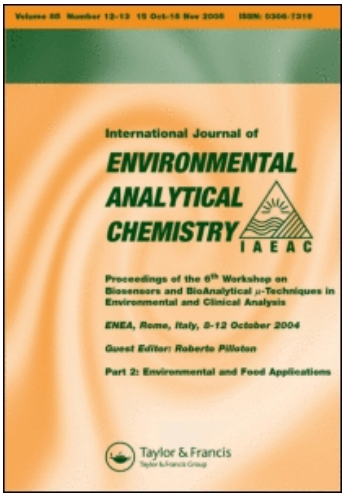

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On the necessity of organic solvent extraction for carbon isotopic analysis of α-cellulose: implications for environmental reconstructions

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On the necessity of organic solvent extraction for carbon isotopic analysis of α-cellulose: implications for environmental reconstructions

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α-cellulose is widely used as a target substance for isotope ratio analysis in environmental reconstructions. Its preparation includes three basic steps: organic solvent extraction, delignification and alkaline hydrolysis. Recent works have suggested omission of the first step. We have made a detailed comparison in carbon isotope ratio of α-cellulose with or without organic solvent extraction using 32 consecutive tree ring and 30 subfossil peat samples. These samples were exposed to three different chemical treatments: with organic solvent extraction as the first step ($Cell_{OE}$), without organic solvent extraction ($Cell_{NOE}$), and with organic solvent extraction as the final step ($Cell_{NOE/OE}$). The third treatment is used to test if organic extractives can be completely removed or if their solubility in organic solvents has been altered by delignification and alkaline hydrolysis. In tree rings and peat, $\delta^{13}C_{Cell\,NOE}$ was always significantly different from $\delta^{13}C_{Cell\,OE}$, but the trends were not the same. In tree rings, $\delta^{13}C_{Cell\,NOE}$ was always more negative than $\delta^{13}C_{Cell\,OE}$ by $-0.31 \sim -0.01$‰. In contrast, $\delta^{13}C_{Cell\,NOE}$ in peat could be more negative or more positive than $\delta^{13}C_{Cell\,OE}$ by $-3.08 \sim 0.27$‰. The third chemical treatment resulted in different patterns. For tree rings, $\delta^{13}C_{Cell\,NOE/OE}$ was still more negative than $\delta^{13}C_{Cell\,OE}$ by $-0.36 \sim -0.08$‰. However, the differences between $\delta^{13}C_{Cell\,NOE/OE}$ and $\delta^{13}C_{Cell\,OE}$ for peat varied in a more narrow range from −0.58 to 0.61‰, compared to the differences between $\delta^{13}C_{Cell\ NOE}$ and $\delta^{13}C_{Cell\,OE}$. These results exposed a complex chemical evolution behaviour and an incomplete removal of lipids during delignification and alkaline hydrolysis. The mean value, long-term trend and seesaw patterns for a tree ring or peat $Cell_{NOE}$ series were significantly different from those for a $Cell_{OE}$ series, indicating that omission of organic solvent extraction will lead to a biased inference of past environmental conditions.

Keywords: α-cellulose; carbon isotopes; organic solvent extraction; tree rings; peat

1. Introduction

α-cellulose is defined as the portion of holocellulose that is insoluble in 17.5% NaOH solution [1]. Due to its high abundance and high stability, α-cellulose preserved in tree rings, peat bogs and sediments has been used as an optimal material in isotopic ratio analysis for environmental reconstructions [2–6]. Natural α-cellulose is present in plant cell

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walls and elaborately intermixed or interconnected with other compounds, such as lipids, lignin, hemicellulose, etc. Hence, its purification involves complex and time-consuming chemical treatments: organic solvent extraction for the removal of lipids and tannins, delignification with acidified sodium chlorite solution for the removal of lignin, and alkaline hydrolysis for the removal of hemicelluloses and other carbohydrates [1,7]. When α-cellulose preserved in peats or lake sediments is needed, additional chemical treatments (boiling in 5% NaOH and in 4% HCl, and oxyhydroxide leaching) are necessary for the removal of other materials (humic substances, pectins and oxyhydroxides) that will interfere isotope analysis [2,7]. Each step needs several to over ten hours, which limits the number of samples that can be processed under restricted apparatus and labour availability.

Over the past two decades, numerous efforts have been made to shorten the process time, to enhance the simplicity of α-cellulose separation, and to evaluate the suitability of different chemical procedures for the purification of α-cellulose from different plant types (e.g., C_3, C_4, CAM, different genotypes of the same species) or different tissues (e.g. roots, stems, leaves) [8–18]. Recent works [15,17] have shown that organic solvent extraction is unnecessary for the purification of α-cellulose from resinous pine wood samples, based on an on-line continuous flow isotope ratio mass spectrometry (CF-IRMS) technique with a precision better than $\pm$0.2‰. But it is not known whether this is a universal rule for α-cellulose preparation of any sorts of plant or subfossil plant samples (tree rings, peats and sediments). On the other hand, the content and composition of extractives in tree rings formed in different years may be highly variable. The chemical behaviours of these extractives during delignification and alkaline hydrolysis are still unknown. Considering the fact that the interannual variation of tree ring isotope composition is sometimes of the same level as the isotope analytical uncertainty, it is necessary to make a test with a long and consecutive tree ring series as well as with a dual-inlet isotope ratio mass spectrometry (DI-IRMS) technique with a precision better than $\pm$0.05‰.

Organic solvent extraction was also omitted for α-cellulose isolation from peat samples without any feasibility test [19–22]. That might be of high risk for paleoclimatic misinterpretation of isotope data since many more substances in peats are extractable for organic solvents [23]. These extractable materials are partly inherited from original plant residues (peat-forming plants, such as moss, sedge and woody plants), or partly contributed during humification processes of original plant residues (microbially-reworked materials). The latter can also produce a wide variety of compounds with very different chemical properties.

Based on the above considerations, this work aims to make a detailed comparison in carbon isotope ratio of α-cellulose prepared with different chemical procedures (with or without organic solvent extraction), and to test the necessity of organic solvent extraction for the purification of α-cellulose from modern (32 consecutive annual tree rings) and ancient (30 peat samples) plant materials. We also use organic solvents to extract those samples exposed to delignification and alkaline hydrolysis in order to test if extractives can be completely removed or if the solubility of extractives in organic solvents has been altered following these two treatments. This also gives a test if organic solvent extraction as the final step can provide a remedy to those samples on which organic solvent extraction is not used at the first step. So, we can see if we can go back and reanalyse archived samples. Our carbon isotope analysis is based on a high-temperature (850°C) sealed-quartz tube combustion method coupled with a DI-IRMS technique.

2. Experimental

2.1 *Site description and sample collection*

A resinous pine tree (*Pinus koraiensis*) in a forest farm (42°24′ N, 128°6′ E, 750 m.a.s.l.) of the Changbai Mountains in Jilin Province of Northeast China was cut down in 1997. A round disc (about 10 cm thickness) from the trunk was separated. Each ring was arranged to a calendar year by cross dating and the whole disc covered a lifespan between 1862 and 1997. Annual ring materials were isolated from the disc with a scalpel. Thirty-two consecutive heartwood tree ring samples in this study spanned a period from 1908 to 1939. The climate is cold and dry in winter (with a mean temperature of −15.6°C in January), and warm and humid in summer (with a mean temperature of 19.7°C in July). The annual mean temperature, rainfall amount and relative humidity are 3.6°C, 695 mm and 72%, respectively.

Thirty peat samples were recovered with a Russian corer from a profile (42°20′ N, 126°22′ E, 614 m.a.s.l.), located in Huinan County of Jilin Province. Samples were sealed into plastic bags and immediately carried to a lab of the Northeast Normal University of China (situated in Changchun, the capital of Jilin Province) for drying in an oven at 50°C. Previous work had shown that these samples covered a ^{14}C age ranging between 600 and 5000 years BP [24]. The climate feature is similar to that of the tree ring sampling site. The annual mean temperature, rainfall amount and relative humidity are 4.1°C, 704 mm and 72%, respectively.

2.2 *α-cellulose preparation*

The general technical procedure used in this work is presented in Figure 1. Before grinding, peat samples need additional chemical treatments in order to remove humic substances and pectins [2]. The coarse materials and wood fragments were then identified as far as possible and discarded. The predominant plant type in this profile is sedge, but it is impossible to isolate pure sedge residues even under a microscope due to humification [25]. The moss residues are present in the section from 240 to 280 cm, which is not included in this dataset. The grinding for tree ring and peat samples includes two steps, firstly pulverising in a low-speed ($1400\,r\,M^{-1}$) mill for 2 min and then in a high-speed ($10000\,r\,M^{-1}$) mill for 2 min to 120 mesh (125 μm). It is well known that different plant species or different chemical components in the same plant are very heterogeneous in carbon isotope composition [26]. This milling procedure ensures the homogeneity in chemical and isotopic compositions of each sample, preventing an artificial bias when a sample is separated into two or several aliquots. Thus, in this study the isotopic discrimination effects caused by variations in plant species or components among all the aliquots of the same sample can be represented as negligible [27,28]. Subsequently, each sample was divided into two fractions: one for standard α-cellulose extraction including organic solvent extraction as the first step (so-obtained α-cellulose is defined as $Cell_{OE}$), and the other without organic solvent extraction ($Cell_{NOE}$). $Cell_{NOE}$ were divided into two aliquots, one for carbon isotope analysis, the other was extracted with organic solvents as the final step ($Cell_{NOE/OE}$) (Figure 1).

The standard chemical procedure for the purification of α-cellulose includes the following three steps [1,7]. First, organic solvent extraction was processed in a Soxhlet system for 6 h with a 2 : 1 mixture of benzene to methanol, and another 6 h in acetone. Second, delignification was carried out for 6 h in an ultrasonic bath at 70°C with glacial

acetic acid-acidified sodium chlorite solution [10]. Every 25 min the acidified sodium chlorite was added. Lastly, alkaline hydrolysis was ultrasonically processed for 45 min in a 10% NaOH solution at 80°C, and another 45 min in a 17% NaOH solution at room temperature [10]. Samples at this stage were rinsed with deionised water three times, diluted with acetic acid (10%), and washed six times with deionised water. Then, they were dried overnight in an oven at 50°C.

2.3 *Carbon isotope ratio determination*

Above-treated α-cellulose (2 mg) and 3 g copper oxide wire were loaded into a quartz tube (9 mm O.D.). The tube was sealed in a high-vacuum line (3×10^{-2} Pa), and heated to

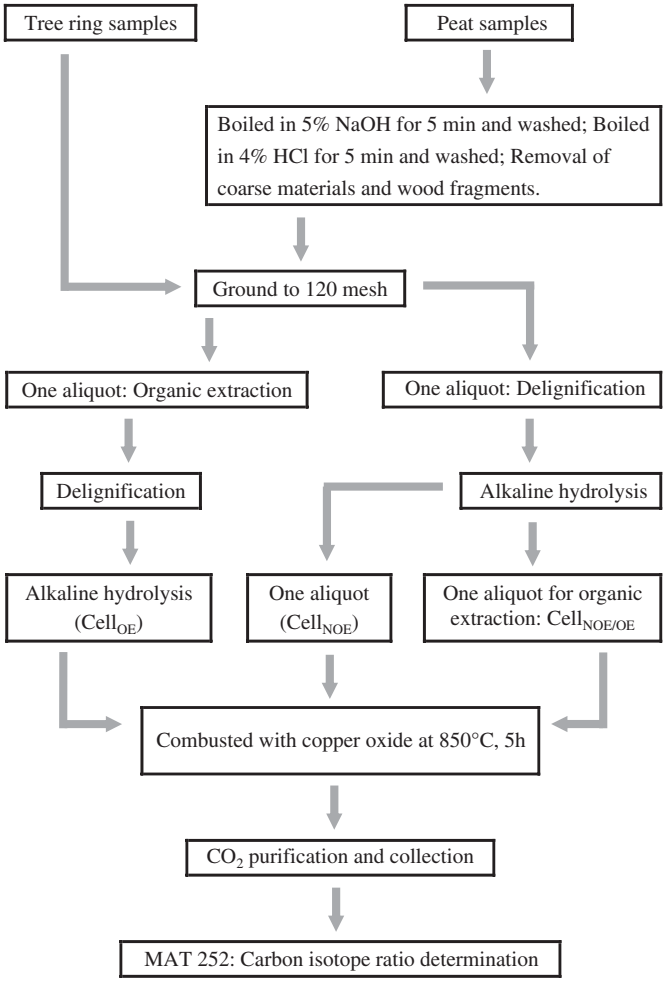

Figure 1. Flow scheme showing the technical procedures from separation and purification of α-cellulose in tree ring and peat samples to carbon isotope ratio determinations. Refer to the text (Section 2.2) for the details about organic solvent extraction, delignification and alkaline hydrolysis.

850°C in an electrical resistance furnace for 5 h [29]. At that temperature, copper oxide is releasing O_2 that completely oxidises all the carbon inside the tube to CO_2. Tube and copper oxide were pre-heated at 850°C for 1 h prior to their use with samples. CO_2 was cryogenically purified in a high-vacuum line, and its $^{13}C/^{12}C$ ratio was determined in a MAT 252. The data were reported with traditional 'δ' denotation relative to the Vienna Pee Dee Belemnite (VPDB). The total analytical precision was better than ±0.05‰. Replicate analyses of IAEA-C3 cellulose (batch of cellulose produced in 1989 from one season's harvest of ca. 40-year-old trees and prepared by W.G. Mook and J. Plicht) displayed a mean $\delta^{13}C$ value of -24.93 ± 0.02‰ ($n = 5$) with a range between −24.95 and −24.91‰. That is comparable to the IAEA consensus value of -24.91 ± 0.49‰ [30]. We analysed five different tree ring α-cellulose samples ($Cell_{OE}$) in 2004, and re-analysed these five samples in 2006. Repeated analyses displayed a mean difference of 0.07 ± 0.02‰ ($n = 5$ pairs).

3. Results

3.1 *Tree rings*

As mentioned above, natural α-cellulose is always intermixed with other chemical components. Hence, pure α-cellulose is a theoretical concept and practically unavailable. It should be bore in mind that α-cellulose is the operationally defined standard to which all methods try to approximate and is needed for consistent interpretations of environmental conditions. Since the standard procedure removes impurities to the largest extent, the α-cellulose so obtained is most closely similar to pure α-cellulose. Thus, the $\delta^{13}C$ values for $Cell_{OE}$ can be used as a yardstick to which the $\delta^{13}C$ values for $Cell_{NOE}$ or $Cell_{NOE/OE}$ are compared. The interannual $\delta^{13}C$ values for tree ring $Cell_{NOE}$ and $Cell_{NOE/OE}$ were nearly identical ($r^2 = 0.991$, $n = 32$, $p < 0.001$), but for some samples, the $\delta^{13}C$ values of $Cell_{NOE}$ or $Cell_{NOE/OE}$ did not follow the seesaw patterns of $Cell_{OE}$ (see the shadowed area in Figure 2). Repeated analyses supported those differences (Appendix 1).

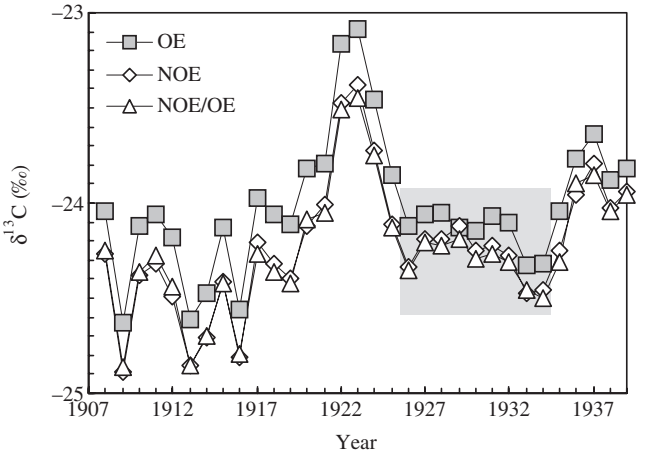

Figure 2. Time series of $\delta^{13}C$ values for tree ring $Cell_{OE}$, $Cell_{NOE}$, and $Cell_{NOE/OE}$. The shaded area indicates that in some points the carbon isotope ratios ($\delta^{13}C$) present different patterns. See the text (Section 2.2) for the definitions of $Cell_{OE}$, $Cell_{NOE}$ and $Cell_{NOE/OE}$.

Table 1. Statistical descriptions of $\delta^{13}C$ values for tree ring $Cell_{NOE}$, $Cell_{NOE/OE}$, $Cell_{OE}$, $Cell_{NOE}$ - $Cell_{OE}$ and $Cell_{NOE/OE}$ - $Cell_{OE}$.

	$Cell_{NOE}$	$Cell_{NOE/OE}$	$Cell_{OE}$	$Cell_{NOE}$ - $Cell_{OE}$	$Cell_{NOE/OE}$ - $Cell_{OE}$
Mean	-24.23 ± 0.35	-24.25 ± 0.33	-24.02 ± 0.35	-0.21 ± 0.07	-0.23 ± 0.07
Range	$-24.89 \sim -23.38$	$-24.86 \sim -23.45$	$-24.63 \sim -23.09$	$-0.31 \sim -0.01$	$-0.36 \sim -0.08$
n	32	32	32	32	32

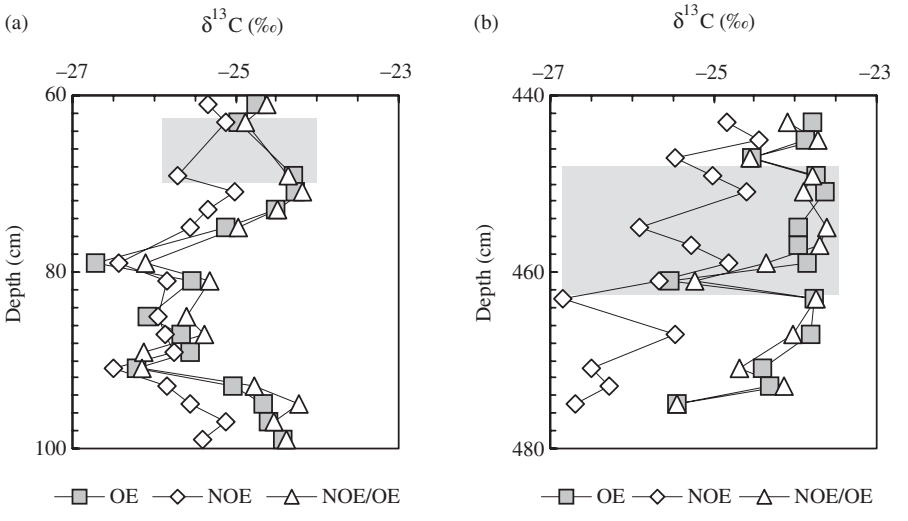

Figure 3. $\delta^{13}C$ values of peat $Cell_{OE}$, $Cell_{NOE}$, and $Cell_{NOE/OE}$ anchored at depth. a: the upper part; b: the lower part. The shaded area indicates that in some points the carbon isotope ratios ($\delta^{13}C$) present different patterns. See the text (Section 2.2) for the definitions of $Cell_{OE}$, $Cell_{NOE}$ and $Cell_{NOE/OE}$.

For environmental reconstructions, the mean value of a proxy (such as $\delta^{13}C$ value in this work) during a historic or geological period represents an average environmental status. Moreover, the mean value is also one of the important parameters for a time series [31]. Thus, it is important to compare the mean $\delta^{13}C$ values and ranges of tree ring α-cellulose with different chemical treatments (Table 1). $Cell_{NOE}$ and $Cell_{NOE/OE}$ nearly had the same mean $\delta^{13}C$ values and ranges, and both presented a negative shift of about 0.22‰ with respect to $Cell_{OE}$ (Table 1). Within the total analytical uncertainty, there is only one sample (i.e. tree ring 1929) for which $Cell_{NOE}$ and $Cell_{OE}$ were of the identical $\delta^{13}C$ value.

3.2 *Peat*

These samples were gathered from the upper (61~99 cm) and the lower (443~475 cm) part of the same peat profile. The former corresponded to a younger period between 600 and 1000 ^{14}C years BP, and the latter an older one between 4000 and 5000 ^{14}C years BP [24].

Unlike tree rings, the inter-sample variations of $\delta^{13}C$ values for peat $Cell_{NOE}$ and $Cell_{NOE/OE}$ did not closely match those for $Cell_{OE}$ (Figure 3). $Cell_{NOE}$ and $Cell_{NOE/OE}$ presented a similar pattern for the upper part ($r^2 = 0.617$, $n = 16$, $p < 0.01$), but not for the

Table 2. Statistical descriptions of $\delta^{13}C$ values for peat $Cell_{NOE}$, $Cell_{NOE/OE}$, $Cell_{OE}$, $Cell_{NOE}$ - $Cell_{OE}$ and $Cell_{NOE/OE}$ - $Cell_{OE}$.

	$Cell_{NOE}$	$Cell_{NOE/OE}$	$Cell_{OE}$	$Cell_{NOE}$ - $Cell_{OE}$	$Cell_{NOE/OE}$ - $Cell_{OE}$
Upper part					
Mean	-25.65 ± 0.43	-25.00 ± 0.69	-25.15 ± 0.74	-0.50 ± 0.44	-0.15 ± 0.27
Range	$-26.50 \sim -25.01$	$-26.14 \sim -24.18$	$-26.71 \sim -24.27$	$-1.43 \sim 0.27$	$-0.58 \sim 0.61$
n	16	16	16	16	16
Lower part					
Mean	-25.55 ± 0.79	-24.22 ± 0.58	-24.18 ± 0.62	1.37 ± 0.74	0.03 ± 0.26
Range	$-26.84 \sim -24.44$	$-25.46 \sim -23.62$	$-25.54 \sim -23.63$	$-3.08 \sim 0.13$	$-0.50 \sim 0.33$
n	14	14	14	14	14

lower part ($r^2 = 0.152$, $n = 14$, $p < 0.20$). Big differences in $\delta^{13}C$ value existed between $Cell_{NOE}$ and $Cell_{OE}$. The uncommon points testified by replicate analyses for peat samples were more clearly observable compared to tree ring samples (see the shadowed area in Figure 3).

The mean $\delta^{13}C$ values and ranges for peat $Cell_{NOE}$, $Cell_{NOE/OE}$ and $Cell_{OE}$ were quite different (Table 2). In contrast to tree ring samples, the typical features for peat are: (1) The difference in $\delta^{13}C$ between $Cell_{NOE}$ and $Cell_{OE}$ embraced a broad range for both the upper and lower parts (Table 2); (2) the difference in $\delta^{13}C$ between $Cell_{NOE/OE}$ and $Cell_{OE}$ was still relatively large, such as from -0.58 to 0.62‰ for the upper part, and from -0.50 to 0.33‰ for the lower part. However, its range was greatly narrowed, compared to that between $Cell_{NOE}$ and $Cell_{OE}$; (3) the $\delta^{13}C$ values for $Cell_{NOE}$ or $Cell_{NOE/OE}$ could be more negative or more positive than those for $Cell_{OE}$, and hence did not present a fixed (always more negative) tendency. Sometimes, the $\delta^{13}C$ values for $Cell_{NOE/OE}$ could be equal to those for $Cell_{OE}$ (Appendix 1). No samples showed equal $\delta^{13}C_{Cell\,NOE}$ and $\delta^{13}C_{Cell\,OE}$ values.

4. Discussion

4.1 *Incomplete removal of extractives by delignification and alkaline hydrolysis*

Like α-cellulose, lignin and hemicellulose, extractives (mainly lipids) are also among plant cell wall chemicals. They are mainly composed of a series of monomers, dimers and polymers, such as fats, fatty acids, fatty alcohols, phenols, terpenes, steroids, resin acids, waxes, tannins as well as many other minor organic compounds [32]. As far as bark extractives are concerned, fats, oils, phytosterols, resin acids, waxes, terpenes, tannins and pectins are included. The extractives content is highly variable, depending on tissue types and tree species, and also related to environmental conditions. For the same tree, bark has a higher extractives content than sapwoods or heartwoods while sapwoods have a lower extractives content compared to heartwoods. Softwoods are generally higher than hardwoods in terms of the extractives content. The North American softwoods and hardwoods were reported of an ethanol-benzene extractives content ranging from 1 to 14% and from 1 to 7%, respectively [32].

Table 3. Linear regression results between $\delta^{13}C$ values of $Cell_{NOE}$, $Cell_{NOE/OE}$ and $Cell_{OE}$.

	$Cell_{NOE}$ vs. $Cell_{OE}$			$Cell_{NOE/OE}$ vs. $Cell_{OE}$		
	r^2	n	Slope	r^2	n	Slope
Tree rings	0.957	32	0.993	0.963	32	1.039
Peat						
Upper part	0.715	16	1.452	0.868	16	0.998
Lower part	0.225	14	0.368	0.827	14	0.965

Bark, leaf, flower and stem can be a source of plant remains in peat. With increased humification, the content of cellulose, hemicelluloses and lignin decreases while their derivatives including humic substances accumulate. Oils, waxes, resins, partially decomposed pigments and humic substances (if not completely removed in last chemical treatment steps) are extractable for organic solvents. Therefore, peat may contain more extractable components and posses far more complex composites compared to tree ring materials. It has been reported that the peat extractives in 4 mires in Finland are comprised of fatty acids, ω-hydroxy acids, 1-alkanols, sterols, alkanes, α, ω-alkanedioic acids, various triterpenoid compounds and a mixture of polymerised compounds and their content can be up to 11% of the dry peat weight [23].

Lipids are depleted in ^{13}C relative to the primary carbohydrates (such as glucose) by $4 \sim 6‰$, due to the isotope effect on a pyruvate dehydrogenase reaction [33]. As mentioned above, lipids are a group of chemicals with different structures and chemical properties. It is questioned whether or not this mixture can be completely decomposed and dissolved during delignification and alkaline hydrolysis. If they are not completely removed, even present in a small amount, then more negative $\delta^{13}C$ values for $Cell_{NOE}$ should be expected. This has been testified on tree ring samples as $\delta^{13}C_{Cell\,NOE}$ is always more negative than $\delta^{13}C_{Cell\,OE}$ by 0.21‰ (Table 1). Although peat samples presented more subtle behaviours, $\delta^{13}C_{Cell\,NOE}$ was still generally more negative than $\delta^{13}C_{Cell\,OE}$ by 0.50‰ for the upper part or by 1.37‰ for the lower part (Table 2). These results give a hint to the existence of lipid materials inside the corresponding samples after delignification and alkaline hydrolysis.

Organic solvent extraction as the final step ($Cell_{NOE/OE}$) resulted in different patterns for tree rings and peat, giving further evidence for the above argument. The correlation between $\delta^{13}C_{Cell\,NOE/OE}$ and $\delta^{13}C_{Cell\,OE}$ is slightly refined for tree ring samples compared to that between $\delta^{13}C_{Cell\,NOE}$ and $\delta^{13}C_{Cell\,OE}$ but greatly improved for peat samples (Table 3). The slope for the plot of $\delta^{13}C_{Cell\,NOE}$ vs. $\delta^{13}C_{Cell\ OE}$ deviates from unity, but the slope for the plot of $\delta^{13}C_{Cell\,NOE/OE}$ vs. $\delta^{13}C_{Cell\,OE}$ is close to unity (Table 3). To some extent, the linear regression shows the similarity of two time series. In the case of $y = ax + b$, if the slope (a) is 1, and then y and x will simultaneously change with the same speed or amount ($a = \Delta y/\Delta x = 1$). If $a = 1$, $b = 0$, and $r = 1$, then these two time series (parameters x and y) are eventually identical ($y = x$). When $a = 1$, $b \neq 0$, and $r = 1$, then two time series has the same seesaw patterns (the differences at each time point are equal). When $r \neq 1$, then two series are less similar (the differences at each time point are not equal), and so on. When a chemical method is used with an improved correlation and a slope close to 1, then it is clear to show an enhanced efficacy of this method since it makes two series more similar. On the other hand, the $\delta^{13}C$ difference between $Cell_{NOE}$ and $Cell_{OE}$ for tree ring and peat samples show a time dependence (Figure 4a: $r^2 = 0.456$,

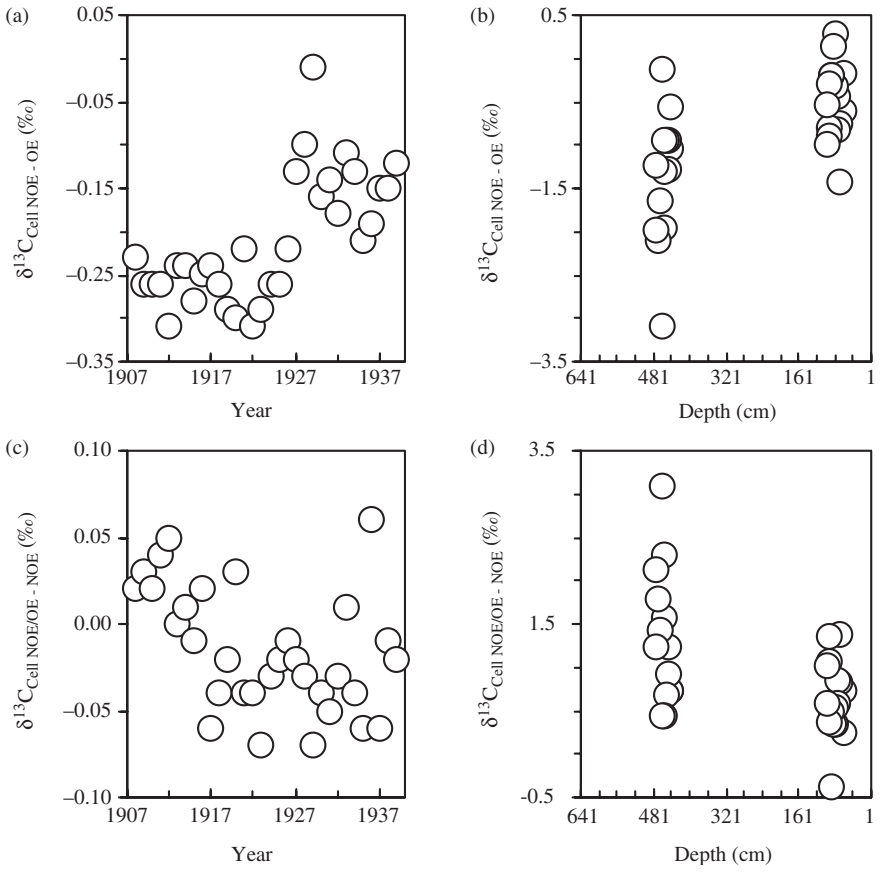

Figure 4. Time dependence of the $\delta^{13}C$ difference between $Cell_{NOE}$ and $Cell_{OE}$ ($\delta^{13}C_{Cell\ NOE\text{-}OE}$: a, b) or between $Cell_{NOE/OE}$ and $Cell_{NOE}$ ($\delta^{13}C_{Cell\ NOE/OE\text{-}NOE}$: c, d). a and c: tree rings. b and d: peat. All data for the upper and lower parts of the peat profile were pooled for the detection of this linear relation. See the text (Section 2.2) for the definitions of $Cell_{OE}$, $Cell_{NOE}$ and $Cell_{NOE/OE}$.

$n = 32$, $p < 0.001$; Figure 4b: $r^2 = 0.372$, $n = 30$, $p < 0.001$), i.e. the older the sample, the more negative the difference. The older tree rings or peat samples may contain higher content of lipids and have more negative $\delta^{13}C$ values with respect to the younger tree rings or peat [23,32], and therefore enhances the chances for lipids to survive delignification and alkaline hydrolysis. This deduction is confirmed by the positive correlation of the $\delta^{13}C$ difference between $Cell_{NOE/OE}$ and $Cell_{NOE}$ with age (Figure 4c: $r^2 = 0.192$, $n = 32$, $p < 0.02$; Figure 4d: $r^2 = 0.260$, $n = 30$, $p < 0.01$). This points to an equal importance of organic solvent extraction for carbon isotope analysis of tree ring and peat α-cellulose.

The linear relation in Figure 4c is still significant though most values for the $\delta^{13}C$ difference between tree ring $Cell_{NOE/OE}$ and $Cell_{NOE}$ are within the total analytical uncertainty. The data within the total analytical uncertainty does not definitively indicate their meaninglessness or meaningfulness. Hence, the significant correlation in Figure 4c may imply that a high-precision DI-IRMS technique is important for the detection of an influence of the incomplete removal of lipids by delignification and alkaline hydrolysis on carbon isotope ratio analysis of α-cellulose.

4.2 *Possible chemical evolution of extractives during delignification and alkaline hydrolysis*

Acidified sodium chlorite releases chlorine dioxide, which is a highly selective oxidising agent with a minimum degrading action on cellulose. Chlorine dioxide oxidises lignin by transferring oxygen to lignin to break up the aromatic rings while itself is reduced to chlorite ion and hypochlorous acid [34,35]. In addition to lignin, chlorine dioxide also attacks a wide variety of organic components, including carbohydrates, ethylenic double bonds, aromatic amino acids, pectic acids, keratins, pigments, phenols, unsaturated acids and so on [36,37]. The reaction of hypochlorous acid with organic chemicals leads to the formation of solubilised chlorinated organic compounds. Phenols and other lipids containing double bonds or carboxyl groups can be oxidised during this stage, but obviously not all lipid materials can be converted to soluble components (typically and mostly low-molecular-weight acids).

During alkaline hydrolysis, a portion of lipids can be saponified by sodium hydroxide. Saponification is the reaction of metallic alkali (such as sodium hydroxide) with some lipids (such as triglyceride). The products of saponification are hydrophilic substances, such as alcohols and carboxylates (soaps). Thus, acids and chlorinated organic matters produced in the delignification stage, and saponified lipids can be removed. However, composites, contents, physical and chemical properties of lipids depend on plant species, growth seasons, tissue types and environmental conditions. Both soluble and hardly soluble chemicals can be equally built during delignification. This will cause two kinds of outcomes: in some samples, lipids can be completely removed by delignification and alkaline hydrolysis, such as that reported in recent works [15,17] or tree ring 1929 in this study (Appendix 1); meanwhile in other samples, lipids cannot be completely removed, such as most samples in this work. The similar theory holds true for peat samples, but the $\delta^{13}C$ difference between $Cell_{NOE}$ and $Cell_{OE}$ is so large that one can postulate that delignification and alkaline hydrolysis have little effects on the removal of peat lipids.

Very close $\delta^{13}C$ values (Figure 4c) between tree ring $Cell_{NOE/OE}$ and $Cell_{NOE}$ indicate that, after delignification and alkaline hydrolysis, some chemical components could not be completely extracted with organic solvents. These components should be of lower $\delta^{13}C$ value with respect to α-cellulose, and different contents in different rings for these refractory components could be assumed. As a result, it is reasonable to conclude that some lipid components transformed by chloride dioxide are still present following delignification and alkaline hydrolysis, but they are not soluble in organic solvents any longer.

When organic solvent extraction as the final step was applied to peat samples, very large positive $\delta^{13}C$ differences (Figure 4d) between $Cell_{NOE/OE}$ and $Cell_{NOE}$ were observed (29 out of 30 samples) while the $\delta^{13}C$ differences between $Cell_{NOE/OE}$ and $Cell_{OE}$ for the same sample were greatly reduced compared to the $\delta^{13}C$ differences between $Cell_{NOE}$ and $Cell_{OE}$ (Figure 3). This implies that a relatively large portion of peat lipids was not transformed by chloride dioxide in terms of their solubility in organic solvents, and still can be removed after delignification and alkaline hydrolysis. Like tree ring samples, most of these lipids should be of lower $\delta^{13}C$ value. On the other hand, the $\delta^{13}C$ values for $Cell_{NOE/OE}$ tend to closely shift towards the $\delta^{13}C$ values for $Cell_{OE}$ in three different modes (Figure 3). One is that $Cell_{NOE/OE}$ and $Cell_{OE}$ for 11 samples are isotopically equivalent within the total analytical uncertainty (see samples with a depth of 63, 69, 71, 73, 91, 97, 99, 447, 449, 463 and 475 cm in Appendix 1). The other two modes are characterised by

depleted or enriched ^{13}C compared to $Cell_{OE}$ for 13 or 6 samples, respectively. Hence, the chemical and isotopic evolution of peat lipids during delignification and alkaline hydrolysis is far more complex than that of tree ring lipids. It appears that two types of lipids, with significantly different $\delta^{13}C$ values, exist in peat samples. Perhaps, these lipids were not inherited from the original plant residues, most probably originated from or transformed by microbial activities. Humic and fulvic acids in a salt marsh were found to be depleted in ^{13}C with respect to parent materials [38]. Microbially-associated carbohydrates (xylose, glucose and galactose) in peat have lower $\delta^{13}C$ values [39]. The alternations in carbon isotopic composition during early diagenesis of peat materials are hard to predict. For example, some amino acids during early diagenesis can become ^{13}C-depleted while others tend to be ^{13}C-enriched [40]. In terms of isotope mass balance, an occurrence of a mass with lower $\delta^{13}C$ values should be followed by an emergence of a new product with higher $\delta^{13}C$ values. These results may give some support to the existence of some lipids or extractives with higher $\delta^{13}C$ values after it has been reworked by delignification and alkaline hydrolysis.

4.3 *Biased inference of past environmental conditions without organic solvent extraction*

Since the main purpose of this paper is to test if the difference in carbon isotope composition exists between celluloses with and without organic solvent extraction, the internal consistency of carbon isotope series of $Cell_{OE}$, $Cell_{NOE}$ or $Cell_{NOE/OE}$, or the association of this consistency with environmental parameters is not discussed here. As far as environmental reconstruction is concerned, three important aspects for a time series of a proxy indicator should be considered: the mean value during a historic or geological period, the long-term trend and the fluctuation (seesaw) pattern [31]. At present, two methods (with or without organic solvent extraction) are simultaneously used for isolation of α-cellulose by different scientific communities. So, we would like to constrain our discussion on $Cell_{NOE}$ and $Cell_{OE}$.

As shown in Table 1, a mean $\delta^{13}C$ difference of 0.21‰ existed between tree ring $Cell_{NOE}$ and $Cell_{OE}$. If a transfer function is constructed separately between $\delta^{13}C_{Cell\,OE}$ or $\delta^{13}C_{Cell\,NOE}$ and environmental parameters, then the influence of this $\delta^{13}C$ difference on the inference of past environmental condition will be numerically obvious. If the used mass spectrometry technique (CF-IRMS or DI-IRMS) cannot detect this difference, then potential errors for the estimation of past environmental conditions are expected. If a qualitative reconstruction is needed, this difference still should be considered.

A long-term trend is present in tree ring $Cell_{NOE}$ series ($r^2 = 0.193$, $n = 32$, $p < 0.02$) while absent in $Cell_{OE}$ series ($r^2 = 0.09$, $n = 32$, $p < 0.20$). This will cause a potential error for the estimation of past environmental conditions.

The first differences for a $\delta^{13}C$ series (recent year minus last year) represent the interannual variations in carbon isotope ratio of tree ring cellulose, which should be influenced by changes in environmental conditions. In some years, this value is very minor (see the shadowed area in Figure 1) and can be detected only by a DI-IRMS technique. Although the $\delta^{13}C$ values for $Cell_{NOE}$ or $Cell_{OE}$ presented a similar variation pattern a pronounced disparity at some points was still observable in the shadowed area of Figure 1. These results seem to demonstrate that organic solvent extraction is necessary for tree ring α-cellulose purification if one wants to capture very weak environmental signals transduced in carbon isotope series.

A potentially biased inference on past environmental conditions will be more severe if a peat $Cell_{NOE}$ series is utilised. Firstly, the mean $\delta^{13}C$ difference between $Cell_{NOE}$ and $Cell_{OE}$ is large for both the upper and lower parts of the study profile (Table 2). Secondly, a linear relation of $\delta^{13}C_{Cell\,NOE}$ with depth is present for the lower part ($r^2 = 0.602$, $n = 14$, $p < 0.01$) but it is absent for $\delta^{13}C_{Cell\,OE}$ series ($r^2 = 0.218$, $n = 14$, $p < 0.10$) (Figure 3b). This, again, will lead to an incorrect tendency deduction of past environmental conditions. Lastly, for some points, the seesaw pattern was completely inverted (see the shadowed areas in Figure 3). If this is connected with environmental changes, then we will get two opposite interpretations. One is based on $Cell_{NOE}$, and the other one is based on $Cell_{OE}$. For example, in the shadowed area of Figure 3b, the ups and downs of $\delta^{13}C_{Cell\,NOE}$ will be regarded as a proxy for abrupt environmental changes. But the $\delta^{13}C_{Cell\,OE}$ displays a very minor fluctuation, which will be interpreted as a rather stable environmental condition. Therefore, organic solvent extraction is more essential to the purification of peat α-cellulose. The standard procedure with organic solvent extraction has been used for peat α-cellulose separation in early pioneering works [2,41,42], but not employed in recent works [19–22]. Perhaps, it would be worthwhile to make a further evaluation on what effects will be exerted on the paleoenvironmental interpretation of those data without organic solvent extraction. It is impossible to make a universal correction on the published $\delta^{13}C_{Cell\,NOE}$ data since the inter-sample variations of the difference between $\delta^{13}C_{Cell\,NOE}$ and $\delta^{13}C_{Cell\,OE}$ covered such a wide range from -3.08 to 0.27‰ that we have no idea on how to arrange a suitable correction value to each $\delta^{13}C_{Cell\,NOE}$. The best way is to make one by one $\delta^{13}C$ determinations on $Cell_{NOE}$ and $Cell_{OE}$ series, and then to ascertain if their mean values, long-term trends and seesaw patterns are significantly different from each other.

5. Conclusions

It is clear from our dataset that a long time series and a high-precision DI-IRMS technique are needed for the detection of an influence of organic solvent extraction on the carbon isotope analysis of α-cellulose. Based on a test on several samples, it is difficult to find a long-term trend in $\delta^{13}C$ series induced by increased lipid residues that are not completely removed by delignification and alkaline hydrolysis. This has been demonstrated in our tree ring and peat $Cell_{NOE}$ series.

Delignification and alkaline hydrolysis can rarely remove all the lipids in a sample. There exists just only one sample in our 62 tested samples in which its $Cell_{NOE}$ and $Cell_{OE}$ were found to be of the same $\delta^{13}C$ value, e.g. tree ring 1929. In contrast, the use of a procedure without organic solvent extraction will produce a $Cell_{NOE}$ time series whose mean value, long-term trend and seesaw patterns are significantly different from those of a $Cell_{OE}$ time series with organic solvent extraction. This misleading time series may lead to a biased inference of past environmental conditions.

The $\delta^{13}C$ series of $Cell_{NOE/OE}$ revealed a complex chemical evolution behaviour of lipids during delignification and alkaline hydrolysis. Organic solvent extraction as the final step did not form a remedy for $Cell_{NOE}$ since the $\delta^{13}C$ values for $Cell_{NOE/OE}$ could be both more positive and more negative than those for $Cell_{OE}$. Based on these observations, we suggest that the traditional standard procedure using organic solvent extraction as the first step be used for the purification and separation of α-cellulose preserved in tree ring

and peat archives in order to obtain a consistent postulation of past environmental conditions.

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References

[1] J.W. Green, in *Methods in Carbohydrate Chemistry*, edited by R.L Whistler (Academic Press, New York, 1963), Vol. III, pp. 9–21.
[2] C.A.M. Brenninkmeijer, B. van Geel, and W.G. Mook, Earth Planet. Sci. Lett. **61**, 283 (1982).
[3] T.W.D. Edwards, W. Graf, P. Trimborn, W. Stichler, and H.D. Payer, Geochim. Cosmochim. Acta **64**, 161 (2000).
[4] S.W. Leavitt, Chem. Geol. **192**, 47 (2002).
[5] M. Saurer, F.H. Schweingruber, E.A. Vaganov, S.G. Shiyatov, and R. Siegwolf, Geoph. Res. Lett. **29**, 10 (2002).
[6] S.J. Birks, T.W.D. Edwards, and V.H. Remenda, Palaeogeogr. Palaeoclimatol. Palaeoecol. **246**, 8 (2007).
[7] B.B. Wolfe, T.W.D. Edwards, R.J. Elgood, and K.R.M. Beuning, in *Tracking Environmental Change Using Lake Sediments: Physical and Chemical Techniques*, edited by W.M. Last and J.P. Smol (Kluwer Academic Publishers, Dordrecht, 2001), Vol. 2, pp. 1–28.
[8] S.W. Leavitt and S.R. Danzer, Anal. Chem. **65**, 87 (1993).
[9] D.D. Sheu and C.H. Chiu, Int. J. Environ. Anal. Chem. **59**, 59 (1995).
[10] N.J. Loader, I. Robertson, A.C. Barker, V.R. Switsur, and J.S. Waterhouse, Chem. Geol. **136**, 313 (1997).
[11] C. Macfarlane, C.R. Warren, D.A. White, and M.A. Adams, Tree Physiol. **19**, 831 (1999).
[12] O. Brendel, P.P.M. Iannetta, and D. Stewart, Phytochem. Analysis. **11**, 7 (2000).
[13] P.K. Van de Water, Geochim. Cosmochim. Acta **66**, 1211 (2002).
[14] J.B. Gaudinski, T.E. Dawson, S. Quideau, E.A.G. Schuur, R.S. Roden, S.E. Trumbore, D.R. Sandquist, S.W. Oh, and R.E. Wasylishen, Anal. Chem. **77**, 7212 (2005).
[15] K.T. Rinne, T. Boettger, N.J. Loader, I. Robertson, V.R. Switsur, and J.S. Waterhouse, Chem. Geol. **222**, 75 (2005).
[16] M. Haupt and T. Boettger, Anal. Chem. **78**, 7248 (2006).
[17] T. Boettger, M. Haupt, K. Knöller, S.M. Weise, J.S. Waterhouse, K.T. Rinne, N.J. Loader, E. Sonninen, H. Jungner, V. Masson-Delmotte, M. Stievenard, M.T. Guillemin, M. Pierre, A. Pazdur, M. Leuenberger, M. Filot, M. Saurer, C.E. Reynolds, G. Helle, and G.H. Schleser, Anal. Chem. **79**, 4603 (2007).
[18] K.J. Anchukaitis, M.N. Evans, T. Lange, D.R. Smith, S.W. Leavitt, and D.P. Schrag, Anal. Chem. **80**, 2035 (2008).
[19] Y.T. Hong, B. Hong, Q.H. Lin, Y.X. Zhu, Y. Shibata, M. Hirota, M. Uchida, X.T. Leng, H.B. Jiang, H. Xu, H. Wang, and L. Yi, Earth Planet. Sci. Lett. **211**, 371 (2003).

[20] Y.T. Hong, B. Hong, Q.H. Lin, Y. Shibata, M. Hirota, Y.X. Zhu, X.T. Leng, Y. Wang, H. Wang, and L. Yi, Earth Planet. Sci. Lett. **231**, 337 (2005).
[21] H. Xu, Y.T. Hong, Q.H. Lin, Y.X. Zhu, B. Hong, and H.B. Jiang, Palaeogeogr. Palaeoclimatol. Palaeoecol. **230**, 155 (2006).
[22] Y.T. Hong, B. Hong, Q.H. Lin, Y. Shibata, Y.X. Zhu, X.T. Leng, and Y. Wang, Quarternary Sci. Rev. **28**, 840 (2009).
[23] K. Lehtonen and M. Ketola, Org. Geochem. **20**, 363 (1993).
[24] X.J. Sun and S.M. Yuan, in *Loess, Quaternary Geology and Global Changes*, edited by D.S. Liu and Z.S. An (Science Press, Beijing, 1990), Vol. 2, pp. 46–57.
[25] X.T. Leng (private communication), Department of Geography, Northeast Normal University, Changchun, Jilin 130024, China.
[26] R. Benner, M.L. Fogel, E.K. Sprague, and E. Hodson, Nature **329**, 708 (1987).
[27] S. Borella, M. Leuenberger, M. Sauer, and R. Siegwolf, J. Geophys. Res. **103** (D16), 19519 (1998).
[28] S. Borella, G. Ménot, and M. Leuenberger, in *Handbook of Stable Isotope Analytical Techniques*, edited by P.A. Groot (Elsevier, Amsterdam, 2004), pp. 507–522.
[29] F.X. Tao, A.M. Aucour, S.M.F. Sheppard, C.Q. Liu, X.T. Leng, S.L. Wang, G.S. Liu, and W.B. Xu, Chinese J. Chem. **19**, 1089 (2001).
[30] K. Rozanski, W. Stichler, R. Gonfiantini, E.M. Scott, R.P. Beukens, B. Kromer, and J. Plicht, Radiocarbon **34**, 506 (1992).
[31] J.D. Cryer and K.S. Chan, *Time Series Analysis with Applications in R*, 2nd ed. (Springer Science + Business Media, LLC, 2008).
[32] R.M. Rowell, R. Pettersen, J.S. Han, J.S. Rowell, and M.A. Tshabalala, in *Handbook of Wood Chemistry and Wood Composites*, edited by R.M. Rowell (CRC Press, Boca Raton, 2005), pp. 35–74.
[33] E. Melzer and H.L. Schmidt, J. Biol. Chem. **262**, 8159 (1987).
[34] C. Brage, T. Eriksson, and J. Gierer, Holzforschung **45**, 147 (1991).
[35] D.R. Svenson, H.M. Chang, H. Jameel, and J.F. Kadla, Holzforschung **59**, 110 (2005).
[36] G. Gorden, G. Kieffer, and D. Rosenblatt, in *Progress in Inorganic Chemistry*, edited by S. Lippard (Wiley Interscience, New York, 1972), Vol. 15, pp. 201–286.
[37] W.J. Masschelein, *Chlorine Dioxide* (Ann Arbor Science Publishers, Ann Arbor, Mich., 1979).
[38] J.J. Alberts, Z. Filip, M.T. Price, D.J. Williams, and M.C. Williams, Org. Geochem. **12**, 455 (1988).
[39] S.A. Macko, M.H. Engel, G. Hartley, P. Hatcher, R. Helleur, P. Jackman, and J.A. Silfer, Chem. Geol. **93**, 147 (1991).
[40] M. Fogel and N. Tuross, Oecologia **120**, 336 (1999).
[41] L.M. Dupont and C.A.M. Brenninkmeijer, Rev. Paleobot. Palynol. **41**, 241 (1984).
[42] A.M. Aucour, C. Hillaire-Marcel, and R. Bonnefille, Chem. Geol. **129**, 341 (1996).

Appendix. $\delta^{13}C$ values of α-cellulose with different chemical treatments.

Tree rings				Peat			
Year	NOE	NOE/OE	OE	Depth (cm)	NOE	NOE/OE	OE
1908	−24.27	−24.25	−24.04	61	−25.34	−24.62	−24.74
1909	−24.89	−24.86	−24.63	63	−25.12	−24.83	−24.96
1910	−24.38	−24.36	−24.12		−25.15	−24.93	
1911	−24.32	−24.28	−24.06	69	−25.73	−24.37	−24.29
1912	−24.49	−24.44	−24.18		−25.70	−24.33	
1913	−24.85	−24.85	−24.61	71	−25.01	−24.18	−24.27
1914	−24.71	−24.70	−24.47	73	−25.33	−24.49	−24.50
1915	−24.41	−24.42	−24.13	75	−25.56	−24.96	−25.13
1916	−24.81	−24.79	−24.56	79	−26.44	−26.09	−26.71
1917	−24.21	−24.27	−23.97		−26.43	−26.10	
1918	−24.32	−24.36	−24.06	81	−25.84	−25.31	−25.53
1919	−24.40	−24.42	−24.11	85	−25.95	−25.59	−26.08
1920	−24.12	−24.09	−23.82	87	−25.85	−25.37	−25.67
1921	−24.01	−24.05	−23.79	89	−25.75	−26.13	−25.56
1922	−23.47	−23.51	−23.16	91	−26.50	−26.14	−26.21
1923	−23.38	−23.45	−23.09	93	−25.84	−24.76	−25.03
1924	−23.72	−23.75	−23.46	95	−25.56	−24.22	−24.66
1925	−24.11	−24.13	−23.85	97	−25.12	−24.53	−24.59
1926	−24.34	−24.35	−24.12	99	−25.40	−24.38	−24.42
1927	−24.19	−24.21	−24.06	443	−24.82	−24.10	−23.80
1928*	−24.17	−24.22	−24.05		−24.84	−24.08	
	−24.19	−24.22	−24.12	445	−24.44	−23.72	−23.87
1929	−24.15	−24.22	−24.13	447	−25.48	−24.55	−24.53
	−24.09	−24.16	−24.09	449	−25.01	−23.78	−23.74
1930	−24.29	−24.31	−24.15	451	−24.59	−23.90	−23.63
	−24.21	−24.26	−24.03	455	−25.94	−23.60	−23.95
1931	−24.22	−24.28	−24.07		−25.88	−23.63	
	−24.21	−24.26	−24.08	457	−25.27	−23.70	−23.95
1932	−24.26	−24.31	−24.10	459	−24.81	−24.36	−23.86
	−24.30	−24.30	−24.10	461	−25.68	−25.21	−25.54
1933	−24.43	−24.46	−24.33		−25.65	−25.24	
	−24.50	−24.46	−24.38	463	−26.84	−23.74	−23.76
1934	−24.50	−24.52	−24.32	467	−25.46	−24.03	−23.81
	−24.41	−24.48	−24.34	471	−26.49	−24.69	−24.39
1935	−24.25	−24.31	−24.04	473	−26.29	−24.15	−24.31
1936	−23.96	−23.90	−23.77	475	−26.70	−25.46	−25.46
1937	−23.79	−23.85	−23.64				
1938	−24.03	−24.04	−23.88				
1939	−23.94	−23.96	−23.82				

Notes: NOE: without organic solvent extraction. NOE/OE: with organic solvent extraction as the final step. OE: with organic solvent extraction as the first step.
*These samples were replicately analysed.