzer which is coupled to the MS and is described in detail by *Saurer et al.* [1997]. The reproducibility of this method varies between 0.08 and 0.15‰, perhaps depending on the age of the catalysts, which have to be replaced after about 700 samples. With this method we analyzed subsamples of 1.3-1.6 mg.

4. Results and Discussion

4.1. Pooling

It is necessary to average isotope data from several trees to obtain a representative site value. There are two different approaches. One can pool material from different trees prior to analysis, or measure each tree separately and average the results afterward. As the growth pattern of distinct trees can be much different, the results of the two methods will disagree. If the material is pooled prior to analysis, a temporarily rapid growth of one tree enhances the importance of this tree. The influence of pooling samples on the measured isotope value can be seen by measuring $\delta^{13}C$ from several trees from the same site and comparing ring-width weighted and unweighted means. We made this comparison with trees from two sites. From the northern Germany site we have analyzed $\delta^{13}C$ from two oak trees (Li 152 and Li 171) with a 1-year resolution, which differ by up to 2‰. Additionally, we used older measurements (3-year resolution) from *Saurer et al.* [1997] of four beech trees from a site near Twann in the Swiss Jura. For both sites, the weighted and unweighted means show quite the same pattern ($r^2=0.99$, $n=32$ and $r^2=0.94$, $n=18$, respectively), although they exhibit differences of -0.18 to +0.18‰ and -0.22 to +0.13‰, respectively. However, these two examples do not exhibit large and rapid growth rate variations, in contrast to the oak trees from the "Sa" site. Unfortunately, we have analyzed only one tree from the "Sa" site yet. A theoretical discussion of rapid growth rate variations influence is given in section 2.

These numerical experiments show that weighted and unweighted means give significant differences but that the information in both curves is almost the same. Of course, these are only two examples with oak and beech trees, and it is possible that some growth patterns are even more extreme, and hence weighted means could lead to a loss of information due to ring-width variability. To avoid these problems, we suggest measuring each tree separately for periods with large growth variations of any tree and to exclude trees that have a growth pattern that strongly deviates from the site mean over the whole period of analysis. Another disadvantage of pooling prior to measuring is, of course, the loss of any information concerning the intrasite variability. Therefore we suggest to analyze the tree rings from each tree separately every 10 (or 5, or 25, etc., depending on the total length of the record) years, in order to gain an information on the variability. This procedure has already successfully been applied by several authors [e.g., *Leavitt and Lara*, 1994; *Leavitt and Long*, 1992].
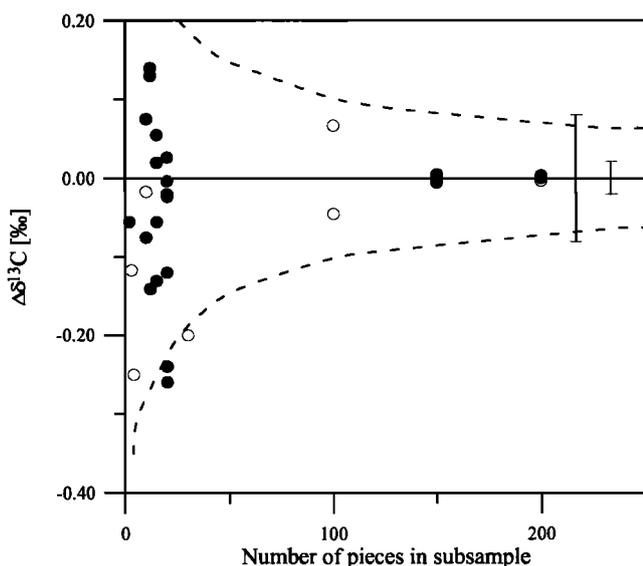
Figure 2. Difference from the accepted value for various sub-samples with known number of cellulose pieces. The mass of the subsamples is almost kept constant within each method. The error bars to the right represent the standard deviation of the on-line (longer bar) and off-line method (shorter line), as determined with measurements of our commercial cellulose. The dashed curves show the expected uncertainty as a function of cellulose pieces as calculated according to section 2 for $\Delta=1‰$ and a probability of 68.3%.

4.2. Homogeneity

Homogeneity depends on the fineness of a sample. To test this dependence, we measured twice or more times the ^{13}C/^{12}C isotope ratio of some coarse-milled samples, from which we weighted single pieces. The standard deviations of the samples are then compared with that of our commercial standard cellulose, which can be assumed as absolutely homogeneous in comparison to our measurement precision, to split the standard deviation into effects from sample inhomogeneity and analysis uncertainty, respectively. In Figure 2 the results are given versus the number of cellulose pieces for the on-line and off-line techniques.

Despite the fact that the number of samples in this study is too low for a statistical analysis, we can see that the samples with fewer cellulose pieces tend to show more scatter. The larger deviations are in agreement with our theoretical calculations shown as dashed lines. Another important result is the generally lower δ^{13}C values of the samples with larger cellulose pieces (i.e., fewer pieces). This confirms our results on standard material that incomplete cellulose extraction gives lower δ^{13}C values (see section 3.2 and Figure 1).

4.3. Cellulose Extraction

The different cellulose extraction steps can be traced measuring the δ^{13}C of different wood constituents of the same samples. First, we compare the δ^{13}C values of birch, beech, oak, and spruce wood after different extraction steps. From each tree species we took wood covering 10-40 years; for oak and spruce we separately analyzed heartwood and sapwood. Table 2a shows these results. Values for wood, TE, holocellulose, and α cellulose are direct measurements. Δ_{CL} have been calculated from TE and holocellulose values and their relative parts in wood as determined from our extractions. Δ_{CL} vary from 1.56 to 4.68‰ (for oak sapwood and beech, respectively), but these large differences are not significant, as can be seen from the large uncertainties, which are a result of the uncertainties of the relative parts of the different wood components.

Secondly, we compare the δ^{13}C patterns of tree ring series of α cellulose, wood without the extractives, and whole wood from two different oak trees as well as from a beech. Table 2b summarizes the differences between δ^{13}C from wood (W), wood without extractives (TE), and α cellulose (C) for an oak of the Swiss "Mittelland" (Sa1) as well as an oak (Li171) and a beech (Li161) from northern Germany. These values agree well with our results (see Table 2a), regarding the fact that the oak and beech wood without the extractives is composed of ~70% holocellulose (with a δ^{13}C almost equal to that of α cellulose) and ~30% lignin (with a δ^{13}C 3.25‰ lower than α cellulose).

We calculated the correlation coefficient as well as the slope of the linear regressions between the δ^{13}C of the different wood constituents (Table 2b and Figure 3). In order

Table 2a. $\Delta\delta^{13}$C for Standard Wood

Tree Species	C-W, ‰	C-TE, ‰	C-Holocellulose, ‰	C-L, ‰	Year AD	Number of Trees Pooled
Birch	1.81±0.06	1.54±0.06	1.03±0.03	2.74±0.68	1989*	~5
Beech	1.68±0.20	1.50±0.20	0.38±0.12	4.68±1.45	1978-1992	2
Oak, heartwood	1.02±0.20	0.86±0.22	-0.34±0.25	3.63±1.43	1930-1970	2
Oak, sapwood	1.64±0.22	0.74±0.22	0.37±0.25	1.56±0.85	1973-1994	2
Spruce, heartwood	1.37±0.20	1.28±0.20	0.14±0.12	3.64±0.94	1950-1960	2
Spruce, sapwood	1.34±0.22	1.08±0.22	-0.15±0.25	3.23±0.91	1987-1996	5

$\Delta\delta^{13}$C between α cellulose(C) and wood (W), wood without the extractives (TE), holocellulose, and lignin (L). Each line corresponds to one large sample containing material from several trees (numbers given in column 7), and from the time period indicated in column 6. Heartwood and sapwood have been analyzed on the same trees. The δ^{13}C values for lignin have been calculated from TE and holocellulose. The other values are direct measurements. The errors are based on replicate measurements of the different samples and estimated uncertainties of our determinations of percentages of the wood constituents. Beech trees germinated in ~1910 and were cored in 1992. Oak trees germinated in ~1730 and were cut in 1994. Spruces germinated in ~1860 and ~1965 (for heartwood and sapwood, respectively) and were cored in 1996.

*The birch material comes from a chamber experiment and was only 1 year old.

Table 2b. Tree-Ring Component Comparison

| Tree	Materials	$\Delta\delta^{13}$C, ‰	r^2	Slope, ‰/‰	n
Oak Li171	C-W	1.12±0.14	0.89 (0.91±0.04)	0.85±0.09 (0.97±0.09)	14
	C-TE	1.01±0.18	0.86 (0.91±0.04)	0.86±0.12 (0.97±0.09)	14
Beech Li161	C-TE	0.99±0.13	0.92 (0.92±0.03)	0.96±0.08 (0.97±0.08)	14
Oak Sa1, latewood	C-W	0.89±0.16	0.99 (0.97±0.01)	0.80±0.03 (0.99±0.05)	16
	C-TE	1.23±0.20	0.90 (0.94±0.02)	1.09±0.08 (0.98±0.05)	26
Oak Sa1, earlywood	C-W	1.12±0.19	0.94 (0.94±0.02)	0.71±0.05 (0.98±0.06)	16
	C-TE	1.24±0.30	0.81 (0.96±0.01)	0.89±0.10 (0.99±0.04)	22

Comparison between δ^{13}C from α cellulose(C) and wood(W) and/or α cellulose and wood after removing of the extractives(TE). Column 3, mean δ^{13}C difference($\Delta\delta^{13}$C) with the standard deviation; column 4, correlation coefficient (r^2); column 5, slope of the linear regression with δ^{13}C of the α cellulose as independent variable with standard error of this slope; and column 6, number of pairs of values (n). The values in parentheses refer to the results of our Monte Carlo simulation over 5000 artificial data sets. (For more details, see text.)

to determine how much of the discrepancies between the δ^{13}C series can be explained by the measurement uncertainty, Monte Carlo simulations were carried out assuming that the δ^{13}C values are Gaussian distributed around our measured values, with a standard deviation of 0.08‰ for α cellulose and 0.11‰ for W and TE. The discrepancies between α cellulose and other constituents are, to a large degree, caused by the measurement uncertainty, only the correlation of wood versus cellulose from oak Sa1 (earlywood as well as latewood), and possibly Li171, give a slope significantly lower than 1 (Table 2b). This means that the δ^{13}C signal from wood is significantly dampened. This fact can be explained by a part of the wood which has a δ^{13}C uncorrelated to that of the α cellulose. A more precise result can be obtained with the correlation between δ_W and δ_C: $\delta_W = (0.80 \pm 0.03) \delta_C - (5.7 \pm 0.6)$‰, $r^2 = 0.986$ (Figure 3b) implying that our measured oak wood is composed of ~80% of material, the δ^{13}C of which varies parallel to that of α cellulose, and 20% of material, the δ^{13}C of which is fully uncorrelated to that of α cellulose. Because r^2 is not significantly different from 1 (see Table 2b), we conclude that δ^{13}C of the extractives is almost constant over the whole measured period. The even lower slope for earlywood confirms our interpretation, as earlywood of oak contains more extractives than latewood. An important question for the use of δ^{13}C in tree rings as climate proxy is whether the significant loss of sensitivity due to the use of bulk wood is negligible or not in comparison with the biological noise. This is the topic of the next paragraph.

Measurements of δ^{13}C on wood, cellulose, or lignin show quite the same variations, but with significant different absolute values (Figure 3a). The discrepancies between the variations of the different series exceed the theoretically expected range of ± 0.09 slightly but not more than what can be explained by the measurement uncertainty. In order to estimate the influence of these effects on whether to use wood or α cellulose for climate reconstructions, we compared our δ^{13}C results with meteorological data. We calculated correlation coefficients for different month-combinations during the

growing season and found no significant difference between the coefficients for wood- and cellulose-δ^{13}C (Table 3). These are good arguments that the extraction of α cellulose is not necessary for climate reconstruction based on δ^{13}C of tree rings, at least for undegraded beech and oak wood. A more detailed interpretation of our δ^{13}C results with climate parameters is in preparation.

Our results confirm the good visual agreement between the wood and the cellulose δ^{13}C variations that several authors have found: Leavitt and Long [1991] measured seasonal δ^{13}C variations of wood and holocellulose of different tree species and found quite parallel curves with a Δ_{CW} of 1.5-2‰ for Douglas firs and ponderosa pine, and Δ_{CW} of 1-2.5‰ (i.e., higher scatter) for pinyon pine and junipers. Wilson and Grinsted [1977] measured seasonal variations of δ^{13}C in two adjacent annual rings of a Pinus radiata for cellulose as well as for lignin. They also found a quite good agreement between the variations of the two components (Δ_{CL} =3.5-3.8‰), but with a temporal shift, in agreement with the results of Fritts [1976], who determined that the lignification of the cells occurs about 1 month after their building. Schleser [1990] found quite analogous height-trends for wood and α cellulose of a beech grown in southern Germany (Δ_{CW} =1.3-2.0‰). Other authors already quantitatively compared the δ^{13}C signals in different wood components. Francey [1986] measured δ^{13}C on wood and cellulose of Tasmanian trees (Phyllocladus aspleniifolius). He found a very good correlation coefficient of 0.96 ($n=7$). Mazany et al. [1980] compared δ^{13}C of lignin and cellulose of a ponderosa pine (Pinus ponderosa) and obtained a correlation coefficient of 0.914 ($n=21$), and a difference between both compounds of Δ_{CL}=(4.11±0.35)‰. Mazany et al. [1980] also compared both δ^{13}C data sets with ring-width indices, which they assumed to represent climate conditions. They concluded that cellulose better records climate than lignin. However, in both studies, each sample represents 10 successive years; this could strongly reduce the influence of material exchange between adjacent rings. In this study we extended these previous
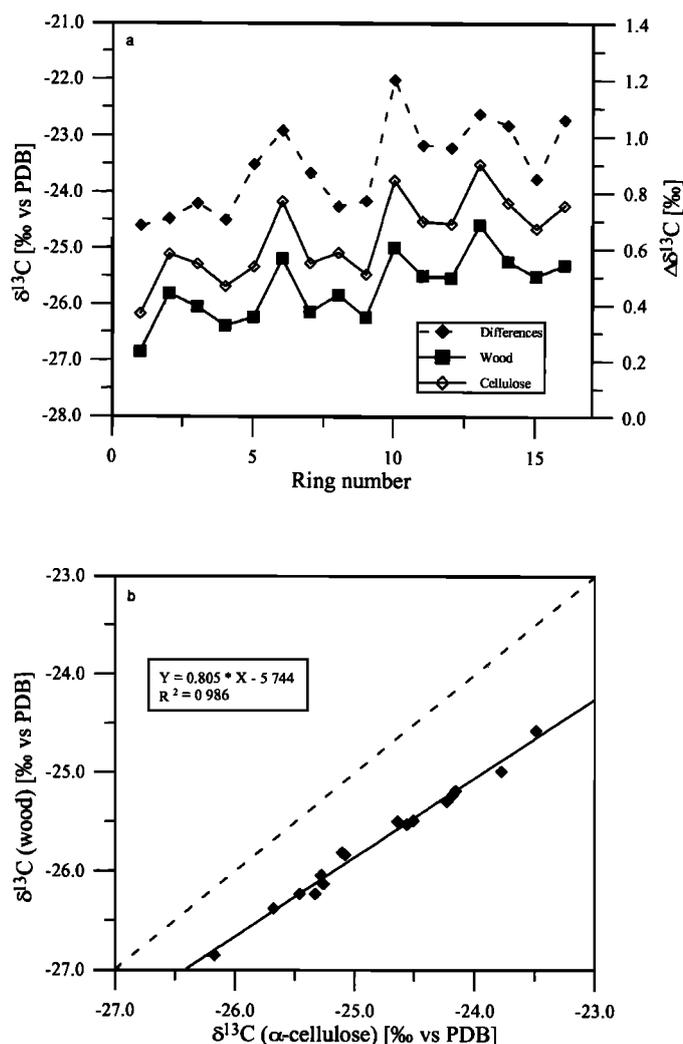
findings with several correlation calculations and significance tests, what is necessary to affirm that δ^{13}C can be analyzed on wood without significant information loss.

5. Conclusions

In view of the development of on-line techniques we have reconsidered the principal steps of stable isotope measurements on tree rings to reconstruct paleoclimate. A prerequisite for a representative isotope ratio for a given site is averaging or pooling wood from different trees and more than one radial direction of each tree. Pooling the material from the different trees prior to milling and measuring will save time and will allow building site chronologies for δ^{13}C of similar quality as in ring-width studies. On the basis of theoretical estimates and measurements we conclude that in most cases it is not necessary to pay much attention on mixing the same amount of material from each tree. Generally, we suggest mixing all available material for each year, except for periods with large growth variations of any tree. For these periods it would be useful to measure each tree separately. If the growth pattern of one tree is very different from that of the others over the whole period of analysis, it is best to measure it separately, or exclude it.

Figure 3. (a) The δ^{13}C from wood (squares) and cellulose (open diamonds) from the same rings of an oak. The difference between both curves (solid diamonds) is also shown (scale to the right). (b) The δ^{13}C from wood is very well correlated with δ^{13}C from α cellulose. The nonunity slope reflects the good correlation between the α cellulose and the differences, as shown in Figure 3a. This fact can be explained by a 20% wood component with an almost constant δ^{13}C. The solid line and the linear equation in the rectangle correspond to the linear regression analysis of the data points. The dashed line represents a one-to-one relation for comparison.

Table 3. Comparison of Correlation-Coefficients Between Cellulose and Wood

Tree	Meteoparameter	Months Averaged	r(Cellulose)	r(TE)	r(Wood)	n	r(C)≠r(TE) ?	r(C)≠r(W) ?
Oak Sa1	daily mean temperature	June-August	0.473	0.433		26	41%	
	1pm temperature	August	0.534	0.525		26	84%	
	precipitation amount	August	-0.283	-0.274		26	87%	
	number of days with >= 0.3 mm precipitation	June-September	-0.435	-0.326		26	4%	
	daily mean relative humidity	July-September	-0.463	-0.527		26	17%	
	1pm relative humidity	August-October	-0.593	-0.541		26	21%	
	sun iradiance	August	0.513	0.477		26	44%	
	evaporation	June	-0.871	-0.851		6	87%	
Oak Li171	daily mean temperature	June-July	0.661	0.734	0.766	14	27%	9%
	precipitation amount	July	-0.770	-0.771	-0.759	14	98%	84%
	number of days with >= 0.3 mm precipitation	July	-0.673	-0.726	-0.726	14	42%	42%
	daily mean relative humidity	July	-0.637	-0.674	-0.680	14	61%	55%
	sky cover	June	0.302	0.149	0.147	14	21%	20%
	sky cover	July	-0.304	-0.415	-0.369	14	32%	57%
Beech Li161	precipitation amount	May	-0.475	-0.583		13	29%	
	daily mean temperature	June-July	0.904	0.933		7	60%	

Correlation-coefficients for linear regression analysis among δ^{13}C of cellulose, TE or wood, and meteodata (from a nearby station). The months, over which the meteodata have been averaged here, are chosen so as to maximize the correlation-coefficients. Columns 8 and 9 give the probability that the assumption r(C) ≠ (TE) or r(C) ≠ (W) is wrong, calculated after *Taubenheim* [1969].

Another important factor is sample homogeneity. We conclude from our calculations that a grain mass of <6 μg should be achieved to ensure an accuracy of 0.15‰. This corresponds to a sieve with 0.15 mm porosity for cellulose and 0.10 mm for wood. The fastest and easiest (but expensive) way would be to couple a milling machine with a sieve system. We can suggest our solution of using a coffee mill (which functions well, even for samples as small as 10 mg wood), and weighing the larger wood pieces to determine how long the samples have to be milled. Furthermore, a fine material is necessary to achieve a complete cellulose extraction. (We find that the material, which is still slightly brown after the extraction, can have a $\delta^{13}C$ value of about 0.2‰ lower than pure α cellulose).

We find that $\delta^{13}C$ of wood is as good a climate proxy as cellulose, at least for undegraded wood from beech and oak trees. This should hold for other tree species as well, but perhaps the extractives from conifers should be removed, as those can have high and variable resin contents. For old wood one has to check whether the cellulose and lignin contents have changed over time. Yet, these findings are valid only for $\delta^{13}C$, and they need to be investigated for $\delta^{18}O$ and δ^2H. If new studies would indicate the necessity of cellulose extraction for $\delta^{18}O$ and/or δ^2H, a solution could be to measure long series of $\delta^{13}C$ on wood and to extract cellulose of part of the samples for $\delta^{18}O$ and δ^2H measurements.

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