SM 5.2.3 Estimating potential effects or implications of variable proportions of wood constituents or contaminants by mass balance equations

The following details a mass balance equation for assessing the potential effects changing proportions of different wood constituents with their various isotope compositions.

The relative percentage of cellulose, lignin and extractives in the corresponding wood is given by f_{cel}, f_{lig} and f_{ext} respectively. In addition, E_{cel}, E_{lig} and E_{ext} shall represent the proportions of a certain element (e.g. C, O or H) in either wood constituent. Under these conditions the overall proportion of element mass, PE_{cel}, in the corresponding wood regarding cellulose (including hemicelluloses) is $PE_{cel} = f_{cel} \times E_{cel}$. Similarly the proportions of element mass from lignin and extractives are $PE_{lig} = f_{lig} \times E_{lig}$ and $PE_{ext} = f_{ext} \times E_{ext}$.

$$\delta^n E_{tW} = \frac{PE_{cel} \times \delta^n E_{cel} + PE_{lig} \times \delta^n E_{lig} + PE_{ext} \times \delta^n E_{ext}}{PE_{cel} + PE_{lig} + PE_{ext}} \quad (1)$$

with

$\delta^n E_{tW}$ δ value of a bulk wood sample, e.g. $\delta^{13}C_{tW}$, $\delta^{18}O_{tW}$, δD_{tW}

PE_{cel}, PE_{lig}, PE_{ext} relative percentage (P) of element (E) of interest (e.g. carbon, oxygen, hydrogen) in cellulose, lignin or extractives

$\delta^n E_{cel}$, $\delta^n E_{lig}$, PE_{ext} original δ value of a sample, e.g. $\delta^{13}C$ or $\delta^{18}O$ value of the cellulose, lignin or extractives, respectively.

Example:

A conifer wood sample may be composed of 65% cellulose, 27% lignin and 8% resin with cellulose, lignin and resin consisting of 44%, 67% and 70% carbon, respectively.

As such follows that the proportions of carbon mass of cellulose, lignin and extractives are: $PC_{cel} = 0.65 \times 0.44 = 0.286$, $PC_{lig} = 0.27 \times 0.67 = 0.181$ and $PC_{res} = 0.08 \times 0.7 = 0.056$.

For simplification it is assumed that secondary plant metabolites lignin and resin have the same $\delta^{13}C$ value, and the isotopic difference to cellulose is 3.5‰. The $\delta^{13}C_{bW}$ of the bulk wood sample is then given by:

$$\delta^{13}C_{bW} = \frac{\delta^{13}C_{cel} - 3.5‰ \times (PC_{lig} + PC_{res})}{PC_{cel} + PC_{lig} + PC_{res}} \quad (2)$$

The offset between cellulose and bulk wood can be calculated:

$$\Delta\delta^{13}C_{bW-cel} = \delta^{13}C_{bW} - \delta^{13}C_{cel} = \frac{-3.5‰ \times (PC_{lig} + PC_{res})}{PC_{cel} + PC_{lig} + PC_{res}} \quad (3)$$

leading to

$$\Delta\delta^{13}C_{bW-c} = -1.59‰$$

This offset between cellulose and bulk wood can be reduced if resin is extracted.

A removal of resin would provide a sample consisting of 70.6% cellulose and 29.4% lignin leading to PC_{cel} = 0.706 x 0.44 = 0.311 and PC_{lig} = 0.294 x 0.67 = 0.196.

Using the same differences in $\delta^{13}C$ between the individual constituents as above, the offset between cellulose and resin-extracted wood ($\Delta\delta^{13}C_{eW}$) is then given by:

$$\Delta\delta^{13}C_{eW-ce} = \frac{-3.5‰ \times PC_{lig}}{PC_{cel}+PC_{lig}} \quad (4)$$

$$\Delta\delta^{13}C_{eW-ce} = \frac{-3.5 \times 0.196}{0.507} = -1.35‰$$

Compared to the offset in $\delta^{13}C$ between cellulose and bulk wood ($\Delta\delta^{13}C_{bW\text{-}cel}$ = -1.59‰) the offset to resin-extracted wood ($\Delta\delta^{13}C_{eW\text{-}cel}$ = -1.35‰) would be reduced slighty by 0.24‰.

Mass balance calculations for testing the implications of potentially contaminating substances

Similar mass balance calculations as shown above can be well applied to test the implications of potentially contaminating substances. A study testing the effects of pencil lead (mixtures of clay minerals with graphite (C) and traces of palm oil; $\delta^{13}C$ = -16 to -28‰) and chalk (calcium sulphate (gypsum, $CaSO_4$ x 2 H_2O) $\delta^{18}O$ = +5 to +8‰) revealed that remnants (up to 1%) of pencil marks and chalk only slightly shifted the original $\delta^{13}C$ or $\delta^{18}O$ values of wood by up to 0.1‰ and -0.18‰, respectively (Schollaen et al. 2017). In addition, many studies apply a mixture of maize starch, water and glycerol (so called non-Newtonian fluid) to the wood surface to penetrate the cell lumens and stabilize the cell walls during microsectioning (Schneider and Gärtner 2013). However, original stable isotope ratios may be altered if these potential contaminants cannot be removed prior to analysis. This is becoming increasingly important as not only tree ecophysiological but also dendroclimatological studies are combining quantitative wood anatomical measures with stable isotope analysis for improved understanding of tree structure and related function (e.g. Szymczak et al. 2014).

SM5.2.4.4 How varying proportions of wood constituents can affect climate – stable isotope relations of bulk wood time series to the better or worse – further details

Hypothetical $\delta^{13}C_{W*}$ records were calculated from two components only, i.e. cellulose (C) and combined lignin and extractives (L+E) using mass balance equations described in 5.2.3 and above in SM5.2.3.

For the calculations a constant offset of about 3.5‰ between $\delta^{13}C$ of cellulose ($\delta^{13}C_C$) and combined, lignin and extractives ($\delta^{13}C_{L+E}$) was chosen. Different $\delta^{13}C_{W*}$ values were obtained by respectively changing the average ratio C/(L+E) of 70/30 to 80/20 and/or 60/40 for individual years or longer time periods. This resulted in offsets between $\delta^{13}C_C$ and calculated $\delta^{13}C_{W*}$ of 0.96‰ (C/(L+E): 80/20), 1.38‰ (70/30) and 1.76‰ (60/40), respectively (given that $\delta^{13}C_C - \delta^{13}C_{L+E} = 3.5‰$).

Potential effects of wood decay

A ratio C/(L+E) of 20/80 was applied for highlighting potential effects of wood decay with a preferential loss of cellulose. Such a modification resulted in an offset of 3.0‰ which matches the reported average value for $\delta^{13}C$ of cellulose versus mummified wood from the Eocene (Hook et al. 2015).

Further details on Figure 5.1

In Figure 5.1A-G time series of $\delta^{13}C_{W*}$ and real $\delta^{13}C_C$ were z-scored (mean = 0; sd = 1) for better illustration and tested for their linear correlation to air temperature of the vegetation period (MJJAS)of the nearby climate station Potsdam.

$\delta^{13}C_C$ is significantly correlated (R^2=0.76) to air temperature of the vegetation period (T_{MJJAS}; Figure 5.1A). Note that, the same coefficient of determination holds for any calculated $\delta^{13}C_W$ as long as the proportion ratio of C/(L+E) and the isotopic difference between cellulose and lignin+extractives are kept stable throughout the records (not shown).

The varying percentage ratios of C/(L+E) used for the calculations from real $\delta^{13}C$ of cellulose are displayed in Figure 5.1D together with the resulting hypothetical time series for $\delta^{13}C_W$. The different curves seem to visually vary in details, only. However, the coefficients of determination are quite different (R^2= 0.299 vs. 0.615) (Fig. 5.1B, C). Calculated $\delta^{13}C_{W*}$ records correlate better than those of $\delta^{13}C_C$ if low $\delta^{13}C$ values (due to low air temperatures) are additionally reduced by lower C/(L+E) ratios and vice versa. In contrast, climate relationship of $\delta^{13}C_W$ is not as good as of $\delta^{13}C_C$ if the C/(L+E) ratio changes in opposite direction, i.e. when $\delta^{13}C_W$ is increasing together with decreasing C/(L+E). This may happen in individual years, periods of several years or along with specific stem sections, e.g. with heartwood and sapwood or wood segments affected by wood decay with preferential loss of cellulose over lignin. The

possible effects exemplified in Figure 5.1E, F and G reveal that increasing C/(L+E) ratios from the inner (heartwood, 60/30) to the outer part (sapwood, 80/20) of a tree-ring sequence can lower the climate relationship of $\delta^{13}C_W$ as compared to $\delta^{13}C_C$ (Fig. 5.1E, F).

SM5.3.2.5 Testing the purity of extracted cellulose

Various vibration modes mainly assigned to cellulose, hemicellulose and lignin dominate the spectral features by absorptions in the 1200–850 cm−1 region.

Wavenumber (cm^{-1})	Stretching vibration	Bending vibration	Wood component	Comment
1725-1738	νC=O		hemicellulose	Unconjucated C=O in xylans
1694	νC=O		resin	
ca. 1640-1650		δH_2O		Absorbed O-H
ca. 1596				Conjugated C-O
1505-1514			aromatic ring lignin (S, G)	
1453-1462 1422-1423		δC-H δC-H	lignins, carbohydrates	C-H deformation in lignin and carbohydrates
1368-1375		δC-H	cellulose, hemicellulose	C-H deformation in cellulose and hemicellulose
1319-1328		δC-H	cellulose, C_1-O vibration lignin (S)	C-H vibration in cellulose and C1-O vibration in syringyl derivates
1266-1268			aromatic ring, νC-O lignin (G)	Guaiacyl breathing, C–O stretch in lignin and for C–O linkage in guaiacyl aromatic methoxyl groups,
1233-1244			aromatic ring, νC-O lignin (S), νC-O hemicellulose	syringyl ring and C–O stretch in lignin and xylan
1153-1158 1104-1122			νC-O, νC-C, νC-O-C in cellulose, hemicellulose; aromatic skeletal in lignin (G+S)	C–O–C vibration in cellulose hemicellulose, aromatic skeletal and C–O stretch
1030-1054				C–O stretch in cellulose and hemicellulose
896-898			cellulose (β linkage)	C-H deformation in cellulose
863-877			lignin (G)	
809-831			Mannan (hemicellulose)	

Table SM5.1: Important IR wavenumbers for assessing the chemical composition of wood and testing the purity of extracted cellulose. After (Harrington et al. 1964; Kačuráková et al. 1999; Marchessault 1962; Pandey and Pitman 2003; Richard et al. 2014 and citations therein). Abreviations: G= guaiacyl structure; S = syringyl structure, β = bending vibration, ν = stretching vibration

A specific band at 1694 cm^{-1} is characteristic of resins occurring only in conifer woods (Rinne et al. 2005).

Extracted α-cellulose samples should show peaks at ~1050 cm^{-1} and 900 cm^{-1}, while peaks around ~1725-1738 cm^{-1} most widely associated with hemicellulose should be absent, just like any peak associated with resin (~1694 cm^{-1}) or lignin (~1505-1514cm^{-1}). Additionally, in

regions free of neutral polysaccharide signatures, characteristic bands of lignin show different relative band intensities, depending on tree species, between 1607–1592 cm^{-1}, and 1300–1200 cm^{-1}, assigned to the aromatic rings vibration associated with stretching C=O, to aromatic ring vibrations, and to C-O stretchings of syringyl (S) and guaiacyl (G) moieties, respectively (e.g. Richard et al. 2014 and citations therein).

Note, lignin is a heterogenous group of highly branched polyphenolic molecules derived from the polymerization of p-coumaryl, coniferyl and sinapyl alcohols (monolignols).

Lignin of conifer trees consists predominately of guaiacyl (G) with minor quantities of p-hydroxyphenyl (i.e. coniferyl alcohol with some contribution of p-coumaryl alcohol). Angiosperm wood usually contains a mixture of G and syringyl (S) with very little p-hydroxyphenyl formed from co-polymerization of coniferyl and sinapyl alcohols (Higuchi 1985).

SM 5.3.3.2 Filter fiber bags and filter bag drum tower

Remarks on Ankom 57 bags

The most commonly used F57 filter fiber bags (Ankom Technology, Macedon, NY, USA) partly disintegrate during the extraction procedure, individual fibres or sheaf tend to fray out and may significantly contaminate cellulose samples when re-used.

We determined isotope values (n = 4) of $\delta^{13}C$ = -28.7±0.6‰ (VPDB) and $\delta^{18}O$ = 16.5±1.3‰ (VSMOW).

Because of this potential issue extracted cellulose should be removed from the bags prior to drying by carefully flushing out the cellulose into a vial with deionized water from a syringe or squeezing bottle.

Details on the PTFE filter bag drum tower

The filter bag drum towers were produced by the fine mechanics workshop of section 4.3 at GFZ Potsdam, Germany. 40 individual drums (2.5cm high) were made from a 100cm long PTFE rod (7cm diameter; ca. 260€). Technical drawings and details on dimensions can be requested from the corresponding author.

References

Harrington KJ, Higgins HG, Michell AJ (1964) Infrared spectra of *Eucalyptus regans* F. Muell. and *Pinus radiata* D. Don. Holzforschung 18:108-117 doi:https://doi.org/10.1515/hfsg.1964.18.4.108

Higuchi T (1985) Biosynthesis of lignin. In: Higuchi T (ed) Biosynthesis and Biodegradation of Wood Components. Academic Press, New York, pp 141-160

Hook BA, Halfar J, Bollmann J, Gedalof Z, Rahman MA, Reyes J, Schulze DJ (2015) Extraction of alpha-cellulose from mummified wood for stable isotopic analysis. Chemical Geology 405:19-27 doi:10.1016/j.chemgeo.2015.04.003

Kačuráková M, Wellner N, Ebringerová A, Hromádková Z, Wilson RH, Belton PS (1999) Characterisation of xylan-type polysaccharides and associated cell wall components by FT-IR and FT-Raman spectroscopies. Food Hydrocolloids 13:35-41 doi:https://doi.org/10.1016/S0268-005X(98)00067-8

Marchessault RH (1962) Application of infrared spectroscopy to cellulose and wood polysaccharides. Pure Appl Chem 5:107–129

Pandey KK, Pitman AJ (2003) FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. International Biodeterioration & Biodegradation 52:151-160

Richard B, Quiles F, Carteret C, Brendel O (2014) Infrared spectroscopy and multivariate analysis to appraise alpha-cellulose extracted from wood for stable carbon isotope measurements. Chemical Geology 381:168-179 doi:10.1016/j.chemgeo.2014.05.010

Rinne KT, Boettger T, Loader NJ, Robertson I, Switsur VR, Waterhouse JS (2005) On the purification of alpha-cellulose from resinous wood for stable isotope (H, C and O) analysis. Chemical Geology 222:75-82 doi:10.1016/j.chemgeo.2005.06.010

Schneider L, Gärtner H (2013) The advantage of using non-Newtonian fluids to prepare microsections. Dendrochronologia 13:175-178

Schollaen K, Baschek H, Heinrich I, Slotta F, Pauly M, Helle G (2017) A guideline for sample preparation in modern tree-ring stable isotope research. Dendrochronologia 44:133-145 doi:10.1016/j.dendro.2017.05.002

Szymczak S, Hetzer T, Bräuning A, Joachimski MM, Leuschner H-H, Kuhlemann J (2014) Combining wood anatomy and stable isotope variations in a 600-year multi-parameter climate reconstruction from Corsican black pine Quaternary Science Reviews 101:146-158