Chapter 5
Stable Isotope Signatures of Wood, its Constituents and Methods of Cellulose Extraction

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Abstract In this chapter, we give some basic information on the chemical and isotopic properties of wood constituents and describe their relative contribution to the isotopic signature of wood. Based on these considerations we review studies that have compared stable isotope signals of wood with those of corresponding cellulose. We exemplify how relationships of wood-based tree-ring stable isotope sequences with climate can be affected by varying proportions of wood constituents like cellulose, lignin and extractives. A majority of benchmarking studies suggests that cellulose extraction may not be necessary. However, based upon existing research, a general statement cannot be made on the necessity of cellulose extraction. Changes in wood composition can particularly influence environmental signal strength during periods of low isotope variability. Cellulose extraction removes any effects from changing wood composition. We present the three established chemical approaches of extraction, outline how to test the purity of isolated cellulose and present user-friendly efficient experimental setups allowing to simultaneously process hundreds of samples in one batch. Further, we briefly address the analysis of stable isotopes of lignin methoxyl groups because of easy sample preparation and its potential additional value for studies on fossil wood.

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5.1 Introduction

At the beginning of tree-ring stable isotope investigations, bulk wood was used without any chemical pre-treatment (e.g. Craig 1954; Farmer and Baxter 1974; Libby and Pandolfi 1974). However, wood is a chemically complex material consisting of various biopolymers (cellulose, lignin, resin etc.) with divergent isotopic signatures (Taylor et al. 2008; Loader et al. 2003; DeNiro and Epstein 1977; Schmidt et al. 1998, 2001; Wilson and Grinsted 1977). Consequently, the use of bulk wood is usually avoided in eco-physiological or climatological stable isotope studies due to potentially changing mass proportions of the different wood constituents relative to each other, different seasonal timing of formation and possible mobility (of extractives) across tree-rings that may cause signal distortion of the tree-ring isotope records. Instead, it is presumed that analysis of one of the major wood constituents, usually cellulose rather than lignin, can ensure isotopic records uninfluenced of changing mass proportions over the life span of a tree. Cellulose (α-cellulose or, holocellulose which is α-cellulose and hemicellulose) is the most abundant and most important structural constituent of any terrestrial plant cell wall and it is most frequently chosen for tree-ring stable isotope analyses. It is preferred over lignin because cellulose is a chemically well-defined macromolecule and remains basically immobile during the lifespan of a tree, i.e. the time of polymerization (not necessarily the time of uptake of inorganic precursors by the tree) is always tied to the formation of the annual tree ring. Furthermore, its isolation is relatively simple involving only a few chemicals. Yet, the traditional procedures of cellulose isolation like those described by Green (1963) were tedious. Offline mass spectrometric analysis was the time limiting step in stable isotope analysis, and thus no major efforts in optimizing the methodology of cellulose extraction had to be made. Modern continuous-flow isotope ratio mass spectrometry permits efficient measurement of large sample numbers using minimal sample amounts (few micrograms) (e.g. Loader et al. 2015; Woodley et al. 2012). Sample preparation has become the limiting step in terms of cost and sample throughput and several studies have used bulk wood material with or without prior testing if the bulk wood (or extractives-free wood) and cellulose isotope values are highly cross correlated and show similar relationship, variability and significance to the environmental or climate dynamics against time series of instrumental data. Nonetheless, these approaches are compromises to circumvent the constraints of classical chemical sample preparation. More efficient extraction techniques were developed capable of processing micro-amounts of sample material, while at the same time ensuring high quality in terms of sample purity and homogeneity (e.g. Andreu-Hayles et al. 2019; Schollaen et al. 2017; Kagawa et al. 2015). Ongoing advances in the dissection tree-rings and/or parts thereof using on- and offline UV-laser ablation or UV-laser dissection microscopes (cf. Chap. 7) have challenged the current development of well-adapted sample preparation techniques (e.g. Schollaen et al. 2014, 2017).

Several methodologies have been proposed for the isolation of holo- or α-cellulose from wood for isotopic analysis. They differ from one another to a greater or lesser

extent concerning the extraction chemistry applied and/or specific devices and reaction vessels developed for improving efficiency by reducing labor time and costs for consumables and laboratory equipment (Andreu-Hayles et al. 2019; Kagawa et al. 2015; Loader et al. 1997; Schollaen et al. 2017; Anchukaitis et al. 2008). Besides the efforts in improving chemical sample preparation for C, O and H isotope analysis of tree-ring cellulose, C and H isotope analysis of lignin methoxyl groups by GC-C/TC-IRMS (Keppler et al. 2007) has been introduced as a novel approach in stable isotope dendroclimatology with a fast and easy preparation method.

In this chapter, we provide some basic information on the chemical and isotopic properties of wood constituents and describe their relative contribution to the isotopic signature of wood. Based on these considerations we review studies that have compared stable isotope signals of wood with those of corresponding cellulose and discuss why the extraction and use of cellulose instead of wood is of benefit. We address the analysis of stable isotopes of lignin methoxyl groups and its additional value. Last, but not least we describe the most commonly used chemical approaches and efficient experimental setups for extracting cellulose and outline how to test the purity of the resulting cellulose.

5.2 Whole Wood, Resin Extracted Wood, Lignin or Cellulose?

5.2.1 Basic Considerations from Chemical and Isotopic Properties of Wood Constituents

Wood is composed of α-cellulose, hemicellulose, lignin, resin and other extractives that show very different intrinsic isotopic signatures. For carbon, the extent of the general depletion of primary sugars in ^{13}C relative to the atmospheric CO_2 pool due to availability of CO_2 for photosynthesis (Farquhar et al. 1982) is the very foundation of eco-physiological and palaeoclimatological interpretation. Beyond leaf level physiology, photosynthetic intermediates are modified further at various metabolic branching points in primary and secondary plant metabolism. This is due to the involvement of (predominately) kinetic, isotope effects on multiple enzyme reactions during the polymerization or breakdown of precursor substances of the wood constituents (Gleixner et al. 1993; Schmidt et al. 1998). A progressive depletion in ^{13}C can be usually observed with metabolic distance from a metabolic branching point (Schmidt et al. 1993). Accordingly, carbohydrates from primary plant metabolism like sugars, starch, hemi- or α-cellulose normally have heavier isotopic signatures than secondary metabolites (e.g. lignin or fatty acids) that originate from different and rather long metabolic pathways. Hemi- and α-cellulose are usually found enriched in ^{13}C over lignin and fatty acids by 2 to 4 ‰ on average (Table 5.1) (e.g. Robertson et al. 2004; Schmidt et al. 1998 and citations therein). These differences vary with

Table 5.1 $\delta^{13}C$ and $\delta^{18}O$ of lignin and their offsets to corresponding values of cellulose. Only data of pine and oak have been published so far

Species	$\delta^{13}C_{Lig}$ ‰ vs.VPDB	Offset (C – L)	$\delta^{18}O_{Lig}$ ‰ vs. SMOW	Offset (C – L)	Reference
P. glauca	*NA*	*NA*	+13.5 ± 1.5	10.6	Gray and Thompson (1977)[a]
Quercus spp.	*NA*	*NA*	+22.1 ± 3.5	6.9	Barbour et al. (2001)[a]
Pinus spp.	*NA*	*NA*	+21.4 ± 5.4	6.4	Barbour et al. (2001)[a]
P. halepensis	−25.2 ± 0.6	*2.5*	+22.7 ± 2.4	8.6	Ferrio and Voltas (2005)[a]
Q. petraea	−27.17 ± 0.8	2.41	NA	NA	Robertson et al. (2004)
P. radiata	−28.8 ± 1.2	ca. 3.5	NA	NA	Wilson and Grinsted (1977)
P. ponderosa	−21.0 ± 0.8	4.1	NA	NA	Mazany et al. (1980)
Q. robur	ca. −23.8	ca. 3	NA	NA	Loader et al. (2003)

NA = Not analysed

[a] Isotope data of lignin obtained gravimetrically from combined mass balance calculations and $\delta^{18}O$ analyses of solvent-extracted wood, hemicellulose and a-cellulose or holocellulose

tree species, tree age, tree organ (e.g. leaf/needle, sapwood, heartwood etc.,) and site conditions.

The majority of comparative stable isotope studies on different wood constituents were on carbon and conclusions drawn cannot simply be transferred to hydrogen or oxygen isotopes. Oxygen and hydrogen atoms of chemical wood constituents basically originate from water and, their isotopic signatures are characterized by various exchange reactions of their different precursors at leaf level and beyond (cf. Chaps. 10 and 11 for details), potentially leading to insignificant correlation of $\delta^{18}O$ data of cellulose and lignin (Gray and Thompson 1977).

Cellulose is generally enriched in ^{18}O by ~+27 ± 4‰ versus leaf water due to an equilibrium isotope effect between carbonyl groups and water (Sternberg 1989). Hemi- and α-cellulose were found to have rather similar oxygen isotopic compositions (Gray and Thompson 1977; Richard et al. 2014).

Generally, the $\delta^{18}O$ of aromatic compounds like lignin attains values of around + 12‰ (vs. V-SMOW) (Schmidt et al. 2001) revealing significantly lower $\delta^{18}O$ values than cellulose. However, to our knowledge no data from direct $\delta^{18}O$ measurements on chemically extracted lignin do exist, as the extraction procedure (hydrolysis with 72% H_2SO_4 at 20 °C, (Klason 1911; TAPPI 1988) usually applied in $\delta^{13}C$ studies

may affect $\delta^{18}O$ of remaining acid-insoluble lignin. Estimates of $\delta^{18}O$ of lignin were obtained gravimetrically from combined mass balance calculations and $\delta^{18}O$ analyses of solvent-extracted wood, hemicellulose and α-cellulose. The calculated $\delta^{18}O$ values of lignin were found quite variable and the offsets in $\delta^{18}O$ between cellulose and lignin appear to be larger and more variable than the offsets reported for $\delta^{13}C$ (Table 5.1).

A similar picture likely holds for hydrogen stable isotopes. $\delta^{2}H$ values of cellulose tend to be higher by 30 to 40‰ than those of corresponding bulk wood due to significantly lower $\delta^{2}H$ values of lignin than those of cellulose (Gori et al. 2013). $\delta^{2}H$ values of wood ($\delta^{2}H_w$) collected from various sites between 69°N and 1°S of the equator were always found to be higher ($\delta^{2}H_w = -141$ to -29‰ vs. V-SMOW) than $\delta^{2}H$ ($\delta^{2}H_L$) obtained from corresponding lignin methoxyl groups ($\delta^{2}H_L = -325$ to -153‰ vs. V-SMOW) (Keppler et al. 2007).

While the general differences between the stable isotope values for various wood constituents are a consequence of enzyme-specific fractionations at various metabolic branching points involved in their biosynthesis, the definite extent of isotopic shifts depend on flux rates at metabolic branching points as well as the isotopic signature and the pool sizes of precursor substances which can vary with changing ambient environmental conditions (for details cf. Schmidt et al. 2001, 2003; Keppler et al. 2007; Schmidt 1999).

5.2.2 The Isotope Signatures of Wood as a Result of Relative Contributions of Its Individual Constituents

5.2.2.1 Cellulose and Lignin

The major and also minor wood constituents derived from primary and secondary plant metabolism hold intrinsic differences in their stable isotope C, O and H signatures. Their relative contribution to the isotopic composition of bulk wood depends on the extent of isotopic difference and relative mass contribution of individual constituents.

Hemi- and α-cellulose together form the largest part within wood (on average 65–75%). They are composed of ca. 45% carbon, 6% hydrogen and 49% oxygen, whereas lignin contains around 60–70% carbon, 6–7% hydrogen and 20–30% oxygen, depending on the relative contribution of monolignols and degree of methoxylation. The lignin content of different woody species can vary between 15 and 36% of the dry weight of wood (e.g. Kürschner and Popik 1962; Pettersen 1984). However, within the same species variability appears to be lower. For pine trees, a range from 25 to 30% has been observed (Zobel and van Buijtenen 1989). On average, gymnosperms have a slightly higher lignin content than angiosperms. Within the same plant lignin can vary also in quantity and composition between different cell types and tissues (Agarwal and Atalla 1986; Boudet 2000). For example, wood formed at the top

of a mature conifer typically has a higher lignin content than wood from further down the stem (Zobel and van Buijtenen 1989). The overall lignin quantity and its composition of different alcohol monomers can also vary depending on location in the cell wall, developmental state of the cell and tissue, and the influence of environmental stress (Zobel and van Buijtenen 1989). Hence, the relative lignin content can vary radially within a tree ring, i.e. from earlywood to latewood (Wilson and Wellwood 1965; Lanvermann et al. 2013; Fergus et al. 1969; Gindl 2001; Fukazawa and Imagawa 1981). Also, radially across the trunk heartwood was found to contain significantly more lignin and less cellulose than sapwood e.g. in *P. abies* (Bertaud and Holmbom 2004) or in *Tectona grandis* (Narayanamurti and Das 1955). This may particularly affect the significance of time series of eco-physiological or climatic signals in tree-ring stable isotope sequences.

5.2.2.2 Extractives

Besides cellulose and lignin, extractives, i.e. nonstructural substances that are soluble in organic solvents or water, represent an additional contribution of carbon, oxygen and hydrogen in bulk wood. They are supposed to be rather mobile within the wood and resin or fatty acids can have highly variable carbon and hydrogen contents of up to more than 70% with oxygen contributing not more than around 20%. Extractives in sapwood, often starch, simple sugars or lipids, are generally considered to be energy reserve materials for the tree and carbohydrate and lipid extractives are believed to be converted to compounds during heartwood transformation such as phenols and terpenes (resin) in rather variable amounts contributing to a passive defense to prevent attack by wood destroying insects and fungi (Keith 1969; Hillis 1987; Friedman et al. 2019; Schmidt 1999; Taylor et al. 2002, 2007). Extractives may show a very wide range of δ-values, however, as most of them derive from secondary plant metabolism it can be assumed that their stable isotope signatures are considerably depleted as compared to cellulose (Schmidt 1999), but stable isotopes of extractives also revealed significant correlations, at least with respect to carbon (Taylor et al. 2007, 2008). The few studies that have compared bulk wood and extractives-free wood have found no difference or shifts of only up to +0.3‰ on average in $\delta^{13}C$ after removal of extractives (e.g. Harlow et al. 2006; Richard et al. 2014; Ferrio and Voltas 2005). With respect to oxygen positive as well as negative shifts were obtained from *P. pinaster* wood from multiple sites with an average of $+0.24 \pm 0.6$‰ (Ferrio and Voltas 2005). From these studies it can be derived that the effects of extractives on the overall isotopic signature of wood might be negligible. However, the amount of extractives in wood can be highly variable. In heartwood of various pine species contents ranging between 5–62% (5–34% *P. sylvestris*, 15–62% *P. nigra*) were found, whereas their content was found fairly stable in sapwood (3–5%). A similar, but smaller radial gradient was found in young trees ranging from 7% (central wood) to 2.5% (outermost rings) (Kurth 1933), and also the chemical properties of extractives can differ between sapwood and heartwood (Keith 1969; Hillis 1987). This can add to the potential differences in lignin and cellulose contents between heartwood and

sapwood resulting in isotopic trends that may mask ecological or climatological long-term information. Extractives obtained from broadleaf woody species growing in temperate climates can constitute up to 10% of dry weight and up to 20% in certain tropical tree species (Pettersen 1984). Besides the general differences found between tree species and gradients across the trunk, the content of extractives can vary in relation to particular environmental incidents such as fire or drought which may induce, for example, resin production or may act as part of the trees' defense mechanism against microbial attack (e.g. Hall 1993; Guest and Brown 1997).

5.2.3 *Estimating Potential Effects or Implications of Variable Proportions of Wood Constituents*

Prior to any test measurements the variability of the stable isotope composition of wood due to changing proportions of different wood constituents with their various isotope compositions can be estimated by simple exercises using mass balance equations as demonstrated by e.g. Richard et al. (2014) or Schleser et al. (2015). SM5.2.3 details a general equation for calculating the δ value of carbon, oxygen or hydrogen of bulk wood from the relative mass proporations of cellulose, lignin and extractives and their respective isotopic signature. An example calculation of $\delta^{13}C$ of bulk wood ($\delta^{13}C_{bW}$) for a hypothetical conifer sample is also given. Assuming an average composition of 65% cellulose, 27% lignin and 8% resin and a constant difference of 3.5‰ between cellulose and secondary plant metabolites lignin and resin (i.e. assuming the same $\delta^{13}C$ value for lignin and extractives) mass balance calculation results in an offset of $\delta^{13}C$ of cellulose ($\delta^{13}C_c$) to $\delta^{13}C_{bW}$ ($\delta^{13}C_c - \delta^{13}C_{bW} = 1.59‰$) that is well around the mean of real values observed (Table 5.2a). Compared to $\delta^{13}C_{bW}$, resin-extracted wood ($\delta^{13}C_{eW}$) is calculated slighty less negative by 0.24‰ ($\delta^{13}C_c - \delta^{13}C_{eW} = 1.35‰$). Such an example indicates that the contribution of extractives like resin to the overall stable isotope value of wood may be negligible if their mass fraction of carbon, oxygen or hydrogen from these extractives make up only a small percentage of the wood and/or if the isotope values of these fractions are mainly in the ranges of the major wood constituents cellulose or lignin. Perhaps more important than the influences of extractives are changing relative proportions of the major constituents cellulose and lignin. As outlined above (Sect. 5.2.2), the proportion of cellulose can increase up to 80% with the lignin content decreasing down to 20% (e.g. in reaction wood). This would lead to an offset of ($\delta^{13}C_c - \delta^{13}C_{eW} = 0.96‰$). It has to be emphasized that in this example a constant isotopic difference between cellulose and lignin of 3.5‰ has been assumed, however, this isotopic difference not only differs between species (Table 5.1), but potentially changes within a tree and may vary with time, site conditions and wood preservation, as demonstrated in Sect. 5.2.4.3 and Figs. 5.1 and 5.2.

Similar mass balance calculations as for carbon isotopes may suggest that the potential influence of varying cellulose to lignin proportions on $\delta^{18}O$ and δD values

Table 5.2 Overview of comparitive studies on stable isotopes of carbon (a), oxygen (b) and hydrogen (c) in wood and cellulose. See key to abbreviations at the end of the table

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
(a) carbon isotopes						
C – bW	JM(E^1)/Browning (1967)	*P. ponderosa*	NA	0.8–0.96	10 yr and 6 yr blocks, CE820-1045, 22 samples	Mazany et al. (1980)
$\delta^{13}C_C$ and $\delta^{13}C_{Lig}$ correlate strongly with each other, $\delta^{13}C_C$ shows better correlation with regional tree-ring index than $\delta^{13}C_{Lig}$						
C – bW	JW/Green (1963)	*J. deppeana*	0.9–1.9	0.86	6 intra-annual samples, CE1978-79	6 intra-annual samples, CE1978-79
Similar intra-ring patterns of $\delta^{13}C_{bW}$ and $\delta^{13}C_C$						
C – bW	JW/Green (1963)	*P. menziesii*, *P. ponderosa*	1.5–2.0	NA	2 yrs, 4 samples per yr, CE1908-1909	Leavitt and Long (1991)[c]
Similar intra-ring patterns of $\delta^{13}C_{bW}$ and $\delta^{13}C_C$						
C – tW	JM(E^2)/Green (1963)	*P. menziesii*	ca. 2	NA	20 yrs, 3 samples per yr, CE1962-1981(SW*), n = 60	Livingston and Spittlehouse (1996)[c]
C – eW			ca. 1	NA		
Offset rather stable, half of the difference between $\delta^{13}C_{bW}$ and $\delta^{13}C_C$ accounting for extractives						
hC – bW	JM/Leavitt and Danzer (1993)	*P. ponderosa*	0.65SEW, 2.25SLW; 2.28HEW, 2.78HLW		4 replicates per factorial combination, sapwood/heartwood and earlywood/latewood analysed	Marshall and Monserud (1996)
		P. menziesii	1.75SEW, 2.00SLW; 1.66HEW, 2.02HLW			
		P. monticola	2.13SEW, 2.64SLW; 1.65HEW, 2.40HLW			

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
Offsets differed between earlywood and latewood, and between heartwood and sapwood; cellulose extractions were necessary						
hC – bW	JW(E^2)/Brenninkmeijer (1983)	*Betula sp.*	1.81	NA	5 saplings, 1-yr old, pooled, 1989	Borella et al. (1998)
		F. sylvatica	1.68	NA	2 trees, pooled, 1978–1992	
		Quercus sp.	1.02HW	NA	2 trees, pooled, 1930–1970, HW	
		Quercus sp.	1.64SW	NA	1973–1994, SW	
		P. abies	1.37HW	NA	2 trees, pooled,1950–1960, HW	
		P. abies	1.34SW	NA	5 trees, pooled, 1987–1996, SW	
		Oak Lil171	1.12 + −0.14	0.94	1 tree, n = 14	
		Oak Lil171	1.01 + −0.18	0.93	1 tree, n = 14	
		Oak Sa1	0.89 + −0.16	0.995	1 tree, n = 16, LW	
		Oak Sa1	1.01 + −0.20	0.95	1 tree, n = 26, LW	
		Oak Sa1	0.89 + −0.19	0.97	1 tree, n = 16, EW	
		Oak Sa1	1.01 + −0.30	0.90	1 tree, n = 22, EW	
		Beech Lil61	0.99 + −0.13	0.96	1 tree, n = 14	
Good agreement between $\delta^{13}C_{bW}$ and $\delta^{13}C_{hC}$ with similar relationships to climate, if wood is not decayed. Extractives from conifer wood should be removed prior to analysis. Slopes of regression significantly different from 1 except for *Q.* latewood						

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
αC – bW	dHCl/Wallis et al. (1997) (modified)	*P. pinaster* *P. radiata*	0.3–1.6, Ave. = 0.8	0.94	n = 81 with 3–5 replicates, no information on tree age, SW or HW	MacFarlane et al. (1999)
		E. globulus	0.2–1.3 Ave. = 0.8	0.93		
hC – bW	JM/(Green 1963)		0.8–1.3	0.95		
hC – eW	E^2		0.5–1.0	0.96		
Strong and consistent correlation between $\delta^{13}C_{bW}$ and $\delta^{13}C_C$ and $\delta^{13}C_{\alpha C}$; dHCl method removes heC						
αC – bW	dHCl/MacFarlane et al. (1999)	*P. pinaster* *P. radiata*	0.87	0.96	10 trees, planted in 1966 (SW*); pooled wood samples, n ≈ 25	Warren et al. (2001)
The difference between $\delta^{13}C_{bW}$ and $\delta^{13}C_{\alpha C}$ was similar for all treatments, cellulose extraction not necessary						
hC – bW	JM(E^2)/Leavitt and Danzer (1993) (no NaOH treatment)	*P. latifolius,* *A. falcatus,* *O. capensis,* *P. longifolia,* *C. viridifolium,* *C. africana*	ca. 1	0.92	44 wood samples from 6 tropical tree species	West et al. (2001)
hC extraction necessary in studies with low sample size examining small isotopic shifts. Away from regression, no pattern in the dispersion of residuals found						
C – fbW	JM/Wiesberg (1974)	Unknown gymnosperms	0.1–3.9	0.06	n = 6, fossil wood, early Miocene	Bechtel et al. (2002)[c]

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
C – fbW	JM/(Bechtel et al. 2002)	Unknown gymnosperms	3.1–4.6	0.9	n = 7, fossil wood, middle Miocene	Bechtel et al. (2007b)[c]
C – fbW		Unknown gymno- and angiosperms	2.5–4.0	0.97	n = 14, fossil wood, late Miocene	Bechtel et al. (2003a)[c] Bechtel et al. (2007a)[c]
C – fbW		Unknown gymnosperms	1.4–3.6	0.7	n = 8, fossil wood, Pliocene (2.58–5.33Mio a)	Bechtel et al. (2003b)[c]
Higher isotopic difference of ca. 3.5‰ between cellulose and fossil wood ascribed to wood decomposition						
αC – bW	JM/Loader et al. (1997)	*Q. robur*	ca. 1	0.981, 0.876 $p < 0.01$	2 trees, CE1946-2000, 55 years, LW	Loader et al. (2003)
		Q. robur (bog oak)	ca. 1	0.965 $p < 0.01$	1 tree, BCE2340-2361, 20 years, LW	
$\delta^{13}C_{bW}$ shows higher correlations to climate variables than $\delta^{13}C_{\alpha C}$, $\delta^{13}C_{bW}$ may be used to provide evidence of inter-annual variance. Some lower-frequency variance could be induced by differential decay						
hC – bW	JW/Sohn and Reiff (1942)	*Fagus sylvatica*	0.5–1.8	0.94	3yrs, 279 intra-ring samples, CE1994-1996	Helle and Schleser (2004)
Offset between $\delta^{13}C_{bW}$ and $\delta^{13}C_{hC}$ can be different between years. Overall slope of regression different from 1 (0.86)						

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
hC – bW	JW(E^2)/Leavitt and Danzer (1993)	*Q. cerris* *F. ornus* *P. radiata*	0.04 n.s 0.09 n.s ca. 1.1	0.72 0.57 0.84	3yrs, CE1999-2001 (SW*), n = 44, juvenile trees, 6–7yrs old 3yrs, CE1999-2001(SW*), n = 14 3 tree rings, CE1999-2001 (SW*), n = 6, juvenile trees, 22–24yrs old	D'Alessandro et al. (2004)
$\delta^{13}C_{bW}$ is suitable for ecophysiological studies. Slope of regression significantly different from one-to-one relationship						
C – bW	dHCl/modified after MacFarlane et al. (1999)	*C. odorata*	1.73 ± 0.08 n = 37	0.87 $p < 0.001$ (all data)	52 samples from tropical species; 10-yr periods pooled	Hietz et al. (2005)[c]
		S. macrophylla	1.01 ± 0.23 n = 16			
Original dHCl method requires additional $NaClO_2$ treatment to fully remove lignin						
C – bW	JW/Sohn and Reiff (1942)	*Q. sp.* (bog oak)	0.6–2.1	0.67	n = 22, 5-yr blocks, BCE137-193	Sass-Klaassen et al. (2005)[c]
Similar intra-ring patterns of $\delta^{13}C_{bW}$ and $\delta^{13}C_C$						
hC – bW	JM(E^1)/Leavitt and Danzer (1993)	*Rhizophora mucronata* (mangrove)	0.97 ± 0.03	0.98 $p < 0.001$	2 trees, n = 27, SW	Verheyden et al. (2005)[c]
Slope of regression not significantely different from one. $\delta^{13}C_{bW}$ can be used in studies of sapwood of *R.mucronata*						

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
hC – bW	JW(E^2)/Leavitt and Danzer (1993)	*P. halepensis*	1.2	0.97	23 sites, 4–6 trees per site, 25yrs, CE1975-1999(SW*), SfT approach	Ferrio and Voltas (2005)
hC – eW	E^2		1.0	0.49 $p < 0.05$		
$\delta^{13}C_{hC}$, $\delta^{13}C_{eW}$ and $\delta^{13}C_{bW}$ show significant correlations to climate variables*						
αC – bW	dHCl/Cullen and MacFarlane (2005)	*C. glaucophylla*	1.17	0.64	Chronology with replication of 3 to 11 trees; 20yrs, CE1979-1999 (SW*)	Cullen and Grierson (2006)
$\delta^{13}C_{bW}$ does not record climate in the same way as $\delta^{13}C_{\alpha C}$; $\delta^{13}C_{\alpha C}$ is the temporally more stable proxy						
hC – eW	JW(E^2)/Leavitt and Danzer (1993)	44 species from the US, angiosperms and conifers	1.07 ± 0.09[b] range 0.5–1.9	0.98	2 samples per species, various tree portions (stem and branch wood)	Harlow et al. (2006)[c]
			1.32 ± 0.10[b] range 0.3–1.6	0.98		
hC extraction is unnecessary for most analyses. Simple solvent extraction suitable for many applications. Slopes of regression calculated from different models sometimes significantly different from 1						
hC – eW	JW(E^2)/Leavitt and Danzer (1993)	*P. menziesii*	1.48 ± 0.54	0.91	Groups of 3 consecutive rings, 6 trees, 4 SW samples per tree (12 tree rings, CE1990-2001)	Taylor et al. (2008)
Significant correlations between $\delta^{13}C_{hC}$, $\delta^{13}C_{eW}$ and $\delta^{13}C$ of extractives. hC extraction may not be necessary if low concentration of extractives						

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
hC – bW	JW(E^2)/Leavitt and Danzer (1993)	*Q. petraea*	1 ± 0.7	0.92	2-yr-old saplings, 29 samples	Eglin et al. (2008)[c]
hC – eW			0.9 ± 0.7	0.97		
$\delta^{13}C_{eW}$ and $\delta^{13}C_{hC}$ show practically identical variations; presence of extractives slightly alters variability of $\delta^{13}C_{bW}$ compared to $\delta^{13}C_{hC}$						
αC^5 – eW	JW(E^4)/Loader et al. (1997)	*Larix cajanderi*	1.83–1.99	0.88–0.97, $p < 0.05$	4 trees, >400yrs old, CE1880-2004	Sidorova et al. (2008)[c]
			1.41–1.50	0.80–0.91, $p < 0.05$	CE900-1000	
Offset not stable in time, during some periods $\delta^{13}C_{eW}$ showed other and partly stronger relationships to climate variables than $\delta^{13}C_{\alpha C}$						
αC^5 – bW	JW/Loader et al. (1997)	*Larix gmelinii*		0.75, $p < 0.05$	8 trees, >180yrs old, CE1864-2006, annual resolution, material pooled	Sidorova et al. (2009)
$\delta^{13}C_{bW}$ data showed significant relationship to climate, whereas no climate signals were found in $\delta^{13}C_{\alpha C}$						
hC – bW	JW/Kürschner and Popik (1962)	*Pinus nigra ssp.*	1.2 ± 0.5	0.71	80 yrs, pooled record, CE1905-1985, 5 trees, >200 yrs old	Szymczak et al. (2011)
Offset declining from pith to bark; climatic conditions best reflected in $\delta^{13}C_{hC}$; extraction of cellulose avoids insufficient sample homogenization						

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
C – bW	JW/Loader et al. (1997)	*Carapa guianensis*	0.2 to 1.6	0.88	1 tree, 47 intra-annual samples	Pons and Helle (2011)[c]
		Goupia glabra	0.7 to 1.5	0.89	1 tree, 49 intra-annual samples	
Similar intra-ring patterns of $\delta^{13}C_{bW}$ and $\delta^{13}C_C$						
αC^2 – bW	JW(E^3)/Loader et al. (1997)	*P. radiata*	ca. 1	0.99	Seedlings; greenhouse experiment	Roden and Farquhar (2012)[c]
		E. globulus	ca. 1.5	0.98		
Slopes of regression not significantly different from 1:1 relationship, αC extraction unnessecary						
C – bW	JW/Gaudinski et al. (2005)	*L. cajanderi*	0.1 to 2.1	0.96	67 yrs, CE1940-2007	Tei et al. (2013)[c]
Climatic information can be satisfactorily obtained using $\delta^{13}C_{bW}$						
C – bW	no details given	*P. abies*	1.35	0.96 $p < 0.001$	1 tree, annual growth rings, n = 60, CE1973-2006 (SW*)	Sohn et al. (2013)
Bulk wood samples rather than extracted cellulose can be used for isotope analysis as sample sizes were limiting						

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
C – bW	JW/Loader et al. (1997)	*P. abies*	ca. 1.4	0.91, 0.88, 0.83 $p < 0.001$	3 sites, 10 trees each site, 4 radii each tree, individual tree rings pooled; CE1860-2009; CE1933-2009; CE1936-2009	Gori et al. (2013)
Extraction of cellulose is unnecessary for trees from the studied sites. High correlation between $\delta^{13}C_{bW}$ and $\delta^{13}C_C$. Climate signal best preserved in stable isotope ratios of wood						
αC – bW	JW/Sternberg et al. (1989)	*P. sylvestris*	0.8 to 1.5	0.95	2 trees, 48 samples	Edvardsson et al. (2014)[c]
$\delta^{13}C_{\alpha C}$ and $\delta^{13}C_{bW}$ are strongly correlated and both show lower isotope values at the onset of growth depressions						
hC/αC – bW	JW(E^2)/Jayme (1942); Wise (1945), different variants	*Q. petraea*	1.2(hC), 1.3(αC)	NA	From adult trees, not further specified	Richard et al. (2014)
		P. deltaoides,	0.8(hC), 0.7(αC)	NA		
		P. pinaster	1.2(hC), 1.4 (αC)	NA		
		F.sylvatica	1.2(hC), NA (αC)	NA		
No difference in offset of $\delta^{13}C\alpha C$ and $\delta^{13}C_{hC}$ to $\delta^{13}C_{bW}$ observed						
αC – bW	BM/Brendel et al. (2000)	*P. sylvestris*	1.09 ± 0.09	0.96 $p < 0.001$	4 trees, individual tree rings of 4–8 radii pooled, SW, CE1989-2009	Mischel et al. (2015)

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
$\delta^{13}C_{bW}$ records climate in a similar way as $\delta^{13}C_{\alpha C}$. Cellulose extraction may not be mandatory for studies on *P. sylvestris* from investigated site						
αC^3 – bW	JW(E^4)/Green (1963)	*F. sylvatica*	1.45 ± 0.07	0.72 $p < 0.05$	5 sites, 3 trees per species, 2 radii each, 20 years of sapwood, CE2001-2010 (SW*)	Weigt et al. (2015)
		Q. robur	1.84 ± 0.18	0.85 $p < 0.05$		
		A. alba	1.79 ± 0.0.09	0.92 $p < 0.05$		
		P. abies	1.73 ± 0.0.05	0.79 $p < 0.05$		
αC^3 – eW	E4	*P. menziesii*	1.75 ± 0.10	0.91 $p < 0.05$		
$\delta^{13}C_{bW}$ from sapwood is as useful as $\delta^{13}C_{\alpha C}$ in environmental studies at short-term scale. Variable response of *Q.* requires further investigations						
hC – bW	JW/Wieloch et al. (2011)	*Cariniana micrantha*	0.8 to 2.0	0.96[b], moving corr. 0.05 to 0.98	1 tropical tree, 253 consecutive yrs, CE1755-2007, SW and HW	Schleser et al. (2015)[c]
High correlation from a subset or subperiod cannot necessarily prove its validity for a longer isotopic record						
αC – fbW	BM/Brendel et al. (2000); JW(E^2)/Loader et al. (1997)	*Piceoxylon*	ca. 3.0	NA	Mummified fossil wood, 2 samples per species, Eocene (53.3 ± 0.6 Ma)	

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
αC – eW	E^2		ca. 3.2	NA		
JW method for cellulose extraction from mummified fossil wood favored over BM						
C – bW	BM/Brendel et al. (2000)	*P. cembra* *P. abies* *L. decidua*	0.6–1.6	0.96	Total n = 36, CE1975-2010 (SW*)	Wieser et al. (2016)[c]
Bulk wood samples rather than extracted cellulose can be used for isotope analysis						
αC – bW	JW(E^2)/Leavitt and Danzer (1993) and Sternberg et al. (1989)	*C. tomentosa*	1.2	NA	2 trees per species, ca. 80–100 yrs old; 7 indiv. rings sampled per tree between CE2000-2011(SW*)	Guerrieri et al. (2017)[c]
		Q. rubra	0.1	NA		
		T. canadensis	0.9, 1.5	NA		
		P. rubens	0.9	NA		
		Q. prinus	1.3	NA		
		P. echinata	1.9	NA		

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
		Ave. all species	1.1	0.62 $p < 0.001$		
αC extraction not necessary for climate reconstructions, both $\delta^{13}C_{bW}$ and $\delta^{13}C_{\alpha C}$ showed significant correlations with climate variables; partly stronger correlations of $\delta^{13}C_{bW}$ with climate data						
αC – fbW	BM/(Gaudinski et al. 2005)	Unknown gymno- and angiosperms	1–4.4 Ave. 2.5	0.98	n = 38, Eocene, Miocene, Oligocene	Lukens et al. (2019)
A strong linear correlation exists between $\delta^{13}C_{bW}$ and $\delta^{13}C_{C}$ in both deep-time and modern wood samples (augmented data from literature[c]), suggesting that either substrate can provide a reliable record of environmental conditions						
αC – bW	BM/modified after Gaudinski et al. (2005) and Anchukaitis et al. (2008)	*P. deltoides*	ca. 0.5–2	0.54	7 trees, indiv. rings of 2 cores pooled, n = 9 to 42; 39 to 220 yrs old; longest sequence: CE1791-1972	Friedman et al. (2019)
Increasing offset between $\delta^{13}C_{\alpha C}$ and $\delta^{13}C_{bW}$ from pith to bark due to decreasing long term trend in $\delta^{13}C_{bW}$						
hC – bW	JW/(E^2Loader et al. (1997)	*P. massoniana*	1.26 ± 0.23	0.74 to 0.98 $p < 0.01$	5 site, 216 trees, CE1982-2014 (SW*)	Gu et al. (2020)
αC^6 – bW			1.31 ± 0.19			
$\delta^{13}C_{bW}$, $\delta^{13}C_{\alpha C}$ and $\delta^{13}C_{hC}$ display uniform year-to-year variations and common significant climatic signals						
Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C-W) ‰	Correlation r	Remarks	Reference
(b) oxygen isotopes						
αC – bW heC – bW	JW(E^1)/Green (1963)	*P. glauca*	4.99 ± 1.1	0.63	1 tree, 5 yr blocks, CE1890-1968, n = 16	Gray and Thompson (1977)

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C-W) ‰	Correlation r	Remarks	Reference
Good response of $\delta^{18}O_{bW}$ to seasonal air temp., but higher correlations of $\delta^{18}O_{\alpha C}$ with temp.; $\delta^{18}O_{heC}$ not significantly different from $\delta^{18}O_{\alpha C}$						
αC^1 – bW	JW(E^2)/Borella et al. (1998)	*Quercus sp.*	4.25 ± 0.55	0.81	1 tree; individual tree-rings, 15yrs, CE1971-1986(SW*), latewood	Borella et al. (1999)
Some climatic information may be lost if $\delta^{18}O_{bW}$ is analyzed instead of $\delta^{18}O_{\alpha C}$						
bW	no extraction	*Abies alba*	NA	NA	4 trees, LW only, CE1840-1997	Saurer et al. (2000)
Significant correlations of $\delta^{18}O_{bW}$ with climate data						
αC^2 – bW	JW(E^3)/Barbour and Farquhar 2000	*Quercus spp.*	3.7 ± 2.0	NA	16 (*Q.*) and 26 (*P.*) samples from around the world; SfT approach	Barbour et al. (2001)
		Pinus spp.	3.9 ± 1.4	NA		
αC extraction unnecessary for isotope studies looking at correlations with site parameters						
hC – bW	JW(E^2)/Leavitt and Danzer (1993)	*P. halepensis*	ca. 4	0.49	23 sites, 4–6 trees per site, 25yrs, CE1975-1999 (SW*); SfT approach	23 sites, 4–6 trees per site, 25yrs, CE1975-1999 (SW*); SfT approach
hC – eW			ca. 4	0.41 n.s		

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C-W) ‰	Correlation r	Remarks	Reference
$\delta^{18}O_{bW}$ show no significant correlations to climate variable, whereas $\delta^{18}O_{hC}$ do						
αC – bW	dHCl/Cullen and MacFarlane (2005)	*C. glaucophylla*	7.59	0.68	Chronology with replication of 3 to 11 trees, 20yrs, CE1979-1999(SW*)	Cullen and Grierson (2006)
$\delta^{18}O_{bW}$ does not record climate in the same way as $\delta^{18}O_{\alpha C}$; $\delta^{18}O_{\alpha C}$ is the temporally more stable proxy						
C – bW	JW/Loader et al. (1997)	*F. sylvatica*	5.1	0.46 $p < 0.01$	6 trees (2 cores, each) per species, CE1916 –1950	Battipaglia et al. (2008)
		A. pseudoplatanus	4.4	NA		
Cellulose extraction necessary to investigate climate signals from $\delta^{18}O$ of broad-leaved species						
αC – eW	JW(E^4)/Loader et al. (1997)	*L. cajanderi*	3.51–3.99	0.56–0.92, $p < 0.05$	CE1880 – 2004, 4 trees, >400yrs old, CE900-1000	Sidorova et al. (2008)
			2.97–3.43	0.68–0.78, $p < 0.05$		

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C-W) ‰	Correlation r	Remarks	Reference
Offset not stable in time, during some periods $\delta^{18}O_{bW}$ data showed stronger relationship to climate than $\delta^{18}O_{\alpha C}$						
αC^5 – bW	JW/Loader et al. (1997)	*Larix gmelinii*	NA	0.61, $p < 0.05$	8 trees, >180yrs old, CE1864-2006, annual resolution, material pooled	Sidorova et al. (2009)
$\delta^{18}O_{bW}$ and $\delta^{18}O_{\alpha C}$ do not show similar climatic signals. $\delta^{18}O_{bW}$ data showed significant relationship to climate, whereas $\delta^{18}O_{\alpha C}$ did not						
hC – bW	JW/Kürschner and Popik (1962)	*Pinus nigra ssp.*	4.8 ± 0.72	0.77	5 trees, >200 yrs old, 80 yr-long pooled record, CE1905-1985	Szymczak et al. (2011)
Offset declining from pith to bark; climatic conditions best reflected in $\delta^{18}O_{hC}$; hC extraction avoids insufficient sample homogenization						
αC^2 – bW	JW(E^3)/Loader et al. (1997)	*P. radiata*	ca. 4	0.92	Seedlings, greenhouse experiment	Roden and Farquhar (2012)
		E. globulus	ca. 7	0.77		
Slopes of regression significantly different from 1:1 relationship; αC extraction required						
C – bW	JW/Loader et al. (1997)	*P. abies*	3.9, 4.0, 3.3	0.67, 0.75, 0.78 $p < 0.001$	3 sites, 10 trees each site, 4 radii each tree, individual tree rings pooled; CE1860-2009; 1933–2009; 1936–2009	Gori et al. (2013)
Extraction of cellulose unnecessary. Significant correlations found between $\delta^{18}O_{bW}$ and $\delta^{18}O_C$; Climate signal best preserved in $\delta^{18}O_{bW}$						

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C-W) ‰	Correlation r	Remarks	Reference
eW	E^2	*Tectona grandis*	NA	NA	7 trees, 107 yr chronology, not pooled, CE1900-2007	Schollaen et al. (2013)
Significant correlations of $\delta^{18}O_{eW}$ with climate data						
C – bW	no details given	*P. abies*	5.9	0.82 $p < 0.001$	1 tree, annual growth rings, n = 30, CE1973-2006(SW*)	Sohn et al. (2013)
bW samples rather than extracted cellulose can be used for isotope analysis as sample sizes were limiting						
αC – bW	BM/Brendel et al. (2000)	*P. sylvestris*	5.58 ± 0.23	0.89 $p < 0.001$	4 trees, individual tree rings of 4–8 radii pooled, CE1989 – 2009(SW*)	Mischel et al. (2015)
$\delta^{13}C_{bW}$ records climate in a similar way as $\delta^{13}C_{\alpha C}$. Cellulose extraction may not be mandatory for carbon isotope analysis						
αC^4 – fbW αC^4 – feW	BM/Brendel et al. (2000); JW(E^2)/Loader et al. 1997	*Piceoxylon*	15.5 17	NA	Mummified fossil wood (53.3 ± 0.6 Ma), 2 samples	Hook et al. (2015)
Cellulose extraction from fossil wood after JM preferred over BM						
αC^3 – bW	JW(E^4)/Green (1963)	*F. sylvatica*	4.95 ± 0.14	0.5 $p < 0.05$	5 sites, 3 trees per species, 2 radii each, 20 years of SW, CE2001-2010	Weigt et al. (2015)
		Q. robur	5.12 ± 0.07	0.41 $p < 0.05$		

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C-W) ‰	Correlation r	Remarks	Reference
		A. alba	5.22 ± 0.13	0.84 $p < 0.05$		
		P. abies	5.31 ± 0.11	0.71 $p < 0.05$		
αC^3 – eW	E^4	*P. menziesii*	5.44 ± 0.11	0.75 $p < 0.05$		
$\delta^{18}O_{bW}$ or $\delta^{18}O_{eW}$ of tree rings from sapwood are as useful as $\delta^{18}O_{\alpha C}$ for studying environmental effects at short term scale. Variable response of $\delta^{18}O_{bW}$ of *Q. robur* requires further investigations						
αC^6 – bW	BM/Gaudinski et al. (2005)	*P. densiflora*	3.8–4.0	0.92	31 stem disks from 13 sites, 5 outermost tree rings (pentads) were sampled	Lee et al. (2015)
$\delta^{18}O_{bW}$ is suggested for use as proxy for the geographical origin of Korean Red Pine. $\delta^{18}O_{bW}$ shows similar correlations to $\delta^{18}O$ of surface water than $\delta^{18}O_{\alpha C}$* ($r^2 = 0.5$ vs. 0.53). Approx. 50% of $\delta^{18}O_{bW}$ explained by surface water isotopic composition						
C – bW	BM/Brendel et al. (2000)	*P. cembra* *P. abies* *L. decidua*	4.4–4.0	0.85	Total n = 18, CE1975-2010 (SW*)	Wieser et al. (2016)[c]
Bulk wood samples rather than extracted cellulose can be used for isotope analysis						
αC – bW	JW(E^2)/Leavitt and Danzer (1993) and Sternberg et al. (1989)	*Carya tomentosa*	4.3	NA	2 trees per species, ca. 80–100 yrs old; 7 individual tree rings sampled per tree between CE2000-2011(SW*)	Guerrieri et al. (2017)
		Quercus rubra	3.8	NA		

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C-W) ‰	Correlation r	Remarks	Reference
		Tsuga canadensis	6.2, 6.5	NA		
		Picea rubens	5.6	NA		
		Quercus prinus	4.9	NA		
		Pinus echinata	6.2	NA		
		Ave. all species	5.6	0.73 $p < 0.001$		
αC extraction not necessary for climate reconstructions, both $\delta^{18}O_{bW}$ and $\delta^{18}O_{\alpha C}$ showed significant correlations with climate variables; partly stronger correlations of $\delta^{18}O_{bW}$ with climate data						
Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
(c) hydrogen isotopes						
C – bW	JW/Loader et al. (1997)	*P. abies*	ca. 30–45	0.66−0.88 $p < 0.001$	3 sites, 10 trees each site, 4 radii each tree, individual tree rings pooled; CE1860-2009; CE1933-2009; CE1936-2009	Gori et al. (2013)
Extraction of cellulose unnecessary. Significant correlations found between δD_{bW} and δD_C; Climate signal best preserved in δD_{bW}						

(continued)

Table 5.2 (continued)

Compounds compared	Extraction after[a]	Tree species/sample name	Offset (C−W) ‰	Correlation r	Remarks	Reference
αC^6 – bW	BM/Gaudinski et al. (2005)	*P. densiflora*	40–43 ± 6	0.89 to 0.92	31 stem disks from 13 sites, 5 outermost tree rings (pentads) were sampled (SW*)	Lee et al. (2015)
δD_{bW} is suggested for use as proxy for the geographical origin of Korean Red Pine. δD_{bW} show better correlations to δD of surface water than $\delta D_{\alpha C}$* ($r^2 = 0.50$ vs. 0.28). Approx. 50% of δD_{bW} explained by surface water isotopic composition						

Abbreviations: [a] as referred to in publication; C−W = isotope offset between cellulose and wood; bW = bulk wood; eW = extractive-free or resin-free wood; fbW = fossil bulk wood; W = wood, not further specified; hC = holo-cellulose; heC = hemicellulose; C = cellulose, not further specified; αC^1 = 4% NaOH, 70 °C, 24 h; αC^2 = 10% NaOH, 70 °C, 5 h; αC^3 = 15% NaOH, 45 min, 20 °C; αC^5 = 5% NaOH, 2 × 2 h, 70 °C; αC^4 = no NaOH treatment; αC^6 = 17% NaOH, partly various temp.; SW = sapwood; SW* = probably SW, not explicitly indicated in paper; HW = heartwood; SEW = sapwood, earlywood, HEW = heartwood earlywood; SLW = sapwood, latewood; HLW = heartwood, latewood; EW = earlywood; LW = latewood
JW = Jayme-Wise approach, (Jayme 1942; Wise et al. 1946); E^1 = extraction of extractives with benzene/methanol, acetone (mostly using Soxhlet device); E^2 = extraction of extractives with toluene/ethanol; pure ethanol and de-ionized water (mostly using Soxhlet device); E^3 = extraction of extractives with chloroform/ethanol; pure ethanol and de-ionized water (mostly using Soxhlet device); E^4 = extraction of extractives with pure ethanol (24–36 h, at room temperature); dHCL = diglyme-HCL method, (Cullen and MacFarlane 2005; MacFarlane et al. 1999); BM = Brendel-method, (Brendel et al. 2000); NA = Not analysed; SfT = stable isotope-climate relationships from space-for-time approach, not from isotope time series; [b] mean value; [c] compilation of individual data points available in online supplemental material of Lukens et al. (2019)

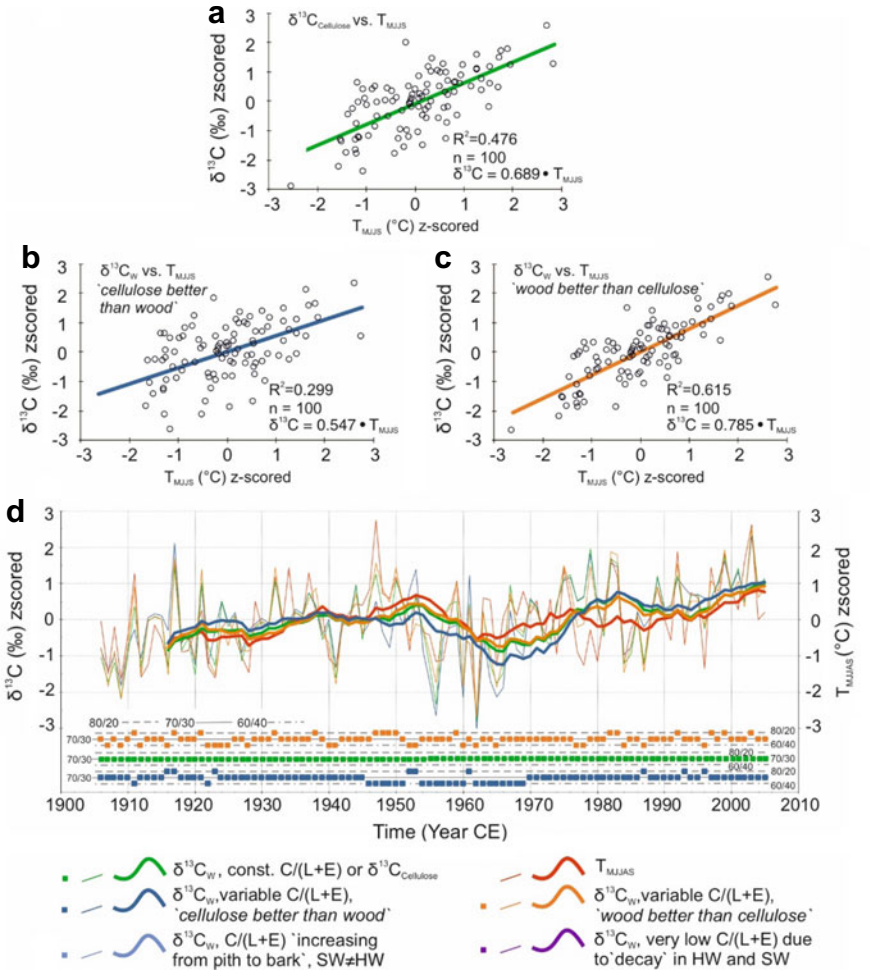

Fig. 5.1 Real $\delta^{13}C$ time series of tree-ring cellulose ($\delta^{13}C_C$, z-scored) and hypothetical time series for wood ($\delta^{13}C_{W*}$, z-scored) calculated from different proportions of wood constituents (cellulose (C), combined lignin and extractives (L + E)) and linear correlations with instrumental climate data. $\delta^{13}C_{W*}$ records were calculated for different C/(L + E) ratios (60/40, 70/30, 80/20) assuming a constant offset of 3.5‰ between C and L + E. Linear correlation of real $\delta^{13}C_C$ to air temperature of the vegetation period (May to September) (**a**). C/(L + E) deviating from 70/30 in individual years or sub-periods causing $\delta^{13}C_W$ to correlate better with climate (**b**) or worse (**c**) than $\delta^{13}C_C$. **d** Corresponding time series of $\delta^{13}CW$ calculated from different C/(L + E). Note, z-scored $\delta^{13}C_W$ with constant C/(L + E) is not different from z-scored $\delta^{13}C_C$. **e** $\delta^{13}C_{W*}$ records calculated from different levels C/(L + E) hypothetically changing from pith to bark (heartwood (HW) to sapwood (SW)) or due to wood decay (C/(L + E) reduced to 20/80). Corresponding correlations to climate (**f**, **g**) show distinct differences to correlation of $\delta^{13}C_C$ (a). See 5.2.4.3 SM5.2.4.3 for further details

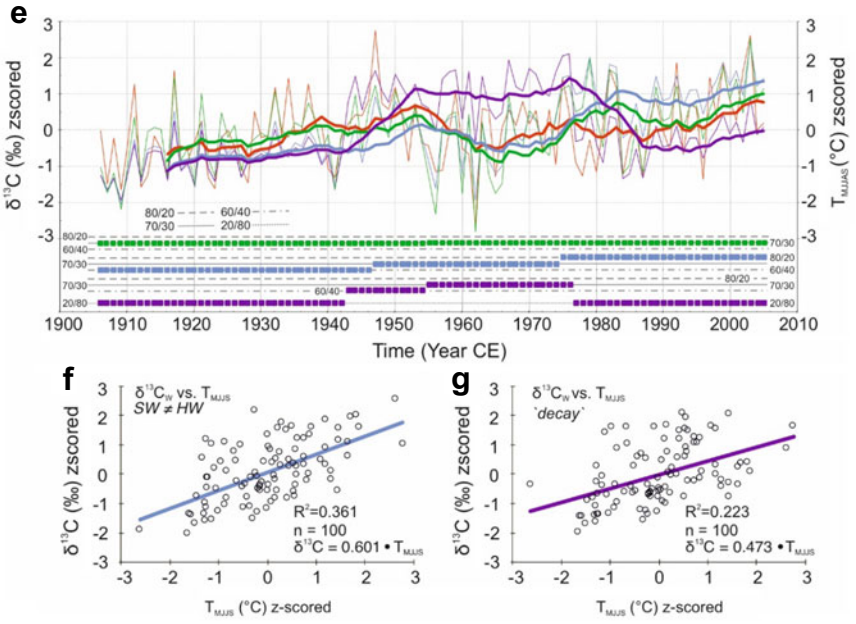

Fig. 5.1 (continued)

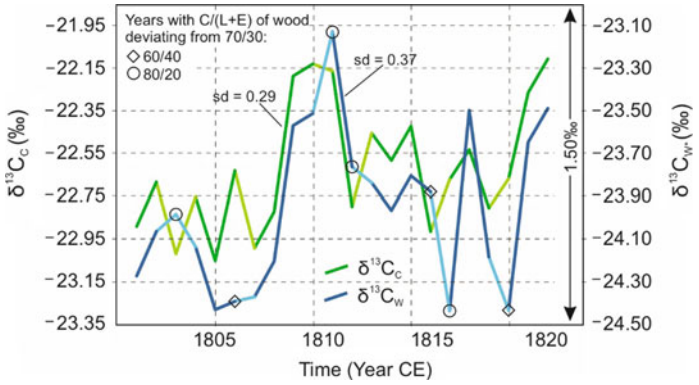

Fig. 5.2 Real $\delta^{13}C_C$ time series (green) from *P.* sylvestris with rather low variability for the time period 1801 to 1822CE (22 years). Hypothetical $\delta^{13}C_W$ (blue) with 7 years deviating from a C/(L + E) ratio of 70/30. Circles: C/(L + E) = 80/20; diamonds: C/(L + E) = 60/40. Besides different year-to-year variability, $\delta^{13}C_{W*}$ can even show inverse trends (bright colored lines). See 5.2.4.3 for details

of wood are likely to be lower than the effects on $\delta^{13}C$ due to the rather low abundances of hydrogen (6–7%) and oxygen (20–30%) in lignin and the generally much higher content of cellulose in wood.

Mass balance calculations can also help to test the implications of potentially contaminating substances, like chalk or from pencil marks (SM5.2.3; Schollaen et al. 2017).

5.2.4 Wood Versus Cellulose—A Review of Tree-Ring Stable Isotope Benchmarking Studies

Stable isotope ratios from cellulose (holo- or α-cellulose) are usually considered to be the benchmark for eco-physiological and climatological studies using tree rings because any variation due to different biosynthetic pathways of wood constituents have been previously removed. However, many studies have tested whether or not stable isotope ratios of wood are equitable in terms of use and quality. A majority of studies investigated carbon isotopes (40, Table 5.2a), twenty comprised oxygen stable isotopes (Table 5.2b) and two studies focused on hydrogen stable isotopes testing whether or not cellulose extraction is necessary for tracing the geographical origin of wood (Gori et al. 2013; Lee et al. 2015; Table 5.2c). Many trials were focusing on rather short periods of time for comparison (Table 5.2, frequently ≤25 years) aiming at extrapolating correlation properties of wood and cellulose to other, usually longer time intervals. Associated to these short assessment periods, a few authors indicated that their studies were constrained to sapwood (SW, Table 5.2a, b) (e.g. Weigt et al. 2015; Verheyden et al. 2005; Taylor et al. 2008), but given the information about tree age and sampled time periods, several other studies were also likely limited to the study of sapwood (cf. SW*; 'Remarks' in Table 5.2a–c). Ten studies clearly indicated that longer time periods (≥50 years) including sapwood (SW) and heartwood (HW) were investigated. The majority focused on bulk wood, whereas some also used extractives-free wood (mostly from conifers) for comparison with cellulose. All of the peer-reviewed publications we found have assessed the differences (offset, C-W) between isotope values of bulk wood (bW) and/or extractives-free wood (eW) and holocellulose (hC) or α-cellulose (αC) for a large number of various species. Holo-, hemi- and α-cellulose are products of primary plant metabolism. Apparently no significant differences do exist with respect to their carbon and oxygen stable isotope signature (Richard et al. 2014; Gray and Thompson 1977). Hence, except for the details given in Table 5.2 no distinction is being made in the following paragraphs.

5.2.4.1 Isotopic Offsets Between Bulk Wood, Extractives-Free Wood and Its Cellulose

Across all studies and species an average offset in $\delta^{13}C$ values between wood and cellulose of around 1‰ was found. Usually, the offsets in $\delta^{13}C$ were ranging between 0.7 and 2‰, very few studies found no (D'Alessandro et al. 2004) or exceptionaly large offsets (Table 5.2a), e.g. ca. 3‰ for fossil wood from the Tertiary geologic period (Lukens et al. 2019 and citations therein; Hook et al. 2015). In a high resolution intra-annual study comprising 279 data pairs across 3 consecutive tree rings, the offset in $\delta^{13}C$ varied between 0.5 and 1.8‰, but it was rather stable within years (Helle and Schleser 2004). Generally, intra-seasonal trends revealed a much higher amplitude (up to 5‰) than the variability that might be induced by potentially changing concentrations of individual wood constituents (e.g. Leavitt and Long 1991; Verheyden et al. 2004). However, some phase shift in the relationship between $\delta^{13}C$ intra-ring curves of cellulose and lignin was observed (Wilson and Grinsted 1977). The resulting consequences for $\delta^{13}C$ of wood were considered to be small with no apparent temporal offset in the climate signal (Loader et al. 2003; Taylor et al. 2008). For more details on intra-annual stable isotope variability see Chap. 15.

Among the ten studies that investigated longer tree-ring sequences, six trials, mostly on $\delta^{13}C$, noted an unstable offset between wood and its cellulose with time. Marshall and Monserud (1996) decided to focus on cellulose after detecting highly variable differences in the $\delta^{13}C$ offset between heartwood and sapwood. Particularly, *P. ponderosa* has revealed a considerably higher offset of wood vs. cellulose in heartwood. A similar difference in isotopic offset was observed for sapwood and heartwood of an oak tree (*Quercus sp.*) (Borella et al. 1998). Schleser et al. (2015) reported a variable offset along 253 consecutive tree rings of tropical *C. micrantha* ranging between 0.8 and 2.0‰ with moving correlations (50 years interval length) conspicuously varying from virtually zero to 0.98 (at an average correlation of 0.96). Friedman et al. (2019) found the offset increasing from pith to bark due to a decreasing trend in $\delta^{13}C$ of wood that was not observed in $\delta^{13}C$ of cellulose. Sidorova et al. (2008) and Sidorova et al. (2009) observed the offsets for both, $\delta^{13}C$ and $\delta^{18}O$, being not stable with time. When compared with climate variables, they found slightly, but consistently better relationships to $\delta^{13}C$ (and $\delta^{18}O$) of wood relative to the correlations of corresponding values of cellulose to climate.

Apart from the particular findings for mummified wood ($\delta^{18}O \geq 15.5$‰, (Hook et al. 2015)), the offsets in $\delta^{18}O$ values between wood and cellulose were found generally ranging from 2.97 to 7.59‰, i.e. a considerable higher variability than for $\delta^{13}C$ was observed.

With respect to hydrogen stable isotopes Gori et al. (2013) and Lee et al. (2015) have found the $\delta^{2}H$ values of wood about 30–45‰ more negative than those of cellulose at a largely similar interannual variability (Table 5.2c).

5.2.4.2 Statistical Relationship Between the Stable Isotopes of Bulk Wood, Extractives-Free Wood and Corresponding Values of Cellulose

Most studies calculated correlation coefficients (r) or coefficients of determination from ordinary least square regression of wood versus cellulose isotope values, and/or applied reduced major axis regression (RMA) or bivariate least squares regression (BLS), for example, to include the measurement errors for calculating confidence intervals (e.g. Verheyden et al. 2005; Harlow et al. 2006). Besides presenting correlation coefficients several authors also examined whether or not slopes of regression were different from one (e.g. Verheyden et al. 2005; Roden and Farquhar 2012; Harlow et al. 2006).

Usually highly significant average correlation coefficients of 0.8 or higher were discovered in the studies comparing $\delta^{13}C$ values of wood and corresponding cellulose. A few authors report medium (D'Alessandro et al. 2004; Weigt et al. 2015; Ferrio and Voltas 2005; Schleser et al. 2015) or insignificant (Guerrieri et al. 2017) relationships. For $\delta^{18}O$ (wood vs. cellulose values), frequently only medium, nevertheless significant, correlation coefficients were discovered, with p values often <0.05, compared to <0.01 or less as was mostly found for $\delta^{13}C$. Whereas one study identified highly variable moving correlations (0.5–0.98; 50 years interval length; rbar = 0.96, n = 253) for a tree-ring $\delta^{13}C$ sequence from a tropical tree (Schleser et al. 2015), no such test has been reported for $\delta^{18}O$ or $\delta^{2}H$, yet, because time series were too short or not existing.

For hydrogen isotope values for bulk wood and its cellulose, Gori et al. (2013) have found highly significant positive correlation coefficients of 0.7, 0.66 and 0.88 ($p < 0.001$) at three different sites. For *P. densiflora* from a network of sites in South Korea a similar relationship was observed ($r = 0.89$ to 0.92) (Lee et al. 2015) (Table 5.2c). These values are slightly lower than those gained for $\delta^{13}C$ (Table 5.2a) and lie within the same order of magnitude as the values determined for $\delta^{18}O$ (Table 5.2b). This may suggest that hydrogen isotope analysis on wood is as useful as on cellulose. However, in these studies exchangeable hydroxyl-bound hydrogen atoms (ca. 30% of hydrogen in cellulose) have not been quantified or removed prior to the isotope measurements. This is assumed to be not necessary for tracing the geographical origin of timber (Lee et al. 2015; Gori et al. 2013), however it is considered to be important if a measure of the hydrogen (water) taken up by a tree during cellulose synthesis is to be obtained in ecological or paleoclimatic studies using tree-ring time series (Loader et al. 2015; Epstein and Yapp 1976; Sternberg 1989).

5.2.4.3 Statistical Relationships of Stable Isotopes of Bulk Wood, Extractives-Free Wood and Corresponding Cellulose to Climate Variables

Several studies tested respective signal strengths of isotope values of wood and corresponding cellulose in relation to climate parameters. This was done by correlating time series of tree-ring isotope data versus instrumental climate variables (e.g. Mazany et al. 1980; Weigt et al. 2015; Guerrieri et al. 2017; Saurer et al. 2000; Gori et al. 2013) or in space-for-time ('SfT', Table 5.2) substitution approaches, by using wood material from trees growing under various eco-climatological conditions at different latitudes and elevations (Barbour et al. 2001; Ferrio and Voltas 2005; Lee et al. 2015; Gori et al. 2013). Barbour et al. (2001) concluded that $\delta^{18}O$ from wood contains the same annual average information on climatological site parameters (temperature, rainfall, weighted $\delta^{18}O$ of rain) as cellulose. Similarly the two comparative studies on hydrogen stable isotopes suggest that bulk wood is as useful as on cellulose for tracing the geographical origin of timber (Lee et al. 2015; Gori et al. 2013). In another space-for-time substitution study on wood fragments of *P. halepensis* from a network of 23 Mediterranean sites across eastern Spain annual means generally failed to adequately reflect the large range of climate seasonality among the sampled sites (Ferrio and Voltas 2005). In contrast to findings of Barbour et al. (2001), only $\delta^{18}O$ values of cellulose revealed weak, but significant correlations to climate variables. Here, the already weak climate signal exhibited by $\delta^{18}O$ of cellulose might be further lowered if the analysis of $\delta^{18}O$ was conducted on wood, probably because of varying proportions of wood constituents in the sample material combined with their different intrinsic $\delta^{18}O$ signatures. Similar to the findings of Ferrio and Voltas (2005), but using $\delta^{18}O$ tree-ring time series from two broad-leaved tree species, a study from the Mediterranean (S-Italy) reported that $\delta^{18}O$ of the cellulose fraction strongly correlated with monthly and seasonally resolved climate data, while the whole wood fraction generally did not (Battipaglia et al. 2008).

Apart from these two studies with insignificant correlations of $\delta^{18}O$ of wood to climate variables statistically significant climate-proxy relationships were generally found for both, wood and cellulose, and for all three isotope species ($\delta^{13}C$, $\delta^{18}O$, $\delta^{2}H$). The rather coherent response to climate can be expected from the similarity that has been generally observed between the isotopic signatures of wood and its cellulose. For $\delta^{2}H$, bulk wood showed even stronger relationships to climate or $\delta^{2}H$ of surface waters than $\delta^{2}H$ of cellulose (Gori et al. 2013; Lee et al. 2015). Also, $\delta^{13}C$ or $\delta^{18}O$ values of wood sometimes showed higher correlations than those of cellulose (Guerrieri et al. 2017; Sidorova et al. 2008, 2009; Loader et al. 2003; Gori et al. 2013), sometimes the opposite was the case, i.e. stronger relationships of cellulose isotopes to climate were reported (Gray and Thompson 1977; Szymczak et al. 2011; Mazany et al. 1980). Furthermore, Cullen and Grierson (2006) found that $\delta^{18}O$ of cellulose provides a temporarily more stable climate proxy. Besides differences in signal strength, $\delta^{18}O$ of bulk wood and corresponding cellulose were also found to respond to different climatic signals (Sidorova et al. 2008) and hence, Sidorova et al. (2009) suggested to analyze both if enough sample material is available.

5.2.4.4 How Varying Proportions of Wood Constituents Can Affect Climate—Stable Isotope Relations of Bulk Wood Time Series to the Better or Worse

The mixed results in terms of the ranking of climate signal strength in wood and cellulose, respectively, may be just coincidence because correlation coefficients of tree-ring stable isotopes in wood or its cellulose with climate variables were often found to be rather close to each other. Yet, variable proportions of wood constituents with their intrinsic isotope signatures might also be relevant to sometimes either strengthen or weaken the climate signature of isotope values of wood relative to cellulose. In order to figure out how the linear relationship of a tree-ring stable isotope sequence with a climate variable can be affected, we have taken an existing $\delta^{13}C$ time series of tree-ring cellulose ($\delta^{13}C_C$) and calculated wood time series ($\delta^{13}C_{W*}$) with different hypothetical proportions of wood constituents for linear correlation with instrumental climate data (Fig. 5.1a–g). The $\delta^{13}C_C$ time series was obtained from six trees (not pooled) of a 230-year old pine (*P. sylvestris*) stand located near (approx. 300 m) the long-term meteorological Station Potsdam Telegrafenberg, Germany (PIK-Potsdam 2020). For simplification the 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 introduced in Sect. 5.2.3 and SM5.2.3. Assuming that cellulose is constantly about 3.5‰ less negative in $\delta^{13}C$ than lignin and extractives three different percentage ratios of C/(L + E), namely 80/20, 70/30 and 60/40 were chosen for calculating $\delta^{13}C_{W*}$.T his resulted in offsets between $\delta^{13}C_C$ calculated $\delta^{13}C_{W*}$ well within the real range of offsets reported in the literature (0.7 and 2‰; Table 5.2a). The ratio C/(L + E) of 70/30 was adopted as default for the majority of calculated $\delta^{13}C_W$ values. Different $\delta^{13}C_{W*}$ curves were obtained by changing this ratio to 80/20 and/or 60/40 for individual years or different periods, e.g. to simulate potential differences between heartwood and sapwood. By changing this ratio for individual years or different periods various $\delta^{13}C_{W*}$ series were obtained reflecting potential responses to extreme years, effects of reaction wood or differences between heartwood and sapwood, as well as wood decay with a preferential loss of cellulose (cf. SM5.2.4.4. for further details).

$\delta^{13}C_C$ is significantly correlated ($R^2 = 0.76$) to air temperature of the vegetation period (T_{MJJAS}; Fig. 5.1a). However, increasing or lowering the average C/(L + E) ratio for some years can cause the resulting $\delta^{13}C_{W*}$ record to correlate better (Fig. 5.1c) with temperature than $\delta^{13}C_C$ or worse (Fig. 5.1b). The coefficients of determination are quite different ($R^2 = 0.299$ vs. 0.615) although the time series for $\delta^{13}C_{W*}$ seem to visually vary in details, only (Fig. 5.1d).

$\delta^{13}C_{W*}$ 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). Changing C/(L + E) over periods of several years or along with specific stem sections the time series for $\delta^{13}C_{W*}$ can show rather deviating trends (Fig. 5.1e−g). 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). Likewise, pronounced cellulose decay can not only statistically weaken correlations to climate (Fig. 5.1g), but also can cause distinct differences in the progression of trends (cf. 1950CE to 1980CE, Fig. 5.1e).

Although this simple exercise is based on the assumption of a constant isotope offset between cellulose and lignin and extractives, both having identical values, it illustrates that varying proportions of wood constituents can well explain the observed differences in signal strength reported for isotope ratios of wood, its cellulose and climate data, respectively (Table 5.2).

Generally, changes of the chemical composition of wood can cause rather larger effects during periods in which the isotopic signature governed by environmental conditions varies little in contrast to periods in which the isotopic signature varies strongly. This is exemplified in Fig. 5.2 showing real $\delta^{13}C_C$ with rather low variability (sd = 0.29) from Potsdam Scots pine for the time period 1801 to 1822CE (22 years). An artificial $\delta^{13}C_{W*}$ was calculated with 7 years deviating from a C/(L + E) ratio of 70/30. The resulting $\delta^{13}C_{W*}$ record shows a higher variability (sd = 0.37) than $\delta^{13}C_C$ and, probably more important, sometimes inverse year-to-year changes. Furthermore, this implies that correlations between wood and cellulose isotope values may be reduced during periods of generally low variability of isotopic signature and high when it is changing considerably from a certain level to another one (Schleser et al. 2015).

The illustrated potential effects might be weakened or even be more prominent if the isotope signatures of the different wood constituents do respond differently to environmental or climatic changes, and not in the same way as assumed for the simple exercise here. In this regard, it should be noted, that conclusions drawn from this exercise on carbon stable isotopes cannot be simply transferred to oxygen or hydrogen, because of different proportions of these elements within cellulose, lignin and extractives and different precursors substances with rather different $\delta^{18}O$ and $\delta^{2}H$ values (Schmidt et al. 2001).

5.2.5 *Benefits of Using Cellulose Instead of Wood*

Before discussing major aspects, the minor benefits of extracting cellulose instead of using wood shall be addressed. Firstly, no carbon transfer across tree-ring boundaries after formation of primary cellulose structures. Secondly, working with cellulose makes the sample homogenization easier. Cellulose can be homogenized rather quickly (ca. 50 samples/hour) with ultrasonic devices after wood slithers or chips of up to 1 mm in thickness and no more than 5 mm in length underwent the chemical extraction procedure (Laumer et al. 2009). Contrastingly, wood material has to be homogenized by grinding, i.e. chopping with subsequent sieving by using certain mills. Although the milling process may only take a few seconds, the necessary cleaning process (vacuum cleaner, compressed air, rinsing with alcohol, etc.)

is usually rather time consuming (ca. 10 samples/hour). Furthermore, sample losses from wood milling are rather high, whereas ultrasonic homogenization virtually leads to no sample loss at all (cf. Sect. 5.3.4 for further details). Thirdly, chemical extraction of cellulose removes contaminants from sampling and handling, e.g. by tree corer or chain saw lubricants and it probably makes an extra removal of extractives obsolete as found for *P. sylvestris* by Rinne et al. (2005). Fourthly, the content of tree-ring cellulose was recently introduced as a potential supplementary proxy in dendroclimatology (Ziehmer et al. 2018).

Major aspects: in theory, the presence of a stable isotopic offset, a significant correlation between isotopes of wood and its cellulose, as well as a slope of regression that is not significantly different from 1 should allow the use of wood instead of cellulose. This seems to be the case for the two studies on δ^2H (Lee et al. 2015; Gori et al. 2013), and for the majority of δ^{13}C and δ^{18}O studies (Table 5.2). However, many authors were usually avoiding generalizing statements as their studies are constrained to certain tree species and/or the specific sites. Nonetheless, a broader validity is suggested simply by the large number of case studies recommending that bulk wood or extractives-free wood (mostly referring to resinous conifers) can be used. This is underlined by two studies that did not assess tree-ring time series but used numerous wood fragments (twigs, branches, stem wood or worked wood) from a wide range of species (Harlow et al. 2006) and/or collected from spatially separated sites along large ecological or environmental gradients (Barbour et al. 2001). Although these studies appear to be very elaborate, Harlow et al. (2006) have investigated 44 different tree species (38 angiosperms; 7 gymnosperms) and Barbour et al. (2001) analyzed 16 samples of different oaks and 26 samples of *Pinus sp.*, it was concluded that cellulose extraction is not necessary for many applications and many wood samples, implying that the conclusions are not unconditional and both studies did not discuss for which species, site conditions or research questions extraction may not be skipped. The uncertainty was indeed highlighted by a Mediterranean site network suggesting that cellulose extraction is required when correlations of isotopes to climate are generally weak so that changes in chemical wood composition can mask the climate signal (Ferrio and Voltas 2005). Other studies from the Mediterranean likewise concluded that cellulose extraction is required for extracting a climate signal from tree-ring δ^{18}O (Battipaglia et al. 2008; Szymczak et al. 2011). In contrast, D'Alessandro et al. (2004) found stable isotopes of bulk wood suitable for ecological studies at their sites in Southern Italy.

In synopsis, the basic question remains whether or not all the studies proposing to skip cellulose extraction are yet adequately systematic in nature, i.e. whether the covered geographical range, species selection, time range and ecological gradients were broad enough for a general conclusive statement. Still, tests seem to be advised for all isotope studies that are not consistent with the framework of the published studies, which is, frequently difficult to define because given site descriptions are lacking in detail.

Many studies that have investigated tree-ring isotope time series of wood and its cellulose and suggest that cellulose extraction is unnecessary have assessed records of no more than 20 to 30 years (e.g. Sohn et al. 2013; Guerrieri et al. 2017; Warren et al.

2001; Weigt et al. 2015), whereas studies on longer sequences were less convincing or even critical about skipping cellulose extraction. They noted some instability in the isotope offsets between wood and its cellulose on inter- and/or intra-annual level, e.g. indicated by variable moving correlations or by slopes of regression being significantly different from a one-to-one relationship (Table 5.2). The results of Schleser et al. (2015), revealing that correlations between $\delta^{13}C$ of wood and cellulose can collapse from >0.9 down to virtually zero for 50-year sub-periods of a 273-year record, raise questions whether it is eligible to extrapolate correlation properties from a certain time interval to any other time interval without considering a weakening of signals.

A probable reason for the differences observed might be that the studies on shorter sequences seem to be largely constrained to sapwood, whereas investigations on the longer sequences were comprising heartwood as well (Table 5.2). Sapwood differs from heartwood by chemical and isotopic properties of extractives (Sect. 5.2.2.2). This might have been relevant in the longer-term studies that did not rely on extractives-free wood and hence, were facing differences from pith to bark. However, apart from varying extractives the cellulose to lignin ratio likewise can be very different in sapwood, heartwood and the transition zone in between. Bertaud and Holmbom (2004) not only found that heartwood of *P. abies* contained significantly more lignin and less cellulose than sapwood, but also the transition zone between heartwood and sapwood had a specific composition, with less lignin and lipophilic extractives than heartwood and sapwood. This can explain, at least partly, the varying offsets observed between isotope ratios of (extractives-free) wood and cellulose, and the exercise above (Fig. 5.1e) has demonstrated that potential effects can well cause different curve shapes or trends (from pith to bark) of isotopes between wood and cellulose, respectively, as was also observed in nature (Friedman et al. 2019; Szymczak et al. 2011). Strictly speaking the available literature indicates that only tree-ring stable isotope ratios of sapwood (untreated or extractives-free in case of resinous conifers) may be used in short-term scale studies. Nonetheless, even in short-term studies oak sapwood have shown variable isotope responses and further investigations were suggested (Weigt et al. 2015; Borella et al. 1998).

Isolation of cellulose excludes any potential issue associated with variability in the relative amounts of wood constituents with their different isotope signatures and related to that particular concern was raised regarding the preferential degradation of cellulose in subfossil and fossil wood under both aerobic and anaerobic conditions (Loader et al. 2003; Borella et al. 1998; Hook et al. 2015; Schleser et al. 1999; Savard et al. 2012; Nagavciuc et al. 2018; Lukens et al. 2019). This may impart a low-frequency signal in bulk wood stable isotope values potentially causing trends that are unrelated to climatic or other environmental changes and cause potential problems when using bulk wood for climate reconstructions from long sub-fossil tree-ring chronologies (McCarroll and Loader 2004). Differential degradation of wood constituents can lead to contrasting isotopic trends in trunks (of same age) buried in bogs or deposited lakes (Savard et al. 2012; Bechtel et al. 2007b; Lukens et al. 2019). However, wood decay from infections by fungi and bacteria can already affect stressed or diseased living trees and probably cause much larger damage than

any degradation commencing after wood-preserving burial. In-depth studies (experiments) on the isotopic effects of cellulolytic enzymes of fungi and/or bacteria are lacking and it is quite uncertain to which extent partial decay results in changes in the isotopic signature of cellulose, which would also degrade the environmental or climatic signal. Savard et al. (2012) could show that highly altered wood from boreal lakes shows a decrease not only in cellulose proportion but also in $\delta^{18}O$ of cellulose, whereas $\delta^{13}C$ ratios were apparently preserved. Despite this one study, there is a need for further research on the effects of different kinds of wood decay, stages of preservation and burial history on the isotopic signatures of wood and its constituents. Loader et al. (2003) and Robertson et al. (2004) suggested to analyze the stable isotope ratios of more resistant lignin to address this issue. Furthermore, the analysis of stable isotope ratios of carbon and hydrogen of lignin methoxyl groups (Mischel et al. 2015; Gori et al. 2013) may also well be used as in case of decayed wood to substitute potentially obscured cellulose isotope data.

5.2.6 The Additional Value of Stable Isotopes of Lignin Methoxyl Groups

Keppler et al. (2007) and Gori et al. (2013) suggested that stable isotope ratios of carbon and hydrogen of lignin methoxyl groups can be used as palaeoclimate proxies. Particularly, because isotope ratios of hydrogen of lignin methoxyl groups are considered not to undergo significant exchange with plant water during metabolic reactions. Carbon and hydrogen isotope ratios are determined on methyl iodide (CH_3I) by GC-C/TC-IRMS (Greule et al. 2009; Greule and Keppler 2011). CH_3I gas is obtained from the reaction of hydroiodic acid (HI, 55–58%) with 2 mg/10 mg ($\delta^{13}C/\delta^{2}H$) of wood at 130 °C for 30 min. This method of sample preparation is rather quick, after equilibration of about one hour aliquots of 10–90 µl of CH_3I can be transferred to the autosampler of the GC-C/TC-IRMS (Mischel et al. 2015). A disadvantage of this method might be that not only lignin methoxyl groups are unclosed, but all methoxyl groups being present in a wood sample. Nonetheless, given the high similarity of lignin and corresponding isotope time series of cellulose (Mischel et al. 2015) parallel analyses may help to identify degraded wood sections not only in terms of reduced cellulose content, but also concerning potentially altered isotopic signatures of the remaining cellulose.

5.3 Cellulose Extraction Procedures, Reaction Devices and Sample Homogenization

5.3.1 Sample Pre-preparation, Wood Cross Sections and Tree-Ring Dissection

Analyzing stable isotope ratios of tree rings requires careful sample collection and precise dissection of tree rings or parts thereof (cf. Chap. 4). In the classical approach, wood material from tree rings dissected by scalpel or rotary tools (e.g. Dremel®) is easiest placed into labeled 2 ml transparent microcentrifuge tubes (e.g. Eppendorf Tubes®) for transport and storage. The wood material should be chopped into small pieces of about 1 to <0.5 mm thickness and length of no more than 5 mm prior to extraction described in Sect. 5.3.2 below. The use of small pieces of wood slivers ensures that the chemicals used are effective and the time needed for ultrasonic homogenization after extraction is minimized (Laumer et al. 2009). The chopping procedure is obsolete if micromilling devices are used for sampling tree-ring material, however, precise tree-ring dissection with a scalpel is usually faster than with a semi-automatic micromilling devices. More recently, approaches to cellulose extraction from wood cross-sections (Sect. 5.3.3.3) were proposed that allow for tree-ring dissection from cellulose laths after extraction (Kagawa et al. 2015; Schollaen et al. 2017).

5.3.2 Extraction Chemistry

Three basic chemical approaches to extract cellulose or α-cellulose are actually applied in stable isotope studies (Fig. 5.3). The most frequently used approaches to extract cellulose are initially based on a procedure described by Jayme (1942) and improved by Wise et al. (1946) (Jayme-Wise method, often referred to as method after Green (1963)). Two other approaches were established but are far less prevalent: the so-called Brendel-method (Brendel et al. 2000) and the diglyme-HCL method (MacFarlane et al. 1999).

If wood samples are not decayed, extraction procedures proposed here usually yield between about 30 to 45% of cellulose, depending on whether α-cellulose or holocellulose is targeted. Assuming that about 200 μg of cellulose are required for a routine mass spectrometric analysis, a minimum of 2 mg of wood material would allow up to 3 individual measurements.

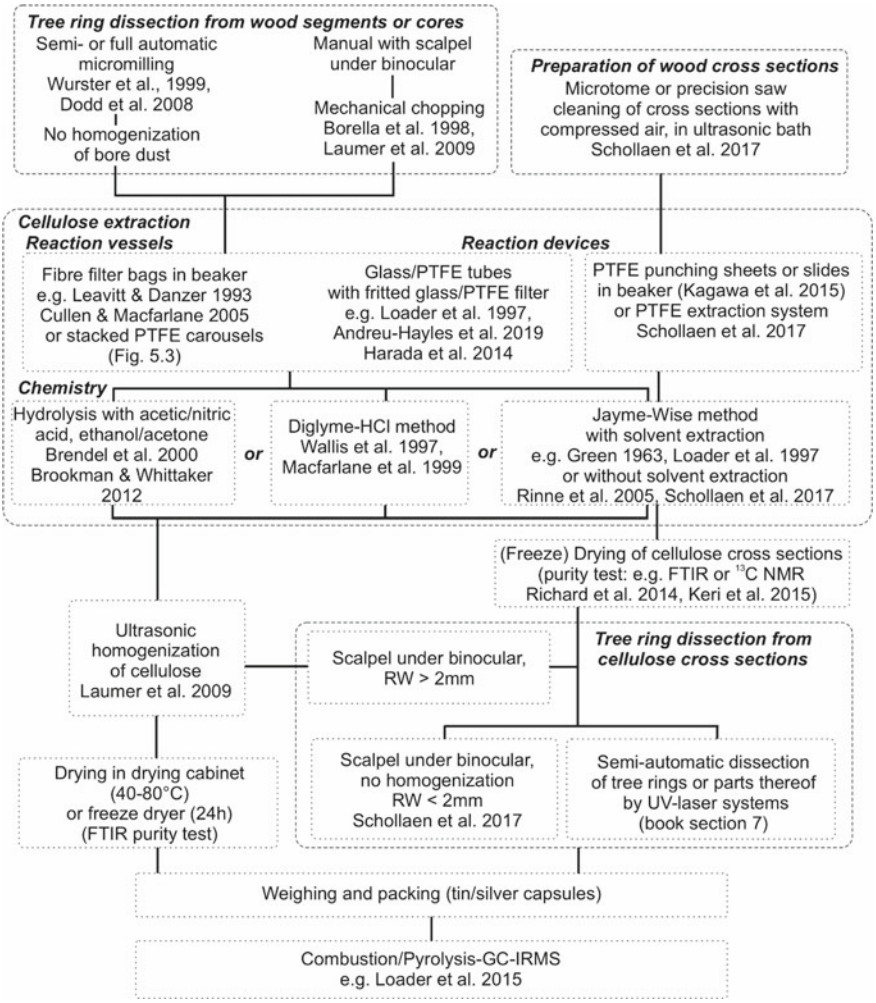

Fig. 5.3 Overview of procedures of tree-ring stable isotope analysis involving cellulose extraction (modified after Schollaen et al. 2017). Note, removal of extractives from resinous conifers and certain tropical tree species is important. However, this is not highlighted as an extra step on the figure, because FTIR purity tests indicate that resin is removed by the bleaching procedure during cellulose extraction even without prior solvent extraction. For details cf. text, as well as key references given in this figure and citations therein

5.3.2.1 Removal of Extractives Prior to Cellulose Extraction

Green (1963) proposed a pretreatment of conifer wood in a Soxhlet apparatus with 2:1 benzene-ethanol and 95% ethanol or 95% ethanol and then ether. He considered a pretreatment to be unnecessary for most non-resinous woods except for tropical

woods. To date, a variety of solvents (ethanol, chloroform, methanol, toluene, deionized water) and solvent-mixtures are applied in isotope studies for removing extractives prior to cellulose extraction (Table 5.2, E^1 to E^4). In most cases a Soxhlet apparatus is used, however, sometimes wood samples are simply treated with ethanol in a beaker for 1 or 2 days (e.g. Schollaen et al. 2013). Note, FTIR purity tests suggest that a solvent extraction step is unnecessary prior to cellulose extraction when applying the Jayme-Wise method (Sect. 5.3.2.4) to resinous conifers (Schollaen et al. 2017; Andreu-Hayles et al. 2019; Rinne et al. 2005).

5.3.2.2 Brendel Method

The Brendel-method applies iterations of acetic acid (80%) and nitric acid (70%) to finely ground wood samples at 120 °C. It has been modified for small samples (Evans and Schrag 2004) and with regards to chemistry (Anchukaitis et al. 2008; Gaudinski et al. 2005; English et al. 2011; Dodd et al. 2008), particularly including sodium hydroxide (NaOH) and extra water rinsing steps to yield pure α-cellulose instead of holocellulose. The modified Brendel-method is supposed to be particularly attractive to non-specialist researchers new to the field of stable isotope dendroclimatology because it requires only basic equipment and reagents and an advantage over other approaches in terms of minimizing losses associated with filtering and changing of reaction vessels (Brookman and Whittaker 2012). However, even experienced researchers can still face problems with the modified extraction protocol (Berkelhammer and Stott 2011). As a disadvantage of this method it was raised that the proposed reaction temperature (120 °C) to be applied is close to (i) the boiling point for 70% nitric acid and (ii) to melting point of polypropylene mircrocentrifuge tubes or Eppendorf© vials. Brookman and Whittaker (2012) proposed to digest the samples at lower temperature (115 °C) and provided an update of this method.

5.3.2.3 Diglyme-HCL Method

The diglyme-HCL procedure as originally proposed by Wallis et al. (1997) was adapted for tree-ring stable isotope analysis by MacFarlane et al. (1999). It applies a 1:0.25 mixture of diglyme (1-Methoxy-2-(2-methoxyethoxy)ethane) and 10M HCl to ground wood samples at 90 °C (shaking water bath) for 1 h. Depending on the reaction vials used residual cellulose is obtained after cooling by gravity filtration (MacFarlane et al. 1999) or centrifugation and discarding of the supernatant (Hietz et al. 2005). Recovery of cellulose is faster when the wood samples are put into heat-sealed filter bags (e.g. type F57, pore size <30 μm, Ankom Technology, NY) for subsequent chemical treatment in a beaker (Cullen and MacFarlane 2005). In adaptation to tropical tree species (*Cedrela odorata*, *Swietenia macrophylla*) Hietz et al. (2005) added an extra step of acidified $NaClO_2$ (10%, 12 h, 70 °C) ensuring complete removal of lignin.

5.3.2.4 Jayme-Wise Method

The Jayme-Wise-method is the prevailing approach to extracting cellulose in stable isotope studies. Originally the procedure results in holocellulose and comprises 3–6 iterations of delignification with sodium chlorite ($NaClO_2$, 1%) acidified with acetic acid to pH 3–4. The reaction temperature is set to about 70 °C and every 60 min fresh portions of acetic acid and sodium chlorite has to be added. A fume hood is required because chlorine dioxide is generated during the delignification. After 3–4 h (softwoods up to 6 h) white (sometimes slightly yellowish) holocellulose is obtained after thorough washing with de-ionized water. At present, the number of iterations with $NaClO_2$, concentration of solution, reaction temperature and reaction time vary by author. In isotope studies pH < 4 should not be used because lower pH will cause higher degradation of final cellulose.

In order to remove hemicelluloses (i.e. non-glucan polysaccharides, in particular xylan and mannan) Green (1963) proposed an additional step with sodium hydroxide (NaOH). He described treatments with various concentrations of NaOH (2–18%) and various reaction temperatures (room temperature to 95 °C) to obtain α-cellulose. Hence, several different variants of this extraction procedure are currently used in the various stable isotope laboratories around the globe. They basically refer to Green (1963), Leavitt and Danzer (1993) and/or (Loader et al. 1997).

Pure α-cellulose is usually isolated by a treatment of holocellulose with a NaOH solution (17%) at room temperature followed by repeated washing with deionized water and 1% (w/v) HCl until pH is neutral (e.g. Ziehmer et al. 2018; Loader et al. 1997). However, as indicated in Table 5.2 various other concentrations of the NaOH solution, application times and reaction temperatures were applied intending to isolate α-cellulose (αC^1 to αC^6). Most frequently solutions with rather low NaOH concentrations (4–10%) are used but maintaining the relatively high reaction temperature (70–80 °C) of the preceding delignification step with $NaClO_2$. Some authors applying a low concentration, high temperature application of NaOH describe their product still holocellulose or just cellulose, since it has not always been tested whether or not all the xylan and mannan hemicelluloses were effectively removed from the holocellulose. Loader et al. (1997) reported that sequential treatment with a combination of 10% (w/v) NaOH at 80 °C followed by 17% (w/v) NaOH at room temperature maximized the removal of hemicelluloses. Either way, two studies found the carbon and oxygen isotopic compositions of hemicellulose and α-cellulose to be identical (Gray and Thompson 1977; Richard et al. 2014), so that holocellulose seems to be well suited. More importantly, findings by Rinne et al. (2005) suggest that the 2-step extraction with $NaClO_2$ and NaOH can make an extra solvent extraction step unnecessary.

5.3.2.5 Testing the Purity of Extracted Cellulose

Despite the various chemical procedures applied no systematic differences seem to prevail between them with respect to the isotopic signature of extracted cellulose. This

is because all the different extraction methods established were tested at some point for the purity of the isolated cellulose in comparison to corresponding wood (Richard et al. 2014; Schollaen et al. 2013; Andreu-Hayles et al. 2019; Keri et al. 2015; Kagawa et al. 2015; Brookman and Whittaker 2012). This is usually done by Infrared Spectrometry (FTIR or IR-ATR; e.g. Richard et al. (2014)) or liquid-state ^{13}C NMR (Keri et al. 2015). Infrared spectrometry is the most common method to identify the functional groups of resin, lignin, α-cellulose and hemicellulose. Analyses are usually performed on normalized spectra in the wavenumber region 1800–730 cm^{-1}. A specific band at 1694 cm^{-1} is characteristic of resins occurring only in conifer woods, some FTIR tests indicate that the chemical bleaching during cellulose extraction removes resin even without prior solvent extraction (e.g. Rinne et al. 2005). Further information on relevant FTIR wavenumbers according to literature can be found in SM5.3.2.5.

Apart from IR, liquid state ^{13}C NMR (DEPT-135 ^{13}C NMR) was chosen as an analytical tool by Keri et al. (2015) in a comparative study testing nine variants of the Jayme-Wise extraction method for the isolation of cellulose from wood. The different approaches mainly varied in concentration of $NaClO_2$, NaOH, and application time and reaction temperatures, as well as rinsing with HCl, HNO_3 and/or water. They evaluated the purity, degradation and yield of cellulose and stable isotope ratios of carbon and oxygen. All preparation methods tested resulted in pure α-cellulose samples without hemicellulose and lignin content, and $\delta^{13}C$ and $\delta^{18}O$ measurements revealed similar values, thus indicating that all the published $NaClO_2$ and NaOH chemical protocols based on the Jayme-Wise approach are suitable. It might be also best applied in conjunction with the technical devices described below for handling of large sample numbers (Andreu-Hayles et al. 2019; Kagawa et al. 2015; Schollaen et al. 2017).

5.3.3 Extraction Devices—Or How to Keep Order When Processing Large Numbers of Small Samples

5.3.3.1 From Erlenmeyer Flasks to Custom-Made Filterfunnels

In the early days of stable isotope analysis cellulose was extracted using Erlenmeyer flasks (Leavitt and Danzer 1993). Because fume hoods have to be used during chemical extraction the number of samples to be processed at a time was limited by the limited space provided for the rather large flasks. A major improvement has been made by introducing small borosilicate extraction thimbles with a sintered glass filter near the bottom end (Loader et al. 1997). Batches (25–50) of (labelled) thimbles could be placed into a beaker containing chemical solutions or deionized water for washing. The beaker containing the thimbles was placed into an ultrasonic bath to promote degassing at 70 °C. Removal of chemical solutions or water was achieved by taking out the thimbles from the beaker and vacuum filtration of remaining solvents

from each thimble. This approach was developed further by using filter thimbles with a tapered bottom (Büchner funnels) to make them fit into specially designed PTFE-devices (Wieloch et al. 2011; Andreu-Hayles et al. 2019) that allow simultaneous drainage of 20 interconnected filter funnels from solvents through a single outlet (Fig. 5.4e, f). As commercially available Büchner funnels are relatively expensive and tend to rapidly degrade after a few extractions, custom-made funnels were introduced (Harada et al. 2014; Andreu-Hayles et al. 2019). A borosilcate filter disc is secured between tubings made of glass or PTFE and silicone rubber (Fig. 5.4a, b). Properly labelled custom-made funnels can be placed also into beakers (Fig. 5.4c, d). For further details cf. Andreu-Hayles et al. (2019) and citations therein.

5.3.3.2 Filter Fiber Bags

Apart from filter funnels to take up wood material for extraction porous bags are utilized. The samples can be processed simultaneously in a Soxhlet apparatus and/or a beaker glass. A variety of bag materials, e.g., Polytetrafluoroethylene (PTFE), fibre glass are utilised. After the bags have been filled with the samples they are usually heat-sealed. Individual encoding of the bags is complicated since they cannot be simply inscribed with a pencil or felt-tipped pen. Therefore, they are either labelled by cutting out bits and pieces in different shapes from their borders or expensive pre-numbered bags are used. After one time application bags normally need to be replaced.

A handy way of processing a high number of samples within filter fiber bags while maintaining sample organisation and ensuring ease of chemical exchange and washing is to use a filter bag drum tower (FBDT) that is designed to hold filter fiber filter bags in place during cellulose extraction a 2000 mL beaker (Fig. 5.5). A FBDT contains up to five PTFE sample drums with 25 individually labelled slots placed on top of each other (Fig. 5.5a). Each slot can hold a single filter bag and unlike other methods, the bags do not require inscription. The entire FBDT can be lifted out of the beaker and placed into another one with fresh chemical solution or for neutralizing with boiling water (Fig. 5.5b, c, e).

Individual sample filter bags can be developed by cutting commercially available Ankom F57 filter bags (Fig. 5.5d) into up to 5 individual sample bags (depending on the size of the samples in question) and closed using an inexpensive Polythene Heat Sealer device or soldering iron. The FBDT provides a means of completing cellulose extraction in a single, economical unit which can be heated within a beaker on a single hotplate (Fig. 5.5b) or multiple beakers within a large water bath (Fig. 5.5f). In an optimal arrangement (Fig. 5.5f), five fully equipped beakers can be placed within an ultra-sonic water bath, resulting in 625 samples to be processed in one batch. See SM5.3.3.2 for details on the FBDT and remarks on F57 filter bags.

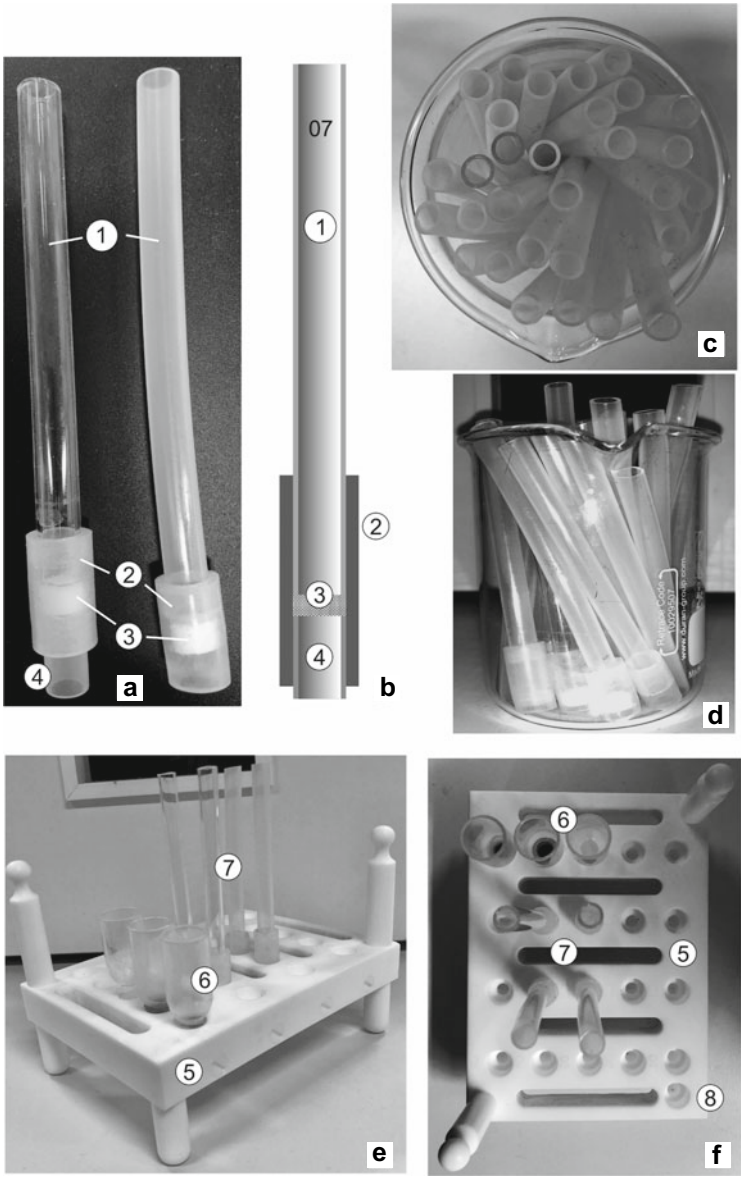

Fig. 5.4 Custom-made funnel-filter assembly (**a**, **b**): (1) Glass or PTFE tube, (2) silicone tube, (3) borosilcate filter, (4) PTFE support tube for fitting into PTFE extraction device (**e**, **f**). **c**, **d** Several labelled filter funnels placed into a beaker for cellulose extraction without special PTFE device. (5) PTFE extraction device for placing into a water bath. (6) Borosilicate Büchner funnels positioned in PTFE extraction device with slots for up to 20 samples. (7) Custom-made funnels positioned in slots, sealed by silicone tubing. Slots are interconnected by channels leading to an outlet slot allowing simultaneous draining of all filter funnels. For details see Sect. 5.3.3.1, as well as Wieloch et al. (2011), Harada et al. (2014), Andreu-Hayles et al. (2019) and citations therein

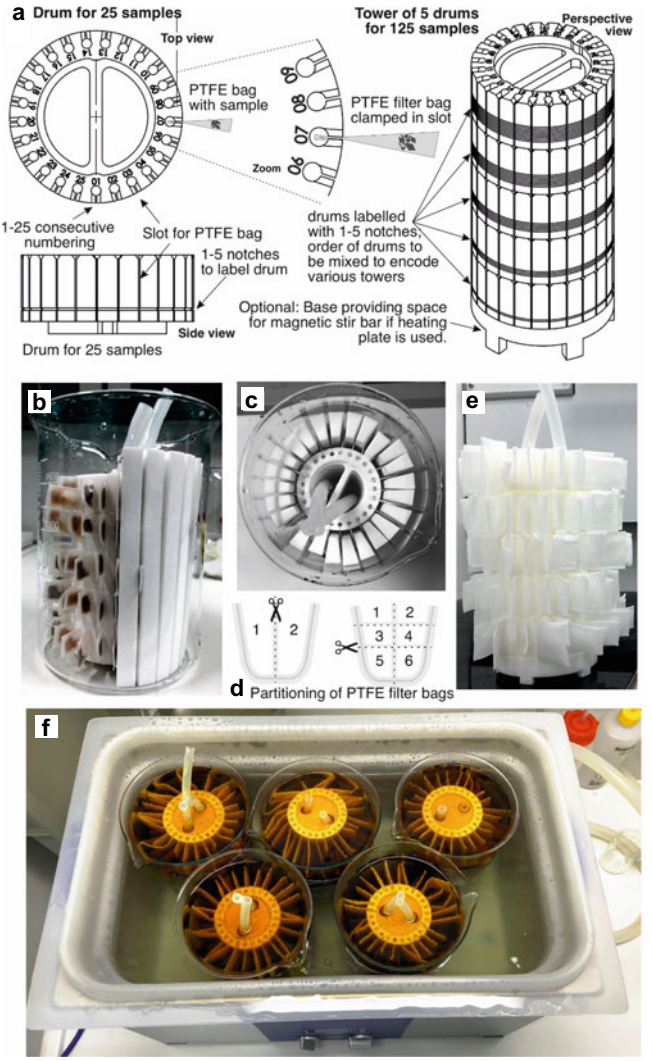

Fig. 5.5 Filter bag drum tower (FBDT) for holding fiber filter bags in place during cellulose extraction. **a** Sketch drawings showing the design of individual PTFE sample drums for up to 25 filter bags. Up to five labeled PTFE sample drums can be combined forming a tower that can be placed into a 2000 mL beaker (**b**, **c**). FBDTs can be swapped between beakers while maintaining sample organisation by using flexible silicone tubings ensuring ease of chemical exchange and washing (**e**). PTFE segments can be placed vertically between the sample bags along the entire tower (**b**, **c**) to reduce the volume of chemical solution required to ca. 800 mL (in a 2000 mL beaker; 6.4 mL/samples). **d** Individual sample filter bags can be developed by cutting commercially available filter bags up (depending on the size of the samples in question) and using an inexpensive Polythene Heat Sealer device or soldering iron. **f** Five fully equipped beakers placed within an ultra-sonic water bath (Elmasonic S300H, Elma-Schmidbauer GmBH, Singen, Germany) allowing simultaneous treatment of 625 samples

5.3.3.3 Cellulose Extraction from Wood Cross-Sections

Loader et al. (2002) made a first attempt to extract cellulose directly from standard increment cores (5 mm Ø). However, this approach was not pursued further, because clear identification of boundaries of narrow tree ring was found difficult to achieve as the cores tend to shrink and twist during extraction and subsequent drying procedure. Instead, Li et al. (2011) reported a technique to extract cellulose from wood cross-sections of 3.5–4 mm thickness (wood laths, Fig. 5.6) using a special perforated PTFE casing to prevent cellulose splines from breaking apart. This method was developed further by Nakatsuka et al. (2011) and Kagawa et al. (2015), by utilizing special containers made of PTFE punching sheet to prevent disintegration of cellulose

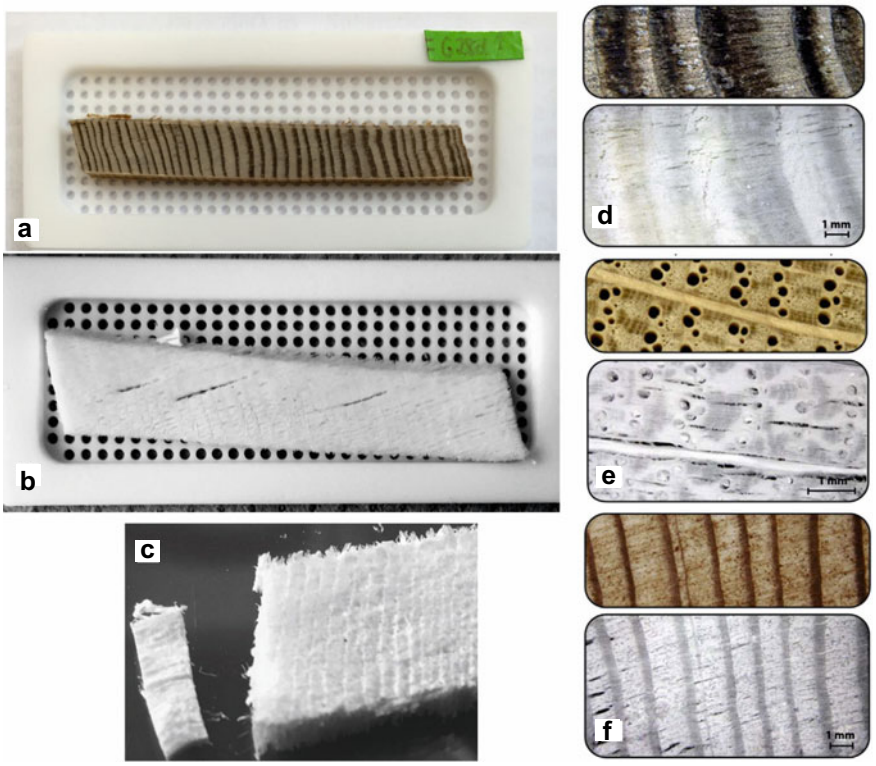

Fig. 5.6 Cellulose extraction from wood cross-sections in custom made PTFE containers (70 mm × 30 mm). **a** Sub-fossil wood cross-section from pine (*P. sylvestris*, Binz, Switzerland). **b** Cellulose cross-section (ca. 3–4 mm thick). **c** Tree ring dissected from a cellulose cross-sections (10 mm wide, 3 mm thick). **d** Wood and cellulose cross-section from a degraded subfossil pine sample. **e** Wood and cellulose cross-section from oak (*Q. petraea*). **f** wood and cellulose cross-section of larch (*L. decidua*). The upper pictures of each species show wood cross-sections while the lower pictures display the cellulose cross-sections obtained. Tree-ring structures remained clearly visible after the cellulose extraction process. d, e, f modified after Schollaen et al. (2017)

laths (see also Xu et al. 2011). Based upon on the idea of extracting cellulose from wood laths, Schollaen et al. 2017 presented an improved semi-automated cellulose extraction system in conjunction with a comprehensive guide of manual (with scalpel, Fig. 5.6c) or semi-automatic (UV-Laser systems; cf. Chap. 7) sample preparation.

The chemical procedure is not different to methods described above (Sect. 5.3.2), however, wood sample preparation and tree-ring dissection of tree rings from cellulose cross-sections are special. Figure 5.6a, b show wood and white cellulose cross-sections of well-preserved sub-fossil *P. sylvestris* (ca. 14 k a BP) from Switzerland. Tree-ring boundaries can be well identified allowing binocular-aided tree-ring dissection with a scalpel (Fig. 5.6c). Tree rings of a partly decayed wood samples (Fig. 5.6d) are not so clearly visible. However, tree-ring structures of modern oak (Fig. 5.6e) and larch (Fig. 5.6f) are well preserved after the cellulose extraction process. For further details cf. Schollaen et al. 2017 and citations therein.

5.3.4 *Homogenization of Micro Amounts of Cellulose Samples*

As mentioned above, one of the advantages of extracting cellulose is that sample homogenization is simpler. Cellulose can be homogenized rather quickly (100 samples/hour or more) by ultrasonic devices (e.g. UP200s, Hielscher Ultrasonics, Germany) (Laumer et al. 2009). Contrastingly, wood material, if not obtained from full- or semi-automatic micromilling devices (e.g. Dremel® rotary tools) has to be homogenized by grinding after dissecting tree rings using a scalpel. Usually (modified) coffee mills (e.g. Borella et al. 1998; Szymczak et al. 2011), ultra-centrifugal mills (ZM200, Retsch GmbH, Haan, Germany) (e.g. Wieloch et al. 2011) or ball mills (e.g. mixer mill MM200, Retsch, Haan, Germany) (e.g. Weigt et al. 2015) are utilized. Although the grinding process itself may only take a few seconds, the necessary cleaning process is usually rather time consuming and sample losses may be high (Laumer et al. 2009).

Homogenization by ultrasonic cracking is virtually without sample loss because sample material does not have to be transferred between storage vials and any special homogenization vessels. Sample material is kept in suspension with water during ultrasound treatment and vacuum freeze-dried thereafter. Laumer et al. (2009) tested and confirmed that neither cellulose soaked in water and stored overnight nor cellulose treated ultrasonically for different time periods showed a significant difference in $\delta^{18}O$ from untreated cellulose. No such tests were yet performed with respect to hydrogen isotopes.

5.4 Concluding Remarks

Many of the studies that have compared stable isotope ratios in wood and its cellulose suggest that cellulose extraction is not necessary. However, this is not without constraints. Removal of extractives from resinous conifers and certain tropical tree species is required in any case. Studies indicate that this is provided by the chemical bleaching during cellulose extraction even without prior solvent extraction. Furthermore, the basic question remains whether or not the benchmarking studies were broad enough for a general conclusive statement with regard to geographical range, species selection, ecological gradients or time range. Still, tests seem to be advised for environmental or climatological isotope studies, maybe except for those at short-term scale focusing on sapwood. The climatic or ecological signal of a tree-ring stable isotope sequence can be strengthened or weakened by variable relative amounts of wood constituents. In particular, during periods of low isotope variability signals may be weakened or even inverted. Radial changes in relative amounts of wood constituents from pith to bark, e.g. due to conversion of sapwood into heartwood or wood degrading processes can cause trends in isotope chronologies from wood that are unrelated to trends in the desired environmental signal. Hence, varying cellulose to lignin ratios may be critical when analyzing long-term climate trends from old living trees (across heartwood and sapwood) and/or potentially decayed trunks. It may not be required for obtaining information on extreme events such as droughts or high-rainfall years, when large year-to-year isotopic shifts potentially make effects of varying wood constituents insignificant. Nonetheless, a moisture signal in tree-ring $\delta^{13}C$ from wood may be enhanced if the fraction of ^{13}C-depleted secondary metabolites, like lignin or fatty acids, decreases/increases with dry/wet conditions relative to ^{13}C enriched primary products like cellulose or starch. Correlations may be weakened if the opposite occurs, and the stable isotope signal and wood composition respond differently to certain environmental changes.

Differences in the proportion of the various wood constituents are not the main source of isotope variability within a tree stand. The use of cellulose is likely not reducing this variability (McCarroll and Loader 2004), but changing wood composition adds to the noise in tree-ring stable isotope signals from wood. Hence, cellulose extraction is of benefit. A preceding Soxhlet procedure to remove extractives seems obsolete and homogenization of cellulose is much faster than grinding wood samples. The experimental setups for extraction have dramatically improved over the last decade with respect to handling and costs. Several guidelines for chemical extraction procedures are available. They basically represent modifications of three different chemical approaches (Jayme-Wise, Brendel-, diglyme-HCL method) that are well approved. Any non-specialist or specialist researcher can establish and/or improve their own guideline in accordance to the technical framework of their laboratory, available budget and research aim. Last, but not least, further investigations of wood constituents and their isotope signatures as a function of a tree's life, i.e. across sapwood and heartwood, in response to climate variables, as well as with respect to wood and cellulose decay are advised. Both, tree-ring stable isotope time

series from wood, as well as from cellulose have revealed nonstationary relationships with climate variables. They may respond differently under particular environmental conditions in the surrounding of a tree, such as weather anomalies, changing length in seasonality, tree competition etc. Consequently, the analyses of isotope data from cellulose, wood and lignin methoxyl groups could in part point to different environmental signals, help to assess effects of wood decay and, hence, provide an added value. In parallel to stable isotope analysis, measurements of the chemical composition of wood could enable a potential correction of isotope signals or ensure that changing wood composition is insignificant relative to the variability of the isotopic signals.

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