

Comparison of δ^{13} C and δ^{18} O from cellulose, whole wood, and resin-free whole wood from an old high elevation *Pinus uncinata* in the Spanish central Pyrenees*

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ABSTRACT

 $δ^{13}$ C and δ^{18} O values from sapwood of a single *Pinus uncinata* tree, from a high elevation site in the Spanish Pyrenees, were determined to evaluate the differences between whole wood and resin-free whole wood. This issue is addressed for the first time with *P. uncinata* over a 38-year long period. Results are also compared with published isotope values of α-cellulose samples from the same tree. The differences in δ^{13} C and δ^{18} O between whole wood and resin-free whole wood vary within the analytical uncertainty of 0.3 and 0.5 ‰, respectively, indicating that resin extraction is not necessary for sapwood of *P. uncinata*. Mean differences between cellulose and whole wood are 0.9 ‰ (δ^{13} C) and 5.0 ‰ (δ^{18} O), respectively. However, further analyses of different species and other sites are needed to evaluate whether the findings reported here are coherent more generally.

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1. Introduction

An increasing number of studies employ stable carbon and oxygen isotope values from tree-rings as climate proxies [1–9]. Most of these studies determined $\delta^{13}C$ and $\delta^{18}O$ values of α -cellulose in tree-rings. Furthermore, isotope signatures of whole wood and lignin were also measured and compared to those of α -cellulose [10–16]. Due to the time-consuming extraction of α -cellulose from tree-rings, whole wood was tested in several studies for its applicability as a climate proxy. According to the results of existing studies, cellulose shows higher $\delta^{13}C$ and $\delta^{18}O$ values than contemporaneously formed whole wood [10–12,14,15]. However, $\delta^{13}C$ and $\delta^{18}O$ values of whole wood and cellulose seem to cohere well at inter-annual (high-frequency) timescales [11,12,14,17,18].

Several studies analysed and compared the isotopic compositions of the different wood components. Sidorova et al. [10] found significantly variyng isotope residuals between cellulose and whole wood over longer timescales. Other work revealed differing climate

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signals between cellulose and whole wood samples [10,12,16–18], while Borella et al. [11] reported larger variations of δ^{18} O values in whole wood than in cellulose. These effects are not coherent among studies. Hence a clear recommendation whether stable isotopes should be derived from cellulose or whole wood samples is still missing.

In some of the existing studies, the resin and soluble components were extracted from the wood samples [10,11] whereas in other studies, the samples were forwarded without any treatment [12,14,17]. The resin and soluble components are not bound to single treerings and could isotopically exchange with wood-cells after their formation [19], which could result in biasing the isotopic signal. Consequently, the question arises whether the also time-consuming extraction of resin and soluble components is necessary and to which extent δ^{13} C and δ^{18} O results are affected. Up to now, only few studies assessed this issue. Harlow et al. [20] compared δ^{13} C of whole wood, extractive free whole wood and holocellulose from a wide range of species (44) and found an average difference of 0.25 ± 0.09 %. They recommended removing resin and soluble components to avoid uncertainties due to changing amounts of extractives. However, the extraction of cellulose was not identified to be necessary. A further study by Ferrio and Voltas [18] analysed the differences of δ^{13} C and δ^{18} O values from whole wood, extracted wood, holocellulose and lignin, and tested their potential as significant proxies. Using Pinus halepensis from 23 different sites, they found a significant mean difference between whole wood and extracted wood of 0.3 % (δ^{13} C), but significant difference in δ^{18} O were absent. The study indicated that cellulose extraction is not necessary for $\delta^{13}C$, although resin and extractives could add noise to the climate signal. For δ^{18} O, the authors recommended cellulose extraction because only the time series from this wood component contained a significant climate signal.

Here, we address some of these issues in a pilot study using sapwood samples from a single pine tree (*Pinus uncinata*) containing a high amount of resin compared to other tree species. This is the first study comparing δ^{13} C and δ^{18} O values over 38 years from whole wood and resin-free whole wood from a 334-year-old high elevation *P. uncinata* individual tree. The objective of this study is to analyse whether a significant difference exists between untreated whole wood and resin-free whole wood samples in both δ^{13} C and δ^{18} O data. The isotopic values of α -cellulose from the same tree, published in Konter et al. [7], were compared to the results from this study in order to evaluate these differences.

2. Material and methods

2.1. Material

For this study, a single 334-year-old pine tree (*P. uncinata*) from the Spanish central Pyrenees was used. The tree was sampled in 2010 along with 22 other pines of which 5 specimens were selected and analysed for incorporated climate signals in stable carbon and oxygen isotope data from α -cellulose [7]. The sampling site near Lake Gerber at the northern border of the D'Aigüestortes Estany de Sant Maurici National Park is located at an elevation of 2400 m above sea level. Four cores (5 mm in diameter) per tree were taken at breast height for tree-ring width (TRW) analyses. Two of these cores were used for α -cellulose extraction [21–23] in a previous study [7]. These two cores were combined and homogenised for annually measurements of δ^{13} C and δ^{18} O values at the GFZ in Potsdam. The analytical long-term reproducibility is ±0.1 ‰ [VPDB, Vienna PeeDee Belemnite] for δ^{13} C and ±0.3 ‰ [VSMOW, Vienna Standard Mean Ocean Water] for δ^{18} O (details in Konter et al. [7]). The existing α -cellulose based δ^{13} C and δ^{18} O data are compared with new measurements (whole wood and resin-free whole wood) from the same tree.

The TRW chronology of the five selected trees for isotope analyses highly synchronise (r = 0.88, p < .001) with the TRW chronology of all other trees from the site [7]. While the single δ^{13} C cellulose series of the selected pine correlates at r = 0.77(p < .001) with the mean of the four other trees (over 1971–2009), this correlation drops to r = 0.48 (p < .01) for the δ^{18} O cellulose series, indicating that the latter parameter is less well represented in the single tree considered here. Nevertheless, based on the significant correlations, we assumed the selected tree represents the site on a reasonable level.

2.2. Sample preparation

The remaining two of four cores of the selected tree, which were analysed for their TRW in the study of Konter et al. [7], were used for this study. One core was analysed for δ^{13} C and the other one for δ^{18} O. The average TRW (1971–2009) is 0.61 mm for the core used for δ^{18} O analyses and 0.68 mm for the core used for δ^{13} C analyses. The annually resolved wood series were analysed for δ^{18} O (1971–2009) and for δ^{13} C (1971–2007). These periods of the two cores completely comprise sapwood. The δ^{13} C series do not contain the years 2008 and 2009 because the respective material was lost during sample preparation.

Firstly, several horizontal thin slices were taken perpendicularly to the wood fibres from both cores using a microtome [24], to preserve the remaining part of both cores for the resin extraction. The single tree-rings – early and late wood were not separated – were annually separated from the thin slices using a scalpel. The different thin slices, representing different cell layers, were not homogenised. However, the isotopic composition should not exceed the analytical uncertainty in such a small piece (smaller than the whole core with 5 mm in diameter) of a single tree-ring, as shown by a homogeneity test in a piece of a single tree-ring in a study by Riechelmann et al. [25]

In a second step, resin was extracted from the remaining parts of both cores. For this procedure, cores were rinsed in deionised water for 8 h at 60 °C, while the water was exchanged every 2 h. The samples were exposed to ethanol for 24 h at 60 °C. Ethanol was changed every 3–4 h until discolouration was absent. The samples were then dried at 50 °C in a cabinet dryer. Annual tree-rings were separated using a scalpel. Meaurements of all resin-free wood aliquots were performed from as many pieces as needed for a sample amount of 1–2 mg for δ^{13} C analyses and 100–300 µg for δ^{18} O analyses. These samples were radially cut from the single tree-rings (always containing early and late wood).

For each year of whole wood, one aliquot was measured for δ^{13} C and two aliquots for δ^{18} O. One aliquot of resin-free whole wood each year was used for δ^{18} O and two aliquots for δ^{13} C. These measurements of two aliquots were performed to evaluate the internal precision of the analysis.

2.3. Isotope analyses

For the δ^{13} C analyses, 1–2 mg of wood material were weighed with a Mettler Toledo (Gießen, Germany) XP6 microbalance (±1 µg) and filled in tin capsules. These samples were combusted in a Vario EL III elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) at 1150 °C and measured with an IsoPrime isotope ratio mass spectrometer (GV Instruments Ltd., Manchester, UK) at the Institute of Organic Chemistry (Johannes Gutenberg-University Mainz). We used the reference materials IAEA-CH-6 (sucrose) and IAEA-CH-7 (polyethylene), which were each measured three times during a sample run. The analytical uncertainty is ±0.3 ‰ [VPDB], which is the standard deviation of the IAEA-CH-7 measurements.

For δ^{18} O analyses, 100–300 µg of wood were weighed with a Mettler Toledo (Gießen, Germany) XP26 microbalance (±1 µg) and filled in silver capsules. The samples were pyrolysed in a high-temperature conversion elemental analyser (TC/EA; ThermoFinnigan, Bremen, Germany) at 1450 °C, which was connected to a Zero Blank Autosampler (Costech Analytical Technologies, Valencia, CA, USA). The sampling gas was measured using an Isoprime 100 IRMS isotope ratio mass spectrometer (Isoprime Ltd., Stockport, UK) at the Institute of Geosciences, Johannes Gutenberg-University Mainz. Each of the reference materials (IAEA-601 and IAEA-602) was measured 21 times for this study. The analytical uncertainty is ±0.5 ‰ [VSMOW], which is the standard deviation of the IAEA-601 measurements. All δ^{13} C values are given with respect to VPDB and all δ^{18} O values with respect to VSMOW.

 δ^{13} C and δ^{18} O series from cellulose were provided by the study of Konter et al. [7]; for details of the methods see there. These data series were compared to the whole wood and resin-free whole wood series derived from this study.

For detrending of the isotope series a 10-year fast Fourier transformation (FFT) filter, which is a low pass filter, was calculated using the software Origin. Detrending of the isotope series was applied to emphasise high-frequency variations. Further, the data were analysed by the Student's *t*-test and linear regressions, testing also the significance of the regression line slopes using the software Origin.

3. Results

All four δ^{13} C time series analysed here are based on raw measurements and show a decreasing trend from 1971 to 2007. The two resin-free whole wood series and the whole wood series do not reveal differences larger than the analytical uncertainty (Figure 1(a)), as confirmed by a Student's *t*-test. The mean difference between the whole wood and cellulose series is larger ($0.9 \pm 0.3 \%$), and clearly exceeds the analytical uncertainty (±0.3 ‰). In general, the cellulose time series show higher δ^{13} C values with a mean of -22.4 % than the whole wood series with a mean of -23.3 %.

The residuals of the whole wood, resin-free whole wood and cellulose series show different slopes (Figure 1(b)) as estimated by linear regression. The residuals between the two resin-free whole wood series show a significant decreasing trend. This occurs as well in the residuals between the whole wood series and one resin-free whole wood series. The residuals between the other resin-free whole wood series and the whole wood series do not show any significant trend. In contrast, a significant increasing trend occurs in the case of the residuals between whole wood and cellulose.



Figure 1. (a) δ^{13} C time series (1971–2007) of whole wood, resin-free whole wood (two values each year), and cellulose. Error bars are 0.3 ‰, except for cellulose (0.1 ‰). (b) Residuals of δ^{13} C between the differently treated series (whole wood, resin-free whole wood, and cellulose) including linear regression lines. (c) δ^{13} C time series of whole wood, resin-free whole wood (two values each year), and cellulose all detrended with a 10-year FFT filter.

However, all series show similar high-frequency variability, which is visible after detrending with a 10-year FFT filter (Figure 1(c)). The correlation coefficients between all δ^{13} C series are significant (p < .001, Table 1(a)) and confirm the common high-frequency variability. The correlation coefficients of the detrended series decrease in comparison to the undetrended series (Table 1(b)), but still share a high-frequency variability.

The mean difference between the δ^{18} O values of the double-measured whole wood series is 0.1 ± 0.3 ‰ (Figure 2(a) and 2(b)). The difference between the whole wood and resin-free whole wood samples is 0.4 ± 0.3 ‰, and remains below the analytical uncertainty of 0.5 ‰. In contrast, a significantly higher mean difference of 5.0 ± 1.2 ‰ is observed between the whole wood and the cellulose series with cellulose showing

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	Whole wood	Resin-free whole wood 1st series	Resin-free whole wood 2nd series
(a) δ ¹³ C			
Resin-free whole wood	0.93		
1st series			
Resin-free	0.95	0.90	
whole wood			
2nd series			
Cellulose	0.89	0.86	0.86
(b) δ^{13} C detrended with a	10-year FFT filter		
Resin-free	0.85		
1st series			
Resin-free	0.89	0.78	
whole wood			
2nd series			
Cellulose	0.77	0.73	0.71

Table 1. (a) Correlation coefficients for δ^{13} C values between all series (whole wood, resin-free whole wood, cellulose). (b) Correlation coefficients for δ^{13} C values between all series (whole wood, resin-free whole wood, cellulose) after detrending with a 10-year FFT filter.

Note: All correlation coefficients exceed p < .001.

higher δ^{18} O values with a mean of 30.7 ‰ than whole wood with a mean of 25.6 ‰. All differences between whole wood, resin-free whole wood and cellulose were tested for significance using a Student's *t*-test, and the results are confirmed. The residuals between cellulose and whole wood show a significant decreasing slope towards recent times, calculated by linear regression, whereas the residuals between the two whole wood and the resin-free whole wood series do not show a significant trend (Figure 2(b).

After detrending with a 10-year FFT-filter, the δ^{18} O series synchronise in the high-frequency domain (Figure 2(c)). This common high-frequency variability is also pronounced in the significant correlation coefficients (p < .01 to p < .001) between the different series (Table 2(a)). The correlation coefficients between cellulose and the other series are lower, but increased after the detrending with a 10-year FFT-filter, confirming a distinct high-frequency relation in the δ^{18} O series.

4. Discussion

4.1. Long-term trends

The decreasing trend in the raw δ^{13} C series results from the decrease of the atmospheric δ^{13} C value, due to anthropogenic burning of fossil fuel [26]. This anthropogenic influence remains untreated in this study, since only the residuals between the differently treated sample series and the characteristic of the long-term trends in the residuals are of interest and would not be altered by applying the same correction procedure to all time series.

Mean differences between whole wood and cellulose series of 0.9 ± 0.3 ‰ for δ^{13} C and 5.0 ± 1.2 ‰ for δ^{18} O, respectively, support results from previous studies [10,11,14,17]. The significant trends of the residuals between cellulose and whole wood δ^{13} C (increasing)



Figure 2. (a) δ^{18} O time series (1971–2009) of whole wood (two values each year), resin-free whole wood, and cellulose. Error bars are 0.5 ‰, except for cellulose (0.3 ‰). In the two whole wood and the resin-free whole wood series, several data points are missing, due to measuring failure. (b) Residuals of δ^{18} O between the differently treated series (whole wood, resin-free whole wood, and cellulose) including linear regression lines. (c) δ^{18} O time series of whole wood (two values each year), resin-free whole wood, and cellulose all detrended with a 10-year FFT filter.

and cellulose and whole wood δ^{18} O (decreasing) stand out, since all other residual trends are insignificant or likely related to analytical uncertainties.

Similar temporal trends of residuals between cellulose and whole wood were also reported in the study of Sidorova et al. [10]. They explained this differing behaviour with varying environmental factors (response to increasing CO_2 in the atmosphere and in water use efficiency [27]) as well as changes in the ratios of wood components (cellulose, lignin, sucrose, lipids, and starch) and the transition from heartwood to sapwood. The last point can be excluded here because all samples were taken from sapwood. The changes in the long-term trends of the residuals detected in our study, independent of

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	Whole wood	Whole wood	Resin-free	
	1st series	2nd series	whole wood	
(a) δ ¹⁸ Ο				
Whole wood 2nd series	0.96			
Resin-free whole wood	0.96	0.96		
Cellulose	0.52	0.55	0.59	
(b) δ^{18} O detrended with	a 10-year FFT filter			
Whole wood 2nd series	0.91			
Resin-free whole wood	0.81	0.91		
Cellulose	0.78	0.75	0.73	

Table 2. (a) Correlation coefficients for δ^{18} O values between all series (whole wood, resin-free whole wood, cellulose). (b) Correlation coefficients for δ^{18} O values between all series (whole wood, resin-free whole wood, cellulose) after detrending with a 10-year FFT filter.

Note: All correlation coefficients indicated in bold refer to p < .001, all in italics to p < .01.

the source, would lead to differences in the calibration of the cellulose or whole wood isotopic composition against climate parameters [10,12,17] and, therefore, to different climate reconstructions from *P. uncinata* for this high elevation environment. Further, the trends observed in this study are opposite for δ^{13} C and δ^{18} O. Several tree species from different locations need to be analysed over periods of several decades to centuries to assess whether such long-term trends in the residuals between cellulose and whole wood are a common feature. This seems to be important when deciding whether cellulose or whole wood should be used for a carbon and/or oxygen isotope ratio-based climate reconstructions in terms of low-frequency trends within the data.

4.2. High-frequency variability

All δ^{13} C and δ^{18} O series, respectively, show coherent patterns, especially in their high-freguency variability [10–12,14,18] (Figures 1(c) and 2(c) and Tables 1(b) and 2(b)). The δ^{13} C and δ^{18} O cellulose series reveal lower correlation coefficients with the whole wood and resin-free whole wood series. A detrending of all series using a 10-year FFT filter results in decreasing correlation coefficients for δ^{13} C between cellulose and the other series (Table 1(b)), which is due to the removal of a common long-term trend inherent to all series caused by the anthropogenic change in atmospheric δ^{13} C. Detrending the δ^{18} O series results in an increase of the correlation coefficients with cellulose and a slight decrease of the correlation coefficients between the whole wood and resin-free whole wood series (Table 2(b)). The correlation coefficients for $\delta^{13}C$ and $\delta^{18}O$, respectively, between the whole wood and resin-free whole wood series are guite high (0.78-0.96), since both time series refer exactly to the same core. The correlation coefficients between these series and the cellulose data are lower, which is probably additionally influenced by the sampling from differing cores of the same tree, because δ^{13} C variations in a single tree-ring range 0.5–1.5 % and 0.5–2 % for δ^{18} O [28]. Nevertheless, the observed correlation coefficients between cellulose and whole wood are within the range of previous studies of δ^{13} C and δ^{18} O [10,14,17,18]. And most importantly, the cellulose, whole wood and resin-free whole wood series contain similar high-frequency variance in both δ^{13} C and δ^{18} O time series. This is an important finding because the year-to-year variations

of the climate and the environment are preserved in the same strength in cellulose, whole wood and resin-free whole wood.

4.3. Implications for sample preparation

Borella et al. [17] revealed only small differences between δ^{13} C from whole wood and resin-free whole wood (0.09-0.27 ‰), in most cases within the analytical uncertainty (0.08–0.15 %). However, the authors suggested a resin extraction for conifer wood. Harlow et al. [20] also compared δ^{13} C from whole wood and extractive free wood from a wide range of species (44) and found a mean difference of 0.25 ± 0.09 ‰ exceeding their analytical uncertainty of 0.1 ‰. The authors recommend an extraction of resin and solvable components to avoid errors due to different amounts of extractives. A further study by Ferrio and Voltas [18] again found significant difference between whole and extracted wood for δ^{13} C (0.3 ‰), but no difference for δ^{18} O. They also recommend an extraction of resin and solvable components for $\delta^{13}C$ and a cellulose extraction for δ^{18} O, due to a more distinct incorporation of climate variations. Our results show that the differences between the whole wood and the resin-free whole wood series range within the reproducibility of our data and the analytical uncertainty for δ^{13} C and δ^{18} O reaching 0.3 and 0.5 ‰, respectively. The uncertainty of δ^{13} C in our study is in the range of the differences between whole wood and resin-free whole wood reported by Harlow et al. [20] and Ferrio and Voltas [18]. Therefore, we probably could not detect differences of up to 0.3 ‰, due to our analytical uncertainty of 0.3 ‰.

Borella et al. [17] also compared the δ^{13} C values of heart- and sapwood from whole wood and resin-free whole wood. For oak, the difference between heart- and sapwood is 0.62 ‰, whereas the difference between heartwood and sapwood for spruce is -0.03 ‰. After resin extraction the differences decrease to 0.08 ‰ for oak and increase to -0.20 ‰ for spruce. Except the differences between heartwood and sapwood for oak, the other values are in the range of their analytical uncertainty. Further, for spruce the difference between heartwood and sapwood should be lower after extraction of the resin and soluble components, which is not the case in the study of Borella et al. [17] probably due to analytical uncertainties. These results show that the pattern is not distinct and further work taking heart- and sapwood into account should be performed in future studies.

The results from our study using sapwood samples from a *P. uncinata* from a high elevation site show that the impact of resin on the δ^{13} C and δ^{18} O of the whole wood is negligible and a resin extraction is not necessary. Also for this issue further tree species from different site locations should be tested on annual resolved decadal or centennial long series to validate these findings.

As reported by previous studies [10,12,15,17,18], stable isotope time series determined in cellulose and whole wood contain climatic information. However, it remains unsolved whether the climate signals are better preserved in cellulose or whole wood. The decision whether cellulose or a specific wood component is measured seems crucial while working with samples from sub-fossil wood, which is potentially influenced by various decay processes [12,29]. Likewise, in the case of physiological studies it is relevant to decide which component is isotopically characterised, specifically whether carbon and oxygen fractionation pathways in different wood components are of interest [30]. For climate reconstructions, which are in most cases composite records of living and relic trees to enhance the temporal length, the absolute isotope values are important because merging series with different absolute isotope values results in biased stacked series by creating artificial or deleting plausible long-term trends. It is confirmed by our study that no clear conclusion could be drawn whether whole wood or cellulose is the better material for isotope studies, which is due to the different long-term trends in the residuals between cellulose and whole wood. To verify the findings of the present pilot study, similar systematic annual resolved isotope time series should be performed for different species and localities.

5. Conclusions

In this study δ^{13} C and δ^{18} O series from whole wood, resin-free whole wood, and cellulose were compared. Cellulose and whole wood samples differ by 0.9 ± 0.3 ‰ for δ^{13} C and 5.0 ± 1.2 ‰ for δ^{18} O. Varying long-term trends in the residuals between cellulose and whole wood isotopic time series of *P. uncinata* indicate that the different wood components might contain varying low-frequency environmental information. Therefore, a clear recommendation to measure cellulose or whole wood for any kind of study cannot be provided from this study. The most significant finding is that the residuals of δ^{13} C and δ^{18} O, respectively, between whole wood and cellulose are not constant over time. More systematic analyses on annual resolution need to be completed, including different species and site locations, to conclude whether this pattern is recurrent.

The differences between whole wood and resin-free whole wood are within the analytical uncertainty of 0.3 ‰ for δ^{13} C and 0.5 ‰ for δ^{18} O. Therefore, the time-consuming resin extraction of *P. uncinata* sapwood is not necessary for δ^{13} C and δ^{18} O analyses. Further studies from different tree species and sites, including heartwood and sapwood on decadal to centennial time series need to be performed to further validate our findings.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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