Chemical Geology 504 (2019) 53–65

Contents lists available at ScienceDirect

Chemical Geology

journal homepage: www.elsevier.com/locate/chemgeo

A high yield cellulose extraction system for small whole wood samples and dual measurement of carbon and oxygen stable isotopes

Laia Andreu-Hayles[a,*], Mathieu Levesque[a,b], Dario Martin-Benito[a,c], Wei Huang[a], Ryan Harris[a], Rose Oelkers[a], Caroline Leland[a], Javier Martin-Fernández[a], Kevin J. Anchukaitis[a,d], Gerhard Helle[e]

[a] Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY 10964, USA
[b] Forest Management Group, Institute of Terrestrial Ecosystems, Department of Environmental Systems Science, ETH Zurich, 8092 Zurich, Switzerland
[c] Forest Research Centre INIA-CIFOR Ctra. La Coruña km. 7.5, 28040 Madrid, Spain
[d] School of Geography and Development and Laboratory of Tree Ring Research, University of Arizona, Tucson, AZ, USA
[e] Climate Dynamics and Landscape Evolution, German Centre for Geosciences, GFZ, Potsdam, Germany

ARTICLE INFO

Editor: K Johannesson

Keywords:
Tree ring
α-Cellulose
Carbon stable isotopes ($\delta^{13}C$)
Oxygen stable isotopes ($\delta^{18}O$)
Continuous flow
High-temperature pyrolysis (HTP)
Isotope ratio mass spectrometry (IRMS)

ABSTRACT

This paper describes devices to extract α-cellulose from small whole wood samples developed at the Lamont-Doherty Earth Observatory Tree-Ring Lab and explains the procedures for chemical extractions and for the dual analysis of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotopes. Here, we provide the necessary steps and guidelines for constructing a cellulose extraction system for small amounts of wood and leaves. The system allows the simultaneous extraction of cellulose from 150 samples by means of in-house filter tubes, where chemicals used for the cellulose extraction are exchanged and eliminated in batches. This new implementation diminishes the processing time, minimizes physical sample manipulation and potential errors, increases sample throughput, and reduces the amount of chemicals and analytic costs. We also describe the dual measurement of $\delta^{13}C$ and $\delta^{18}O$ ratios in tree-ring cellulose using high-temperature pyrolysis in a High Temperature Conversion Elemental Analyzer (TC/EA) interfaced with a Thermo Delta V plus mass spectrometer.

1. Introduction

Measurements of the stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopic composition of the wood of annual tree rings can provide information about the physiological response of trees to environmental conditions and past climate (McCarroll and Loader, 2004). The use of these proxies, alone or in combination with other tree-ring parameters, such as ring widths, maximum latewood density or wood anatomical traits, has advanced our understanding of ecological and climatic systems and their variability in different regions worldwide (Gagen et al., 2011; Andreu-Hayles and Leland, 2014). Due to recent technical advancements for measuring $\delta^{13}C$ and $\delta^{18}O$ in wood material, the analysis of tree-ring stable isotopes is now widely used in ecological, physiological and paleoclimatic studies (Saurer et al., 2004; Treydte et al., 2006; Andreu et al., 2008; Kress et al., 2009; Frank et al., 2015; Andreu-Hayles et al., 2017; Martin-Benito et al., 2017).

Several methodologies have been proposed over the last decades for isolation of cellulose from wood for isotopic analysis (c.f., Green, 1963; Leavitt and Danzer, 1993; Brendel et al., 2000; Schollaen et al., 2017 and citations therein). The objectives of this paper are (i) to provide a detailed description for building a device for the batch extraction of cellulose from small amounts of wood; (ii) to outline a step-by-step comprehensive laboratory protocol for the extraction of α-cellulose from whole wood; and (iii) to describe in detail the dual measurement of $\delta^{13}C$ and $\delta^{18}O$ in cellulose. All of these methods are currently being used at the Lamont-Doherty Earth Observatory, and we provide thorough details of our approach so that other laboratories can easily implement similar facilities and adopt adjusted protocols required for these analyses. Here, we build upon previous approaches (e.g., Wieloch et al., 2011) and incorporate innovative tools to improve laboratory efficiency, allowing for a high sample output, and thus a reduction of laboratory time, materials and costs.

1.1. General methodological review

Stable isotopes in tree rings can be analyzed using whole wood or individual components such as lignin or cellulose. In many cases, α-cellulose has been preferred for stable isotopic analysis, even though it

* Corresponding author at: Tree-Ring Laboratory, Lamont-Doherty Earth Observatory of Columbia University, 61 Route 9W, Palisades, NY 10964, USA.
E-mail address: lah@ldeo.columbia.edu (L. Andreu-Hayles).

https://doi.org/10.1016/j.chemgeo.2018.09.007
Received 15 November 2017; Received in revised form 1 September 2018; Accepted 5 September 2018
Available online 22 September 2018

requires additional laboratory work to isolate the compound, because (i) it is free from mobile compounds such as resin or wax, and thus, in principle, directly linked to the year of formation; (ii) whole-wood can be influenced by variable cellulose-to-lignin ratios due to natural variation within and between trees, as well as differing rates of degradation; and (iii) cellulose can easily be homogenized (Loader et al., 2003; McCarroll and Loader, 2004). The α-cellulose is highly stable and distinct from hemicellulose consisting of non-cellulosic polysaccharides (Green, 1963).

The isolation of α-cellulose requires several sequential chemical acidic and alkaline washes to remove other wood compounds such as hemicellulose, resins or lignin. Prior to α-cellulose extraction, some protocols include the use of solvents, such as a toluene and ethanol, for removing lipids and resins in samples from tree species with high extractive content. However, some studies suggest that such pretreatments are unnecessary (Boettger et al., 2007; Anchukaitis et al., 2008), and that sodium chlorite and sodium hydroxide used during the α-cellulose isolation procedure can effectively remove resins in the process (Rinne et al., 2005).

There are two primary components of the α-cellulose isolation procedure commonly applied using traditional methods in the so-called Jayme-Wise method (see Green (1963) and Loader et al. (1997)). First, lignin is removed using iterative washes of acidified sodium chlorite ($NaClO_2$) to obtain holocellulose. Second, the soluble portion of holocellulose (hemicelluloses and β-cellulose) is removed with sodium hydroxide (NaOH) to leave the insoluble α-cellulose. An alternative approach uses an acetic acid:nitric solution to simultaneously remove lignin and hemicellulose from wood samples (Brendel et al., 2000). Benefits of this 'Brendel method' and its variants (Evans and Schrag, 2004; Anchukaitis et al., 2008; Brookman and Whittaker, 2012), include rapid sample processing time, the ability to process a larger number of samples per time, and a need for potentially less initial wood material. Studies suggest that the traditional and Brendel methods can yield similar results in terms of stable oxygen (Evans and Schrag, 2004; Anchukaitis et al., 2008) and carbon isotopes (Brendel et al., 2000), although significantly different radiocarbon results were reported by Anchukaitis et al. (2008). Moreover, issues regarding C and N additions to the sample and residuals of lipids and waxes have been reported depending on the variant of the Brendel method used (although Anchukaitis et al., 2008 did not find any nitrogen additions), pretreatments and type of tissue analyzed (Gaudinski et al., 2005), as well as significant differences in $\delta^{13}C$ and $\delta^{18}O$ (English et al., 2011). Finally, chemical volume, temperature and reaction time, which should be adjusted to the distinct type of wood from different tree species, were also found to influence the final results (Brookman and Whittaker, 2012). A specific experiment comparing the Jayme-Wise and the Brendel method can be found in Cullen and MacFarlane (2005).

There are multiple procedures in which wood samples are treated using the traditional methodology. One traditional approach is to place labeled filter bags containing individual wood samples in a Soxhlet apparatus or beaker filled with chemicals (Leavitt and Danzer, 1993). These bags are generally made of Polytetrafluoroethylene (PTFE) and although in some aspects can be very convenient (e.g., reduction of the amount of glassware, easy washing and change of chemical solutions by putting the samples from one beaker into another), this method is tedious and there is potential for sample loss (Macfarlane et al., 1999). For instance, the bags are difficult to clean from sticking cellulose remains and need to be disposed after few uses or even just a single use (Wieloch et al., 2011) because labels are etched into the bag and welding seals them. Moreover, they cost more than 1USD each and individually labeling the bags is time consuming. Finally, the bags are prone to graining, which may lead to some contamination of the white cellulose samples when taking them out of the bags: this might not greatly affect $\delta^{13}C$ values, but may influence ^{14}C results (personal comm. K. Treydte & G. Helle). A novel method uses PTFE cases to extract α-cellulose directly from wood laths, which are processed using the same chemical and laboratory procedures, but the remaining α-cellulose is cut along annual ring boundaries after chemical extraction (Kagawa et al., 2015). However, this method is difficult to implement on samples with narrow rings or challenging tree-ring patterns and requires additional tools/equipment such as a cutting room with a microtome. A guideline for extracting α-cellulose directly from wood laths using a new extraction device has recently been published by Schollaen et al. (2017).

Another common approach, which is discussed at length here, is to place wood samples in Büchner funnels where the reagents can be added for a specified period of time, and then to remove the reagents using a vacuum pump (e.g., Loader et al., 1997; Wieloch et al., 2011). In all approaches, hot plates or temperature-controlled water baths are used to heat reagents and samples to necessary reaction temperatures. After chemical extraction, α-cellulose can be further homogenized (Laumer et al., 2009), freeze dried, and then encapsulated for mass spectrometry. For $\delta^{13}C$, tin capsules are used and processed via Elemental Analysis (EA) coupled to an isotope ratio mass spectrometer (IRMS). Cellulose samples are encapsulated in silver capsules for measuring $\delta^{18}O$ using high-temperature pyrolysis in a High Temperature Conversion Elemental Analyzer (TC/EA) coupled to the same IRMS. Recently, some laboratories have successfully measured $\delta^{13}C$ and $\delta^{18}O$ simultaneously using the high-temperature pyrolysis (HTP) approach (e.g., Gehre and Strauch, 2003; Knoller et al., 2005) and even including the simultaneous determination of hydrogen isotopes (Loader et al., 2015). Below, we detail the specific protocols implemented in the Terrestrial Ecology Laboratory at the Lamont-Doherty Earth Observatory (LDEO), including sample preparation, building the system for batch extraction of α-cellulose from wood samples, and the final stable isotope analyses.

2. Methods for cellulose extraction

2.1. Tree-ring sample preparation

Identifying suitable trees and having adequate wood samples for isotopic analysis is critical for determining environmental and climatic responses within a site. Tree core sampling is done in the field using a 5 or 12 mm increment borer from living trees, or occasionally tree disks or cross-sections are cut from dead or subfossil trees. Although sufficient material can usually be obtained from standard 5 mm increment cores, 12 mm cores are sometimes used to ensure enough wood mass for each individual ring when rings are very narrow. When using existing tree-core collections, additional steps are necessary to process the samples. In this case, cores must be first removed from mounts in a heated water bath and glue residue must be removed with fine grit sand papers or by cutting the core surface with a microtome prior to slicing/splitting each ring individually. No pencil marks, glue, chalk or wood dust should remain on the wood samples to avoid potential contamination. Compressed air can be used to remove wood dust. While tests indicated that pencil marks have no significant effect on the $\delta^{13}C$ of tree-ring cellulose, chalk (usually made of gypsum, $CaSO_4 \times H_2O$) can affect the $\delta^{18}O$ of tree-ring cellulose (Schollaen et al., 2017). At LDEO, tree rings are cut along the ring boundary using a surgical blade and placed into labeled 1.5-ml transparent microcentrifuge tubes (e.g. Eppendorf Tubes®) for transport and storage. We recommend processing individual wood samples of 0.5–5 mg in order to ensure enough cellulose for the isotopic analyses. The amounts vary depending on the

species and amount of wood material available for each ring; in practice as little as 0.3 mg and as much as 7 mg have been successfully processed using the protocol described here. After cutting an individual ring, it is necessary to shave the wood material into small pieces of wood. This shaving or chopping procedure is done manually with a surgical blade leading to wood slivers of about < 0.5 mm. The use of small pieces of wood slivers ensures that the chemicals used during the extraction are effective, while facilitating cellulose collection from the filters and minimizing the time needed for homogenization with ultrasound.

2.2. Devices

A polytetrafluoroethylene (PTFE-Teflon) device was especially designed for cellulose extraction, and to improve upon the prototype presented in Wieloch et al. (2011) that proposed using commercially available Büchner funnels. From our experience, the fritted glass disc used as a filter in these commercial funnels degraded after only few cellulose extraction and cleaning cycles. A novel design for custom-made funnels was developed and found to be a cost-effective and durable replacement for previously used Büchner funnels.

2.2.1. Custom-made funnels

Our custom-made funnels are assembled from multiple pieces of tubing (Fig. 1) with materials chosen based on resistance to chemicals and high temperatures, as well as availability and affordability. The filter disc composed of borosilicate glass frit (Fig. 1C) is 10 mm in diameter and 3.5 mm thick with a porosity of 20–50 µm (Ace Glass Incorporated, Vineland, NJ). The filter disc is secured within three

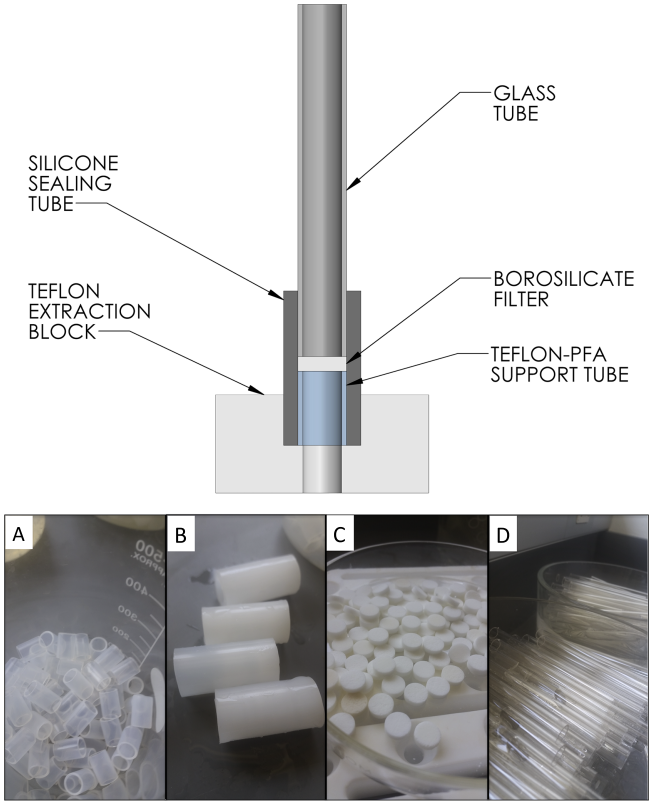

Fig. 1. Above: Funnel-Filter assembly cross-section. Below: Funnel assembly: First, the small Teflon-PFA support tube (**A**) is inserted in the silicone sealing tube (**B**). Second, these two pieces are inserted directly into the sample ports vertically located throughout the PTFE extraction block (Fig. 2). Third, the borosilicate filter (**C**) is located on top of the small PFA tube (**A**) within the silicon sealing tube (**B**). Finally, the glass tubing (**D**) is pushed into the silicon tube (**B**) and located on top of the borosilicate filter (**C**) to complete one funnel.

pieces of tubing of different materials, each providing a distinct function. A perfluoroalkoxy (Teflon-PFA) tube (10 mm outside diameter (OD), 8 mm inside diameter (ID), and 15 mm long) sits underneath the filter, providing support (Fig. 1A). A silicone rubber tube (15.9 mm OD, 9.5 mm ID, and 30.5 mm long) fits snugly within a port in our cellulose extraction device and around the narrower tubes and filter in order to seal the reaction area (Fig. 1B). A glass tube (10 mm OD, 8 mm ID, and 120 mm long) sits above the filter and is used for holding the chemicals during the treatment (Fig. 1D).

2.2.2. PTFE device

The device consists of a PTFE-Teflon block (Fig. 2A). It is 285 mm long, 180 mm wide, and 25 mm tall (Fig. 2B), following the design proposed in Wieloch et al. (2011). PTFE was chosen for its resistance to chemicals and high temperatures. The funnel-filter assembly (Fig. 1) is installed into ports on the cellulose extraction device (Fig. 2) as shown in Fig. 3A. These ports are 15.5 mm in diameter and 10.4 mm deep (Fig. 2C). Beneath the ports there are interconnecting channels with 8 mm in diameter that enable the flow of the chemicals (Fig. 2C).

The PTFE-Teflon extraction device has 50 sample ports evenly spaced across the top of the block (Fig. 2B). Slots were added to the block to ensure an even temperature distribution between sample ports. Each of the long channels that runs through the block were drilled using long drill bits through one side of the PTFE block, leaving a hole at the end of each channel. To seal this hole and to prevent chemicals from flowing directly into the water bath, we tapped a 1/8-inch National Pipe Taper (NPT) thread in each hole and then inserted a 1/8-inch NPT sealing plug into each hole. This plug is made of 316 stainless steel, which is a chemical/corrosion resistant metal with a reasonable cost. Although significantly more expensive, alternatively PTFE plugs can be used to further avoid corrosion.

A vacuum pump (WP6111560, Millipore Chemical duty pump, 115 V/60 Hz) is connected to the outlet in the block (Fig. 3E) to drain the chemicals out of the device through the 8 mm diameter channels located throughout the base of the block underneath each sample port.

Our current device was developed in 2016 to overcome a trapped air problem of our first PTFE device developed in 2013 (Suppl. Fig. 1A). This initial prototype was built with a PTFE-Teflon block of 305 mm long, 135 mm wide, and 50 mm tall. On the top of this block, 36 sample ports were located and evenly spaced to ease funnel installation and filling during the protocol (Suppl. Fig. 1B). Aluminum handles were used for lifting and moving the block. Two of these extraction devices could be fit into the water bath (approximate inner dimensions 635 mm length and 300 mm width), permitting 72 samples to be processed in each extraction run. This initial prototype was flawed in that the long vertical channels (Suppl. Fig. 1C) underneath the funnels trapped air while chemicals were added. The funnel-filter assemblies prevented air from venting through them. As the first funnel was filled with chemicals, the interconnecting channels in the base of the block filled, trapping air underneath each sample port. When the water bath was heated, these pockets of air expanded, forcing chemicals back up through some funnels, occasionally leading to funnel overflow and sample loss.

Our current PTFE device (Fig. 2) is thinner than the initial prototype (Suppl. Fig. 1) in order to reduce the length of the vertical channels (Fig. 2C vs. Suppl. Fig. 1C) underneath each sample port, reducing available volume for trapping air. The interconnection between channels is also reduced; thus each row of channels is connected by a single cross-channel (Fig. 2B) rather than by two (Suppl. Fig. 1B). By filling funnels sequentially from furthest from the outlet to nearest from the outlet, air is forced out toward the outlet tube. These changes have successfully corrected the previous funnel overflow due to trapped air. The thinner PTFE block is also more cost effective than the previous prototype. Moreover, the lighter weight of this block allows for an easier manipulation than the first 36 port block, permitting the use of PTFE handles instead of aluminum handles.

A perforated PTFE plate (2 mm thick) can be also attached near the

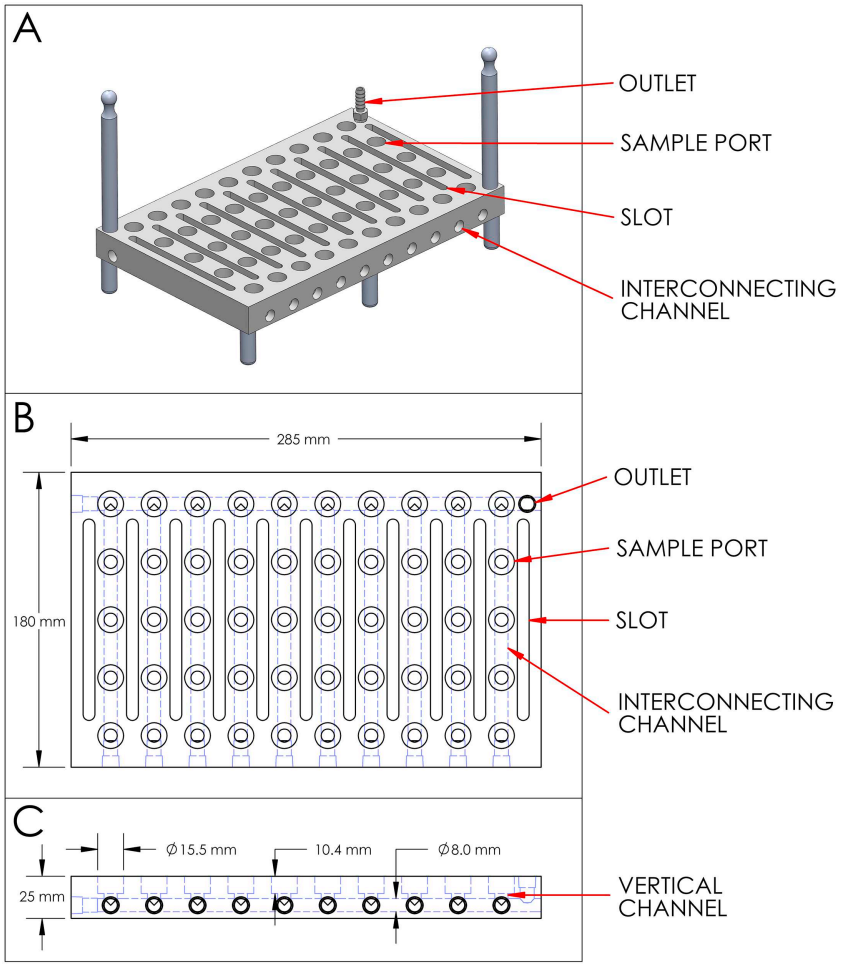

Fig. 2. (**A**) Cellulose extraction polytetrafluoroethylene (PTFE) device with 50 sample ports. Note the holes in one side of the device leading to the interconnecting channels. (**B**) Top view of the cellulose extraction device. Interconnecting channels within the device are indicated by blue dashed lines. The single cross-channel forces air toward the outlet. (**C**) Side view of the cellulose extraction device. Sample ports, vertical channels, and interconnecting channels are indicated by blue dashed lines. The minimal height of vertical channels reduces available volume for trapped air. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

top of the handles to fix the position of the reaction funnels and ease filling of the funnels with the chemical solutions (Suppl. Fig. 2). This additional component has not been implemented at LDEO because the funnels are well fixed through the silicone sealing tubes.

2.2.3. Bath

We used a Grant SubAqua 34Plus digital bath (outer dimensions: 750 mm length, 340 mm width and 270 mm height) as a containment and heat source for our cellulose extraction devices (Fig. 3C). Based on the trials with the initial prototype (Suppl. Fig. 1), it was determined that limiting the number of sample ports to 50 in a single block would be the most effective for managing the extraction protocol. Three of these extraction devices (Fig. 2, Fig. 3B) could be fitted into the working area of the bath (approximate inner dimensions: 635 × 300 mm), permitting 150 samples (3 devices with 50 funnels each) to be processed in each extraction (Fig. 3C) in comparison to 72 samples that could be run with the initial prototype. The versatility of this extraction system has the potential to be used for other chemical analyses with distinct substances and procedures.

2.3. Chemical preparation

We follow the Jayme-Wise chemical procedure of Kürschner and Popik (1962) and Green (1963), modified by Loader et al. (1997) and based on the protocol developed at Swansea University (Neil Loader, personal communication, 2013) for the isolation of α-cellulose (Table 1). For this protocol, it is normally not necessary to use a Soxhlet apparatus with organic solvents for removing resins and similar compounds prior to extraction (Rinne et al., 2005). However, a pretreatment step to remove extractives with organic solvents has been suggested for very resinous species (Lin et al., 2017).

The first and fourth steps are chlorination processes in which the lignin is oxidized by means of bleaching the wood with a chlorine dioxide solution (ClO_2). This is done through the acidification of sodium chlorite ($NaClO_2$) with acetic acid (CH_3CO_2H), effectively setting the pH to 4.5–5. The second and third steps are alkaline reactions to isolate α-cellulose, in which a sodium hydroxide (NaOH) solution hydrolyzes hemicelluloses, such as mannan and xylan, which are later leached to yield α-cellulose (Loader et al., 1997; Boettger et al., 2007). Finally, at the end of the second chlorination a thorough rinse of the cellulose with ultrapure water needs to be done to ensure the removal of all remaining chemicals. For the preparation of the chemical solutions and any water in contact with the inner parts of the funnels, we use ultrapure deionized water. This water has been treated through a process that includes deionization, filtration and a UV system in order to burn up particles and organics (Barnstead Nanopure Diamond ultrapure water system). For cleaning the glassware and the whole extraction system, we use reverse osmosis deionized water (RODI) from our lab faucet. The RODI water is reagent grade water, nominally 18 MΩ in purity, in which calcium is removed first for softening, and then purified through a reverse osmosis filter and deionized.

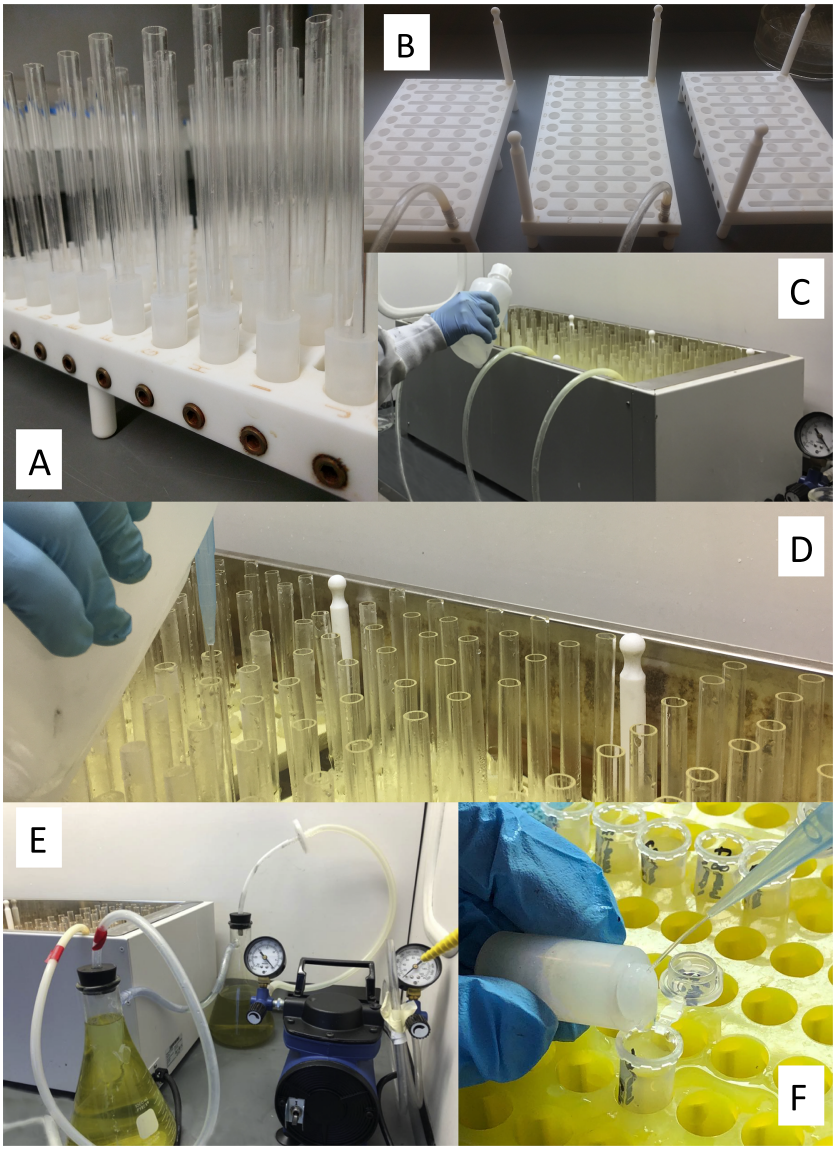

Fig. 3. Pictures of: (**A**) the funnels, drawn in Fig. 1, assembled in the PTFE device; (**B**) the three cellulose extraction devices; (**C**) the Grant SubAqua 34Plus digital bath with the three extraction devices inside; (**D**) zoom of the funnels assembled in the PTFE device located inside the bath; (**E**) Erlenmeyer flask- connected to the vacuum pump and to a PTFE-device; (**F**) 1-ml of ultrapure water poured over the extracted cellulose exposed on the top of the filter.

Table 1
Protocol for the extraction of α-cellulose from wood samples used at the Lamont-Doherty Earth Observatory based upon Kürschner and Popik (1962), Green (1963) and Loader et al. (1997). Rinsing with ultrapure water is required between each step and with boiling ultrapure water for the rinse between step 1 and 2. Step 1 and 2 are performed during the first day, while step 3 and 4 are performed on the second day.

	Step 1: first chlorination	Step 2: first alkaline treatment	Step 3: second alkaline treatment	Step 4: second chlorination
Chemical solution (concentration)	Acidified (pH 4–5) $NaClO_2$	10% NaOH	17% NaOH	Acidified (pH 4–5) $NaClO_2$
Water temperature in the bath	80 °C	70 °C	Room temperature	80 °C
Duration per addition	60 min	45 min	45 min	60 min
Number of repetitions	6	1	1	2
Number of rinses of ultrapure water at Room Temperature (RT) or Boiling Temperature (BT)	After the 6th cycle: 1BT + 4 RT	3 RT	3 RT	After the last cycle: at least 5 RT

2.4. Step-by-step laboratory procedure of cellulose extraction

First, individual parts of the funnels are thoroughly rinsed with RODI water to eliminate potential unwanted materials such as dust that could be deposited during storage and to facilitate the smooth assembly of the system. Second, the assembled funnels are fixed to the extraction device. The funnel extraction system is designed to hold the sample material in a sequential order as outlined in the sketch of the location of the funnels in the devices (Suppl. Fig. 3). Third, samples are recorded in chronological order as they are placed individually into each funnel.

While the funnels are assembled, water in the thermal bath located in a fume hood is warmed to 80 °C in preparation for the first round of $NaClO_2$ (Table 1, Step 1). The water level of the bath must cover about half of the glass tubing part of the funnels during extraction. Electric kettles filled with RODI water can be used in conjunction with the bath's heating system to accelerate the heating process by about 45 min. After loading 150 samples into the filter tubes, each extraction system is placed into the bath in the order portrayed in Suppl. Fig. 3.

Safety goggles are necessary for eye protection during the extraction procedures under the fume hood and while handling chemicals in the lab. Heat-resistant gloves are required for holding wash bottles with boiling water (Table 1, Step 1), and when pouring the NaOH warm solution into each funnel (Table 1, Steps 2 & 3).

Prior to the first extraction, the entire system, including the assembled funnels and the PTFE blocks, is entirely rinsed three times with boiling RODI water and purged with the vacuum pump. The first extraction step consists of a chlorination process that oxides the lignin of the wood material by the generation of chlorine dioxide (ClO_2) (Table 1, Step 1). The reagents of the first chemical solution (acidified $NaClO_2$) are immediately prepared, while the funnels acclimate to bath temperature. In a 1000-ml beaker, 8.56 g of $NaClO_2$ are added to 600 ml of ultrapure water with stirring until the $NaClO_2$ powder is totally dissolved. A graduated cylinder is used to measure 6 ml of glacial acetic acid (CH_3CO_2H) that is immediately poured into the $NaClO_2$ solution. Note that the amount of glacial acetic acid to be added depends on the pH of the ultrapure water used (usually close to 7). A glass-stirring rod is used to slowly mix the reagents. The chemical solution is then transferred to a labeled chemically-resistant wash bottle.

The solution is dispensed sequentially into each funnel using the wash bottle; each funnel should be filled half-way with the solution (Fig. 3C, D). If after the filling, the solution levels of some funnels decrease, refilling will be required to ensure an even distribution of the solution throughout the channels of the system and the same quantity of chemicals inside each funnel. A precision pipette (e.g. Thermo Scientific Finnipipette 2–10 ml) can also be used for pouring precise amounts of liquid in each funnel. The 60-minute timer is initiated once each sample has made contact with the $NaClO_2$ solution. When time expires following each extraction sequence (Table 1), the solution is drained using the vacuum pump connected to a freestanding 1000-ml Erlenmeyer flask (Fig. 3E). Each PTFE-Teflon device is connected to a corresponding drain hose (Fig. 3B). At the end of each hose there is a perforated rubber cap, which connects to the Erlenmeyer-pump framework, to allow for ample suction and removal of used solutions. In order to minimize the total time of the cellulose extraction procedure and maximize efficiency, a new batch of chemical solution for the subsequent extraction is prepared 5 min prior to drainage and set aside inside the fume hood. This process of bleaching the wood with acidified $NaClO_2$ is repeated 6 times totaling about 6 h (Table 1, Step 1).

After the 6th $NaClO_2$ cycle, the samples are rinsed once with boiling ultrapure water and drained with the vacuum pump. Afterwards, a minimum of 4 additional rinses with ultrapure water at room-temperature are necessary to stabilize the pH value of the water in contact with the samples. During this time, the bath temperature is lowered and set to 70 °C in preparation for the following step.

For the first alkaline treatment, which removes non-cristalline cellulose (Table 1, Step 2), a NaOH 10% solution is prepared in a 1000-ml glass beaker by pouring 600 ml of ultrapure water and adding 60 g of NaOH pellets. The NaOH solution is exothermic and highly reactive. Thus, contrary to the $NaClO_2$ sequences, this part of the protocol is particularly time-sensitive. The timer needs to be set at 45 min concurrent with dispensing the solution to avoid unnecessary degradation of the samples. After 45 min, the solution is immediately drained and the samples are rinsed three times with ultrapure water. This concludes the first day of the extraction proceeding (about 8 h). The samples remain submerged in room-temperature ultrapure water overnight to prevent samples from drying out and sticking on the filters and funnels. The bath and extraction devices must be covered overnight to prevent unwanted contaminants accessing the inner part of the funnels.

The second and final day of the extraction proceeding begins with the second and the last alkaline extraction procedure (Table 1, Step 3) that is done at room temperature using NaOH 17% solution. This alkaline treatment leaches carbohydrates such as mannan and xylan from the holocellulose; therefore, this procedure hydrolyzes short-chain hemicelluloses, but retains long-chained α-cellulose molecules (Loader et al., 1997). This step requires some caution because if reaction temperature is not lowered to room temperature considerable amounts of cellulose could be degradated. In a 1000-ml beaker, 102 g of NaOH pellets are added to 600 ml of ultrapure water. The solution should be evenly and sufficiently distributed throughout each filter tube. Immediately after 45 min of exposure, samples are thoroughly drained and immediately rinsed with ultrapure water (3 times). At the same time, the bath temperature is increased to 80 °C once again.

The second chlorination process consists of a bleaching treatment (Table 1, Step 4) and is used because parts of the components extracted during the alkaline process are better washed with an acidic solution. For this last step the water bath is set at 80 °C. The $NaClO_2$ solution is prepared and poured as described previously. After the 60-minute reaction period, the solution is drained from the system and a new $NaClO_2$ solution is prepared for the final chemical cycle. When the last $NaClO_2$ treatment is complete, the chemical solution is drained. The samples remaining in the funnels (α-cellulose) are rinsed a minimum of 5 times with ultrapure water (Table 1). The bath is turned off and all equipment are unplugged before each extraction device is carefully removed from the bath and set aside for disassembly.

Each funnel is pulled from the system individually in chronological order. The label of each sample is retrieved from the sketch that maps the 3 devices (Suppl. Fig. 3). First, the glass tube (Fig. 1D) is carefully separated from the silicon base (Fig. 1B). Using a blunt pipette tip, the filter (Fig. 1C) together with the interior Teflon-PFA tube (Fig. 1A) are slowly pushed from the bottom of the silicon base until the borosilicate filter is aligned with the top of the silicon base. As a result, the extracted cellulose is exposed on the top of the filter. Then, 1-ml of ultrapure water is gently poured over the filter using an air displacement precision pipette making the cellulose gently slide inside a 1.5-ml Eppendorf Tube® for storage (Fig. 3F). Carefully angled over the tubes, the pipette slowly streams the water guiding the cellulose off the glass filter. This method avoids scratching the borosilicate filter and prevents potential contamination of the cellulose samples. If needed, residual cellulose material on the glass filter is carefully collected with tweezers and put in the respective tube. Great care must be taken to avoid the loss of cellulose fibers, especially for samples with little cellulose mass.

Once the cellulose sample is stored in a tube and the lid of the tube is closed, each component of the funnel is immediately dissembled. Each individual part is put in a separate beaker or glassware filled with water. By soaking the funnel parts, potential cross contamination between samples is avoided for the following use. The final cleaning process is also facilitated since remaining cellulose particles do not dry out and stick on the filters and funnels. The remaining cellulose particles (if any) are perfectly visible over the filters and float after sinking the filters inside beakers with water. We thoroughly rinse, soak, and inspect filters after each use. Ultrapure water is used for all the processes occurring inside the funnel and for cleaning the filters and the small Teflon-PFA tube (Fig. 1A, C). The rest of the components such as glass tubing and silicon tubes (Fig. 1B, D) are cleaned using boiling RODI water warmed up with an electric kettle. All the components are soaked in water and several rinses are done before and after scrubbing with a brush or a steel-colander. This approach increases the overall efficiency of our protocol and the lifetime of the filters since it avoids long exposures to strong chemical cleaning solutions (e.g. HCl) and high temperatures in a muffle furnace at 500 °C (e.g., Wieloch et al., 2011).

2.5. Cellulose homogenization

The extracted α-cellulose is homogenized using a 3Qt Fisher FS20 ultrasonic bath to ensure that the small amount (~200 μg) of α-cellulose required for analysis is representative of material from the entire growth ring. Following Laumer et al. (2009), we utilize this homogenization process to break apart the cellulose fibers and convert what appears to be pieces of bleached wood material into a milky suspension of cellulose. Samples in Eppendorf Tubes® are loaded into a small carousel and placed directly into the water – generally a rack of 12 samples can take from 3 to 5 min to homogenize depending on the type of wood and amount of material. Manually shaking the tubes individually or the whole sample-carousel can reduce the amount of time needed for sample homogenization. Some samples might need a longer time to fully homogenize, 10 to 30 min, but this normally occurs when the fibers are too big as wood pieces were not chopped small enough. It is not recommended, however, to expose an individual sample to ultrasound for long periods as the ultrapure water within the Eppendorf Tubes® can warm up and this may potentially impart some ^{18}O exchange between sample and water. Nevertheless, different time exposure of the ultrasound treatments, up to 5 min, does not lead to significant differences in the $\delta^{13}C$ or $\delta^{18}O$ ratios (Laumer et al., 2009).

Following homogenization, the α-cellulose is freeze-dried prior to encapsulation and analysis by an IRMS. The tubes with the samples are transferred in small storage boxes and put in a freezer for at least 24 h to ensure that the samples are completely frozen prior to the freeze-drying process. When samples are frozen, the lids of the Eppendorf Tubes® are individually opened and the storage box is placed into a bench-top freeze-dryer (Labconco Free Zone 6 Liter Freeze Dry System and an Edwards RV3 vacuum pump) for at least 24 h at 47 °F. A freeze-drier dries frozen samples through sublimation, which reduces the $\delta^{18}O$ enrichment that would occur if the samples are dried through evaporation. Dried α-cellulose resembles pure white cotton. Cellulose fibers with harder texture or darker colour (if any) are not used for further analyses to ensure the high quality of the material measured.

The final step in the process of stable isotope preparation is encapsulation. A precise amount of cellulose (0.20 ± 0.01 mg) is weighed on a microbalance and put in 3.2 × 4 mm silver or tin capsules and pressed and rounded with tweezers. The sample capsules are placed in a well-tray and left in a vacuum desiccator (Bel-Art F42074-1118 Secador Vertical Profile Amber 4.0 Auto-Desiccator Cabinet; 120 V, 1.9 cu. ft., Wayne NJ USA) for at least 24 h prior to analysis in the IRMS.

2.6. Purity of alpha-Cellulose

The chemical procedure for extracting α-cellulose described in this paper was adapted from well-known previously evaluated protocols (Kürschner and Popik, 1962; Green, 1963; Loader et al., 1997). Several papers have tested the effectiveness of this proceeding for the isolation of α-cellulose analyzing the spectra of the distinct wood compounds using infrared instruments. Rinne et al. (2005) reported the success of this chemical treatment for extracting resins based on measurements through reflectance Fourier transform infrared (FTIR). Richard et al. (2014) noted that even if some residual compounds could be detected using Attenuated Total Reflection mode (IR-ATR), those were not impacting the $\delta^{13}C$ isotopic ratios; they also reported that some tree species such as oak and beech did not even need the NaOH treatment, so a less intensive chemical procedure can indeed lead to pure α-cellulose. Finally, Kagawa et al. (2015) and Schollaen et al. (2017) described the success of the Jayme-Wise protocol used also here demonstrating that the cellulose extracted from 5 and 6 different tree species, respectively, show the peak related with α-cellulose in the FTIR spectra, while there was mostly an absence of the peaks associated with resin, lignin and hemicellulose. To test the purity of the α-cellulose extracted at LDEO with the Jayme-Wise method we performed FTIR analyses (Suppl. Fig. 4). The samples analyzed include our two standards (primary IAEA-C3 and secondary SAC) and cellulose from 6 different species: white spruce (*Picea glauca*), saltcedar (*Tamarix ramossisima*), red oak (*Quercus rubra*), tulip poplar (*Liriodendron tulipifera*), Siberian pine (*Pinus sibirica*) and polylepis (*Polylepis tarapacana*). Two wood materials were also measured to facilitate the comparison with the spectra peaks that are expected to be absent in the remaining cellulose after the chemical treatment. All the extracted samples show the α-cellulose peaks at ~1000 cm^1 (Li et al., 2011; Harada et al., 2014) and 900 cm^1 (Anchukaitis et al., 2008), while the ~1732 cm^{-1} peak most widely associated with hemicellulose (e.g., Rinne et al., 2005; Harada et al., 2014; Kagawa et al., 2015; Schollaen et al., 2017), was not present or was almost inexistent compared with the oak wood FTIR spectra. None of the cellulose samples show any peak associated with resin such as 1600 cm^{-1} (Anchukaitis et al., 2008; Schollaen et al., 2017) or lignin with the multiple spectra points described in the literature (see details in Suppl. Fig. 4).

3. Dual measurement of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotopes

While the $\delta^{13}C$ ratios in cellulose are classically obtained combusting the samples to CO_2 in an Elemental Analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS) (Pichlmayer and Blochberger, 1988), the $\delta^{18}O$ ratios are measured decomposing cellulose samples to CO using high-temperature pyrolysis in a High Temperature Conversion Elemental Analyzer (TC/EA) coupled to an IRMS (Kornexl et al., 1999). This high-temperature pyrolysis (HTP) technique has also been reported useful for determining $\delta^{18}O$ and $\delta^{13}C$ isotopic ratios at the same time from a single cellulose sample operating at 1400 °C or above (Gehre and Strauch, 2003). Despite the presence of reactive carbon, HTP produces reasonably good $\delta^{13}C$ values as confirmed by higher agreement with measurements obtained by the elemental analyzer system (EA) and the HTP (Knoller et al., 2005).

At LDEO we have implemented the dual measurement of $\delta^{13}C$ and $\delta^{18}O$ through HTP (Gehre and Strauch, 2003; Knoller et al., 2005) coupled to continuous flow (Brenna et al., 1997) and IRMS using a two-point normalization method (see Section 3.3 below) with two certified reference standards ensuring smaller normalization error (Paul et al., 2007).

3.1. High-temperature pyrolysis (HTP)

The HTP procedure implemented at LDEO determines via continuous flow IRMS the relative concentrations of oxygen and carbon isotope molecules in the CO gas obtained by the pyrolytic conversion of the cellulose samples in a TC/EA. The magnetic fields of the IRMS allow for separating the molecules with distinct molecular mass through the three middle cup collectors, of the total of five in the IRMS, which count the electron signals based on molecular masses.

The CO molecules have a distinct molecular weight depending on the isotopic mass of the carbon and oxygen atoms composing the molecule such as $^{12}C^{16}O$ (mass 28), $^{13}C^{16}O$ (mass 29) and $^{12}C^{18}O$ (mass 30), which are the most abundant CO molecules in nature. These isotopologues, which are molecules that differ only in their isotopic composition, are found in a higher proportion as a result from the natural abundance of C atoms (98.93% of ^{12}C; 1.07% of ^{13}C) and of O atoms (99.76% of ^{16}O; 0.205% of ^{18}O; 0.038% of ^{17}O) (CRC Handbook of Chemistry and Physics, 2005).

For each sample, the values obtained from the IRMS are the ratio (R) between the abundance of the heavy isotope and the light isotope (Eq. (1)).

$$\text{ratio (R)} = \left[\frac{\text{abundance of the heavy isotope}}{\text{abundance of the light isotope}}\right] \quad (1)$$

Specifically of interest here, using ratios of mass 28, mass 29 and mass

30, the oxygen and carbon isotopic ratios can be obtained:

$$R_{sample}\ of\ (^{18}O/^{16}O) = \left[\frac{^{18}O_{sample}}{^{16}O_{sample}}\right] \leftarrow \left[\frac{Mass\ 30\ (^{12}C^{18}O)}{Mass\ 28\ (^{12}C^{16}O)}\right] \quad (2)$$

$$R_{sample}\ of\ (^{13}C/^{12}C) = \left[\frac{^{13}C_{sample}}{^{12}C_{sample}}\right] \leftarrow \left[\frac{Mass\ 29^{*}\ (^{13}C^{16}O)}{Mass\ 28\ (^{12}C^{16}O)}\right] \quad (3)$$

*^{17}O correction

Although the CO isotopologues $^{12}C^{16}O$, $^{13}C^{16}O$ and $^{12}C^{18}O$ represent most of the mass 28, 29 and 30, respectively, other combinations of CO molecules with the less abundant ^{17}O also occur in nature, and thus a correction needs to be applied. The Isodat software (Thermo Scientific Isodat) of the IRMS is obtaining the $\delta^{18}O$ and $\delta^{13}C$ ratios using an approach (not shown here) that includes the resolution of several equations and the $\delta^{17}O$ correction (Brand et al., 2010). Specifically, the isotopologues $^{12}C^{17}O$ and $^{13}C^{17}O$ represent about < 7% and < 0.03% of the total mass of 29 and mass 30, respectively:

Mass 28(IRMS cup 2) = $^{12}C^{16}O$

Mass 29 (IRMS cup 3) = $^{13}C^{16}O$ + $^{12}C^{17}O$

Mass 30 (IRMS cup 4) = $^{12}C^{18}O$ + $^{13}C^{17}O$

Therefore, these ^{17}O less abundant CO molecules are also counted in the IRMS collectors. For mass 30, this is not an important concern because the presence of $^{13}C^{17}O$ is very low (< 0.03%) due to the fact that the atoms ^{13}C and ^{17}O are rare in nature (see abundance above). However, the ^{17}O correction is required before using mass 29 to avoid that ^{12}C atoms from the $^{12}C^{17}O$ molecules (< 7%) be considered in the calculation of the ratios (R) of $^{13}C/^{12}C$ (Eq. (3)).

The isotopic data derived from the IRMS (Eqs. (2) and (3)), is expressed using the delta (δ) notation (Eq. (4)) calculated using the ratios of the samples (R_{sample}) in relation to the CO reference gas used (R_{ref}). All the stable isotope results are expressed as per mil (‰):

$$\delta_{sample} = \left[\frac{R_{sample}}{R_{Ref}} - 1\right] \times 1000 \quad (4)$$

Finally, the δ for each sample obtained from the IRMS needs to be calibrated in relation to international standards. The $\delta^{18}O$ relative to the international standard Vienna Standard Mean Ocean Water (VSMOW) and the $\delta^{13}C$ ratios relative to the international standard Vienna Pee Dee Belemnite (VPDB), as shown in Eqs. (5) and (6), respectively. In our case, this is done using a two-point normalization method (see Section 3.3 below) with two certified reference standards that calibrate the δ values of the samples obtained from the IRMS in relation to the reference values of VSMOW and VPDB.

$$\delta^{18}O_{sample} = \left[\frac{(^{18}O/^{16}O)_{sample}}{(^{18}O/^{16}O)_{VSMOW}} - 1\right] \times 1000 \quad (5)$$

$$\delta^{13}C_{sample} = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{VPDB}} - 1\right] \times 1000 \quad (6)$$

3.2. Comparing HTP versus combustion to determine $\delta^{13}C$ ratios

Since the HTP method analyzes five of the six C atoms of cellulose (Knoller et al., 2005), we checked the quality of the $\delta^{13}C$ values of samples obtained through dual measurement of $\delta^{13}C$ and $\delta^{18}O$ through HTP with TC/EA, compared with the $\delta^{13}C$ values of the same samples measured via the combustion method with a Costech elemental analyzer (EA) interfaced with the same continuous flow IV and Thermo Delta V plus mass spectrometer. Our results show very high agreement between the $\delta^{13}C$ values obtained using HTP through TC/EA and combustion through EA (Fig. 4).

For the combustion method, the produced CO_2 gas from the cellulose samples is measured using the three middle cups of IRMS after passing the magnetic field. The CO_2 molecules or isotopologues with the highest abundances in the atmosphere are listed below based on their molecular mass 44, 45 and 46. The values inside the parenthesis are the abundance in % of each CO_2 isotopologue in the atmosphere (Eiler and Schauble, 2004):

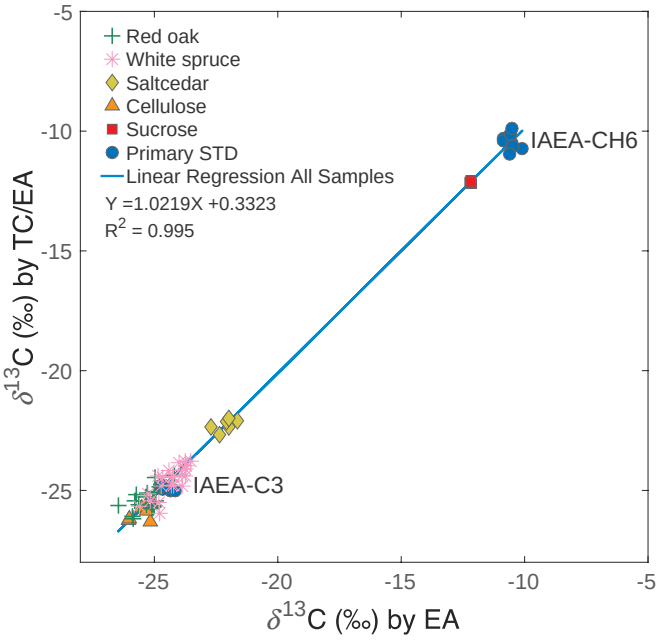

Fig. 4. Plot of $\delta^{13}C$ results obtained by TC/EA versus the respective results from EA. This is a simple way to estimate the difference between two analytical methods. In addition to the primary standards to correct the raw data (IAEA-C3 and IAEA-CH6), this plot shows three types of pure cellulose (IAEA-C3, Sigma cellulose Lot#MKBS2567V, and Sigma cellulose Lot#SLBM6732V), two types of pure sucrose (IAEA-CH6 and Sigma sucrose Lot#SLBQ0671V) and finally cellulose extracted from wood samples of three distinct tree species: red oak (*Quercus rubra*) from New York in USA, white spruce (*Picea glauca*) from Alaska in USA and saltcedar (*Tamarix ramosissima*) from the Taklamakan desert in China. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Mass 44 (IRMS cup 2) = $^{12}C^{16}O_2$(98.4%)

Mass 45 (IRMS cup 3) = $^{13}C^{16}O_2$(1.1%) + $^{12}C^{16}O^{17}O$ (<0.076%)

Mass 46 (IRMS cup 4) = $^{12}C^{16}O^{18}O$ (0.41%) + $^{13}C^{16}O^{17}O$(<0.00085%) + $^{12}C^{17}O_2$(<0.000015%)

Based on the amounts of CO_2 molecules with the heaviest isotope ^{13}C (mass 45) and lighter isotope ^{12}C (mass 44 and mass 46) with the highest abundance in nature, the R of the $^{13}C/^{12}C$ for each sample can be approximately described as follows:

$$R_{sample}\ of\ (^{13}C/^{12}C) = \left[\frac{^{13}C_{sample}}{^{12}C_{sample}}\right] \leftarrow \left[\frac{Mass\ 45^{*}(^{13}C^{16}O_2)}{Mass\ 44\ (^{12}C^{16}O_2) + Mass\ 46\ (^{12}C^{16}O^{18}O)}\right] \quad (7)$$

*^{17}O correction

Note that altough Eq. (7) is useful for understanding how the R of $^{13}C/^{12}C$ are obtained, the Isodat software (Thermo Scientific Isodat) calculates the final isotopic $\delta^{13}C$ values for each sample (Eq. (6)) in a more complex way that also includes an automatic correction of ^{17}O (Brand et al., 2010). The ^{17}O correction is applied to avoid that in the determination of mass 45, that should express the proportion of CO_2 molecules with ^{13}C atoms (e.g. $^{13}C^{16}O_2$), the less abundant CO_2

molecules with ^{12}C atoms (e.g. $^{12}C^{16}O^{17}O$ also with mass 45) is considered. The Isodat sofware is applying an additional ^{18}O correction over mass 46 (Brand et al., 2010).

During the combustion procedure, helium is used as the carrier gas, with a flow rate of 100 ml/min. Samples within the same narrow mass range (0.20 ± 0.01 mg) were loaded into tin capsules and stored in desiccators until analysis. The samples were combusted at 980 °C over a Chromium (III) oxide catalyst in the presence of excess oxygen (25 ml/min). Silvered cobaltous/cobaltic oxide, positioned lower in the quartz combustion tube, ensures complete conversion of sample carbon into CO_2 and removal of residual halogens or sulfur. Any nitrogen oxides are removed by passage over copper wire (650 °C), and traces of water are removed through a magnesium perchlorate trap. The produced CO_2 gas for each sample was separated from other minor gases (if any) through a gas chromatography (GC) column (55 °C), via a continuous flow IV open split device (Thermo Fisher Scientific, Bremen, GmbH) into a Delta V plus isotopic ratio mass spectrometer (Thermo Fisher Scientific, Bremen, GmbH) for isotopic analysis.

In the dual measurement of $\delta^{13}C$ and $\delta^{18}O$ through HTP at LDEO, the samples are loaded into a zero blank autosampler (Costech Company, 100 positions), purged with high purity (Grade 5) helium at 70 ml/min before pyrolysis. The pyrolysis is conducted at 1400 °C using a high-temperature conversion elemental analyzer (TC/EA) (Thermo Fisher Scientific, Bremen, GmbH). The pyrolysis reactor consists of an outer ceramic tube filled with silver wool, quartz wool and a layer of glassy carbon chips upon which lies the inner glassy carbon tube filled up to the level of the hottest zone with glassy carbon chips retained by a silver wool plug. The sample ashes and the molten sample containers are collected in a graphite crucible located in the hottest zone. The pyrolysis reactor and graphite crucible are cleaned and repacked after processing every ~250 samples, but we estimate that it could last up to ~300 samples before a cleaning cycle. A helium flow of 90 ml/min carries the reduction gases through a 1.0 m gas chromatography column (GC) (5 Å molecular sieve, temperature 70 °C) for separating CO and N_2, which have the same molecular mass ($^{14}N_2 = 28$ and $^{12}C^{16}O = 28$). Therefore, this separation through the GC column is necessary because a small amount of N_2 contamination can occur from decomposition of nitrogen-containing or contaminated organic compounds, leakage of the autosampler, or N_2 import with the samples in the silver capsules.

The separated gases are carried by helium flow (90 ml/min) via the continuous flow IV open split device into our Delta V plus IRMS. Three carbon monoxide (CO) reference gas (grade 4.7, Praxair) peaks are run before each sample peak and one CO reference peak after.

3.3. The two-point normalization method

At LDEO, data from the IRMS are corrected using a two-point calibration (e.g., Evans et al., 2016) with two international well-defined reference materials (IAEA) that fully cover the expected δ-range of the samples (Fig. 5). The samples analyzed are referenced to standard materials from the International Atomic Energy Agency (IAEA-C3, IAEA-CH6, IAEA-601 and IAEA-602) and checked with secondary standards from United States Geological Survey (USGS-54) and Sigma Company (Sigma Alpha-Cellulose and Sigma Sucrose). The primary standards are used to extrapolate the values obtained by each particular IRMS in relation to international materials. The secondary standards are used to check the quality and precision of our correction for each run and were chosen because they have similar isotopic range as the cellulose material.

A linear regression (dash line in Fig. 5) to obtain the calibrated isotopic values (δ_{sample}) is calculated based on the true isotopic values of the two international standards and their raw values obtained from the measurements from the IRMS. The regression parameters obtained

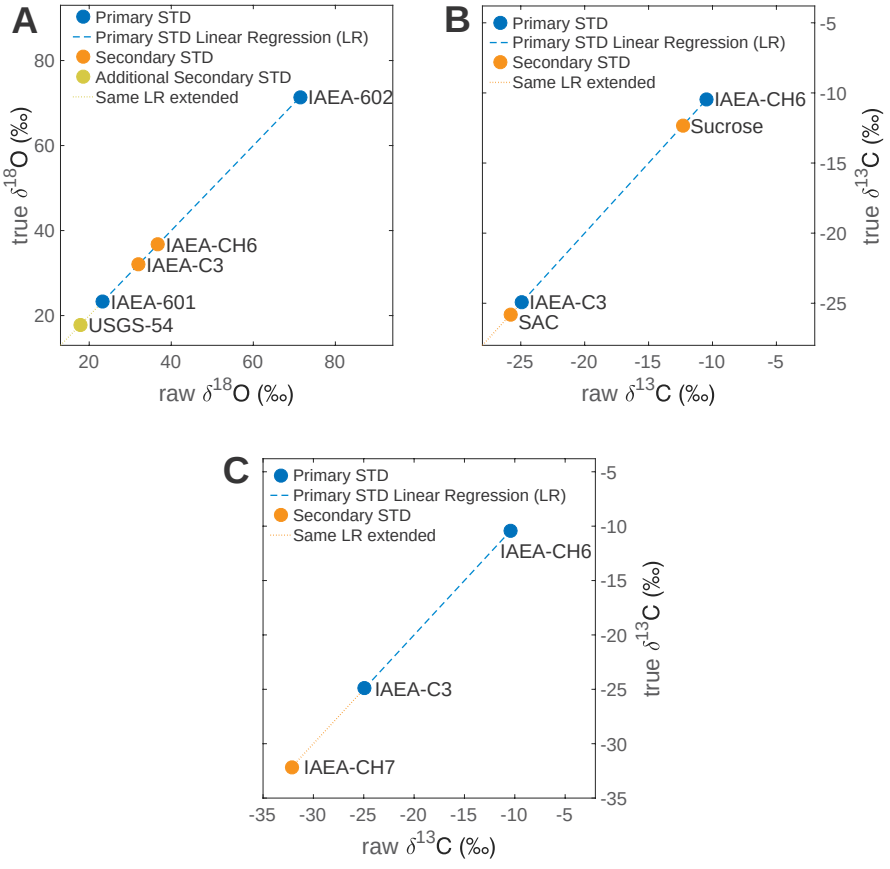

Fig. 5. Two-point calibration method used at LDEO for the dual measurement of $\delta^{18}O$ and $\delta^{13}C$ ratios by pyrolysis (A and B) and for the $\delta^{13}C$ by combustion proceeding (C). The primary standards (STD) are two international well-defined reference materials (IAEA) that properly cover the expected δ-range of the cellulose samples for $\delta^{18}O$ (**A**) and the $\delta^{13}C$ (**B**, **C**) ratios. These regression equations are used to correct the raw values ratios obtained with the IRMS with the true/known δ values from the reference materials from IAEA. The secondary STDs are used to check the quality and precision of our calibration for each run.

(a and b) are applied to all measured raw sample data (δ_{raw}) in order to calculate the final calibrated isotopic values ($\delta_{calibrated}$):

$$\delta^{18}O_{calibrated} = a*\delta^{18}O_{raw} + b \quad (8)$$

$$\delta^{13}C_{calibrated} = a*\delta^{13}C_{raw} + b \quad (9)$$

As a control for this calibration procedure, two secondary standards (with $\delta^{13}C$ and $\delta^{18}O$ values between or close to the two international standards used for normalization) are included several times in each measuring run and are normalized in the same way as the samples.

The primary standards for $\delta^{18}O$ are IAEA-601 and IAEA-602, while for $\delta^{13}C$ are IAEA-C3 and IAEA-CH6. The amounts of 0.20 ± 0.01 mg for the standards (IAEA-C3, IAEA-CH6, USGS-54 and Sigma Sucrose) and 0.37 ± 0.02 mg for the IAEA-601/602 are prepared to yield equivalent peak heights to cellulose samples. Once weighed and encapsulated, samples and standards are stored in a desiccator until analysis. The standards of IAEA-601/602 are stored in a separate desiccator to avoid possible evaporation of benzoic acid. As indicated above, all cellulose samples are weighed within a fixed amount (0.20 ± 0.01 mg) into silver capsules to provide consistent peak heights and preclude the need for size-related (linearity) correction.

Table 2 shows the precision of our primary and secondary standards for both the HTP method to dually measured $\delta^{13}C$ and $\delta^{18}O$ ratios (Table 2A, B, respectively) and for the combustion method to measure $\delta^{13}C$ ratios (Table 2C).

For correcting $\delta^{18}O$ (Fig. 5A), our primary standards are the international standards IAEA-601 (benzoic acid with $\delta^{18}O$ = 23.3‰) and IAEA-602 (benzoic acid with $\delta^{18}O$ = 71.4‰), which are used to anchor unknown sample $\delta^{18}O$ values. Regarding the secondary standards for $\delta^{18}O$, IAEA-C3 ($\delta^{18}O$ = 32.6‰) and IAEA-CH6 ($\delta^{18}O$ = 36.4‰) are used for this purpose because their $\delta^{18}O$ values fall within the $\delta^{18}O$

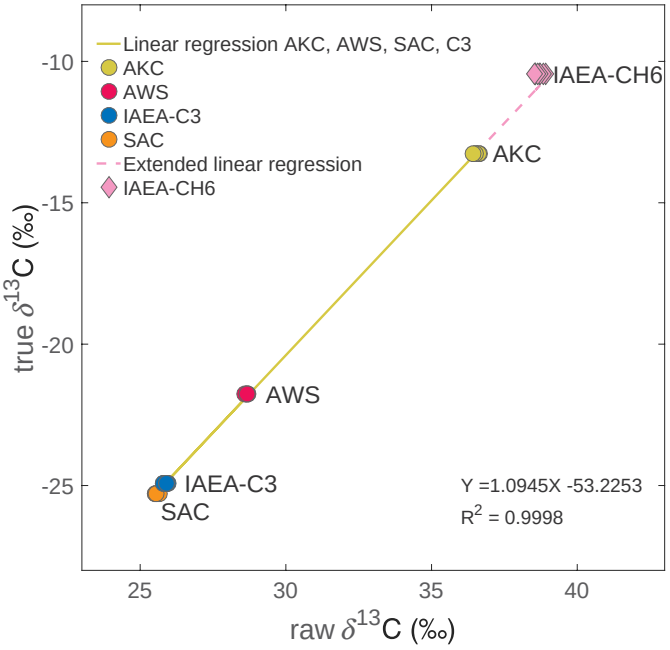

Fig. 6. The $\delta^{13}C$ ratios of the sucrose samples (IAEA-CH6) measured by the HTP method at LDEO plotted together with the $\delta^{13}C$ ratios of four distinct types of cellulose: IAEA-C3, Sigma cellulose (SAC) and two internal secondary standards named AKC and AWS obtained from Alaskan corn and white spruce, respectively (Evans et al., 2016). The $\delta^{13}C$ sucrose values aligned well with the extension (pink dash line) of the regression line (green line) based on AKC, AWS, IAEA-C3 and SAC $\delta^{13}C$ data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
The standards (STD) used for the calibration proceeding at the LDEO laboratory (sample size 0.2 mg cellulose). The number of samples measured (No.), the values (‰) and the precision of the measurements (SD) are indicated for the primary and secondary STD for $\delta^{18}O$ (A) and $\delta^{13}C$ (B) measured by the HTP method via TC/EA; and (C) for the $\delta^{13}C$ values measured using the combustion method via EA. The true values (‰) for all primary STD and CH7 are reported on the IAEA website, while for USGS-54 on the USGS website. Since IAEA does not provide $\delta^{18}O$ values for IAEA-C3 and IAEA-CH6 because they can absorb moisture, the true values listed are from Boettger et al. (2007) and Kornexl et al. (1999), respectively. Regarding the $\delta^{13}C$ of Sigma cellulose and the $\delta^{13}C$ of Sigma sucrose, the true values are averages from our EA analyses. Our long-term averages are listed for all the secondary STD after being calibrated with the linear regression based on the primary STD (Fig. 5).

(A) TC/EA		$\delta^{18}O$			
	STD name	No.	True value (‰)	Long-term average (‰)	SD (precision)
Primary STD	IAEA-601	216	23.3	-	± 0.12
	IAEA-602	177	71.4	-	± 0.38
Secondary STD	IAEA-C3	215	32.6	32.0	± 0.34
	IAEA-CH6	204	36.4	36.7	± 0.28
	USGS-54	31	17.79	17.9	± 0.32
(B) TC/EA		$\delta^{13}C$			
	STD name	No.	True value (‰)	Long-term average (‰)	SD (precision)
Primary STD	IAEA-C3	215	−24.91	-	± 0.14
	IAEA-CH6	204	−10.45	-	± 0.20
Secondary STD	Sigma cellulose	32	−25.43	-25.81	± 0.15
	Sigma sucrose	37	−12.18	−12.28	± 0.29
(C) EA		$\delta^{13}C$			
	STD name	No.	True value (‰)	Long-term average (‰)	SD (precision)
Primary STD	IAEA-C3	10	−24.91	-	± 0.19
	IAEA-CH6	10	−10.45	-	± 0.24
Secondary STD	IAEA-CH7	10	−32.15	−32.32	± 0.30

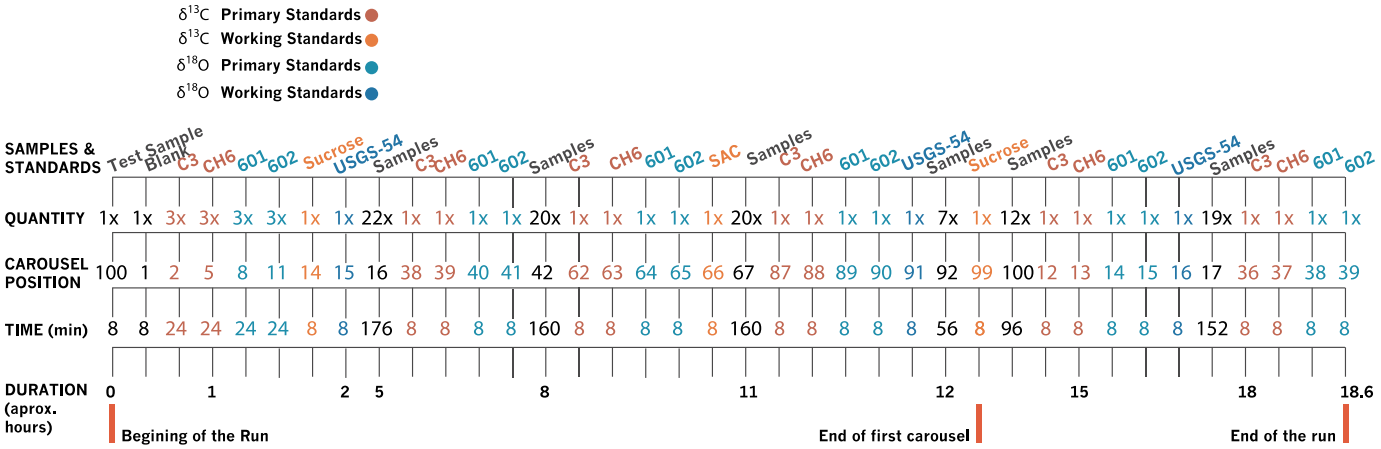

Fig. 7. Sketch showing the sequence of standards and samples for a single run of the HTP procedure of the dual measurement of $\delta^{18}O$ and $\delta^{13}C$ ratios implemented at LDEO. The quantity, numbered position in the carousel and length of the analysis time per sample in minutes are indicated below the type of sample. An overall timeline (in hours) for a complete run before a cleaning cycle is indicated on the bottom. Note that the carousel has 100 holes and that each measurement takes about 8 min, considering the time needed to run three peaks of CO reference gas before each sample and one peak of CO gas after the sample. In total, a complete run consists of 100 sample and 39 standard measurements over an 18.6-hour period.

regression range. IAEA-C3 and IAEA-CH6 are not chosen as standards to anchor $\delta^{18}O$, due to the hygroscopic nature of cellulose and narrow $\delta^{18}O$ cover range (4.7‰ compared to 48.1‰ difference between IAEA-601 and IAEA-602). For the samples with lower (lighter) $\delta^{18}O$ than IAEA-601 (e.g. $\delta^{18}O = 23.3‰$), another standard (USGS54, wood powder with $\delta^{18}O = 17.79‰$ and low N content 0.05%) is analyzed to have a large enough range so that the regression normalization works for all samples.

Note that for $\delta^{18}O$ analyses, we use as secondary standards IAEA-C3 and IAEA-CH6 that are the primary standards for $\delta^{13}C$. This reduces the total number of standards needed in each run.

For correcting $\delta^{13}C$ (Fig. 5B), our primary standards are the international standards IAEA-C3 (cellulose with $\delta^{13}C = -24.91‰$) and IAEA-CH6 (sucrose with $\delta^{13}C = -10.45‰$), which are used to anchor unknown sample $\delta^{13}C$ values. Regarding the secondary standard for $\delta^{13}C$, Sigma cellulose (Lot# MKBS2567V; $\delta^{13}C = -25.43‰$) and Sigma Sucrose (Lot# SLBQ0671V; $\delta^{13}C = -12.18‰$) are used as secondary standards, which are close or within the full range of variability between the two primary standards for $\delta^{13}C$.

For obtaining the $\delta^{13}C$ values with the combustion method (Fig. 5C), the samples were calibrated by two-point regression with the primary standards IAEA-C3 and IAEA-CH6, while IAEA-CH7 (polyethylene $\delta^{13}C = -32.15‰$) was used as a secondary standard.

3.4. The $\delta^{13}C$ ratios in sucrose

Ideally, primary standards should be at least 10‰ apart to give precise calibration. There are no two cellulose standards available on the market with $\delta^{13}C$ values 10‰ apart. However, sucrose IAEA-CH6 has $\delta^{13}C$ value −14.65‰, which is about 14‰ more enriched than IAEA-C3. Both cellulose (polysaccharide) and sucrose (disaccharide) are common carbohydrates, which can be found in many parts of plants. They are composed of the same basic unit, glucose molecule (Suppl. Fig. 5), but it is unknown if they behave the same under the HTP method at 1400 °C. Knoller et al. (2005) and others (Boettger et al., 2007; Woodley et al., 2012; Loader et al., 2015) have discussed C distribution in the cellulose molecule and the potential incomplete reaction effect on the analyzed $\delta^{13}C$ values, comparing the cellulose $\delta^{13}C$ results analyzed both by EA and TC/EA. However, to our knowledge no studies have compared sucrose $\delta^{13}C$.

Our results (Fig. 4; Table 2B) show that sucrose has similar performance when combusted by the HTP method. Even more, additional tests (Fig. 6) also report that sucrose is a good primary standard, coupled with cellulose, to anchor $\delta^{13}C$ of cellulose samples by the HTP method (Fig. 5B). Fig. 6 shows how the $\delta^{13}C$ ratios of the sucrose samples (IAEA-CH6) measured by the HTP method at LDEO are well aligned with a linear regression generated exclusively using four distinct types of cellulose such as IAEA-C3, Sigma cellulose (SAC) and two internal secondary standards obtained after extracting cellulose from Alaskan corn (AKC) and Alaskan white spruce wood (AWS) shared by an independent laboratory (AKC and AWS described in Evans et al., 2016). Although the $\delta^{13}C$ sucrose values are outside the range of the regression curve, the good match with the regression extended to more enriched $\delta^{13}C$ values demonstrates that our HTP method is appropriate for sucrose as well.

3.5. Data quality control

The calibration regression is created with the measurements of the international standards for each run on a daily basis and applied to the raw isotopic data generated by the IRMs. This minimizes the effects of long-term variations in the biases of the mass spectrometer, room temperature and sample preparation, and hence optimizes the offset of correction. The TC/EA reactor is cooled down and cleaned every 250 samples. A total of eight sets of reference standards (IAEA-C3, IAEA-CH6, IAEA-601 and IAEA-602) are run each day (Fig. 7). Three sets of primary standards are run at the start of the daily analysis to test for the drift in the reference gas and stability of the mass spectrometer. For every ~20 samples, one set of standards are inserted to check instrument performance and provide a means for linear-drift correction/checking. Average standard precisions are shown in Table 2. We have not observed any significant reactor/instrument drifting so far, or within analysis precision. Therefore, our system is normally stable within a run and no drift correction is required. However, it is important to conduct these periodic checks with standards (every 20 samples) to detect changes or instability during a run. In case of instrument drifting some corrections can be applied (e.g., Evans et al., 2016). Sigma cellulose (SAC), which has the same molecular structure and similar isotopic values as our samples, is chosen as an internal control to check our long-term analysis precision. SAC is stored together with our samples and standards in a desiccator, and is analyzed in every daily run. Considering the last two years, our long-term precision of SAC was 0.15‰ for $\delta^{13}C$ and 0.25‰ for $\delta^{18}O$.

Acknowledgements

We would like to give special thanks to Neil Loader for kindly

sharing the Swansea protocol for cellulose extraction, as well as Michael N. Evans and Rebecca E. Plummer for their generous guide on finding the suitable CO reference gas for cellulose samples and sharing some standards during our laboratory calibration, including the AKC and AWS α-cellulose secondary standard. We would also like to thank the two anonymous reviewers for providing feedback that greatly improved the manuscript, as well as to Valerie Daux and Michel Stievenard for their useful comments, and Milagros Rodríguez for her support. This research was supported by the Lamont-Doherty Earth Observatory Climate Center grant and by the US National Science Foundation (NSF) grant PLR-1504134, as well as AGS-1702789, OISE-1743738, AGS 15-02150, PLR16-03473, AGS-0959148, AGS-1203818, AGS-1338734 and AGS-1655188. We would also like to acknowledge the project THEMES supported by the BNP-Paribas Foundation. L.A.H. was partially supported by the Columbia University's Center for Climate and Life. D.M-B was partially funded by AGL-2015-73,190-JIN project, and Fulbright-Ministerio de Ciencia e Innovación (Spain) and Marie-Curie FP7-PEOPLE-2012-IE grants (No 329935) postdoctoral fellowships. M.L. was supported by an Early and Advanced Postdoc Mobility Fellowships from the Swiss National Science Foundation (project numbers: P2EZP2_152213 and P300P2_164637). This is LDEO contribution #8259.

Appendix. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemgeo.2018.09.007.

References

Anchukaitis, K.J., Evans, M.N., Lange, T., Smith, D.R., Leavitt, S.W., Schrag, D.P., 2008. Consequences of a rapid cellulose extraction technique for oxygen isotope and radiocarbon analyses. Anal. Chem. 80, 2035–2041. https://doi.org/10.1021/ac7020272.

Andreu, L., Planells, O., Gutiérrez, E., Helle, G., Schleser, G.H., 2008. Climatic significance of tree-ring width and $\delta^{13}C$ in a Spanish pine forest network. Tellus Ser. B Chem. Phys. Meteorol. 60, 771–781.

Andreu-Hayles, L., Leland, C., 2014. Dendrochronology, progress. In: Rink, W.J., Thompson, J. (Eds.), Encyclopedia of Scientific Dating Methods. Springer, Netherlands, pp. 1–12. https://doi.org/10.1007/978-94-007-6326-5_31-1.

Andreu-Hayles, L., Ummenhofer, C.C., Barriendos, M., Schleser, G.H., Helle, G., Leuenberger, M., Gutierrez, E., Cook, E.R., 2017. 400 Years of summer hydroclimate from stable isotopes in Iberian trees. Clim. Dyn. 49, 143–161. https://doi.org/10.1007/s00382-016-3332-z.

Boettger, T., Haupt, M., Knoeller, K., Weise, S.M., Waterhouse, J.S., Rinne, K.T., Loader, N.J., Sonninen, E., et al., 2007. Wood cellulose preparation methods and mass spectrometric analyses of delta C-13, delta O-18, and nonexchangeable delta H-2 values in cellulose, sugar, and starch: an interlaboratory comparison. Anal. Chem. 79, 4603–4612. https://doi.org/10.1021/ac0700023.

Brand, W.A., Assonov, S.S., Coplen, T.B., 2010. Correction for the ^{17}O interference in $\delta(^{13}C)$ measurements when analyzing CO_2 with stable isotope mass spectrometry. Pure Appl. Chem. 82, 1719–1733. https://doi.org/10.1351/PAC-REP-09-01-05.

Brendel, O., Iannetta, P.P.M., Stewart, D., 2000. A rapid and simple method to isolate pure alpha-cellulose. Phytochem. Anal. 11, 7–10. https://doi.org/10.1002/(sici)1099-1565(200001/02)11:1<7::aid-pca488>3.0.co;2-u.

Brenna, J.T., Corso, T.N., Tobias, H.J., Caimi, R.J., 1997. High-precision continuous-flow isotope ratio mass spectrometry. Mass Spectrom. Rev. 16, 227–258. https://doi.org/10.1002/(sici)1098-2787(1997)16:5<227::aid-mas1>3.0.co;2-j.

Brookman, T., Whittaker, T., 2012. Experimental assessment of the purity of α-cellulose produced by variations of the Brendel method: implications for stable isotope ($\delta^{13}C$, $\delta^{18}O$) dendroclimatology. Geochem. Geophys. Geosyst. 13, Q0AI01. https://doi.org/10.1029/2012gc004215.

CRC Handbook of Chemistry and Physics, 2005. Atomic masses and abundances. In: Lide, D.R. (Ed.), CRC Handbook of Chemistry and Physics, Internet Version 2005. 2005 CRC Press, Boca Raton, FL. www.hbcpnetbase.com.

Cullen, L.E., MacFarlane, C., 2005. Comparison of cellulose extraction methods for analysis of stable isotope ratios of carbon and oxygen in plant material. Tree Physiol. 25, 563–569. https://doi.org/10.1093/treephys/25.5.563.

Eiler, J.M., Schauble, E., 2004. (OCO)-O-18-C-13-O-16 in earth's atmosphere. Geochim. Cosmochim. Acta 68, 4767–4777. https://doi.org/10.1016/j.gca.2004.05.035.

English, N.B., McDowell, N.G., Allen, C.D., Mora, C., 2011. The effects of alpha-cellulose extraction and blue-stain fungus on retrospective studies of carbon and oxygen isotope variation in live and dead trees. Rapid Commun. Mass Spectrom. 25, 3083–3090. https://doi.org/10.1002/rcm.5192.

Evans, M.N., Schrag, D.P., 2004. A stable isotope-based approach to tropical dendroclimatology. Geochim. Cosmochim. Acta 68, 3295–3305. https://doi.org/10.1016/j.gca.2004.01.006.

Evans, M.N., Selmer, K.J., Breeden, B.T., Lopatka, A.S., Plummer, R.E., 2016. Correction algorithm for online continuous flow $\delta^{13}C$ and $\delta^{18}O$ carbonate and cellulose stable isotope analyses. Geochem. Geophys. Geosyst. 17, 3580–3588. https://doi.org/10.1002/2016GC006469.

Frank, D.C., Poulter, B., Saurer, M., Esper, J., Huntingford, C., Helle, G., Treydte, K., Zimmermann, N.E., et al., 2015. Water-use efficiency and transpiration across European forests during the Anthropocene. Nat. Clim. Chang. 5, 579–583. https://doi.org/10.1038/nclimate2614. http://www.nature.com/nclimate/journal/v5/n6/abs/nclimate2614.html (supplementary-information).

Gagen, M., McCarroll, D., Loader, N.J., Robertson, I., 2011. Stable isotopes in dendroclimatology: moving beyond 'potential'. In: Hughes, M.K., Swetnam, T.W., Diaz, H.F. (Eds.), Dendroclimatology. Developments in Paleoenvironmental Research, vol 11. Springer, Netherlands, pp. 147–172. https://doi.org/10.1007/978-1-4020-5725-0_6.

Gaudinski, J.B., Dawson, T.E., Quideau, S., Schuur, E.A.G., Roden, J.S., Trumbore, S.E., Sandquist, D.R., Oh, S.W., et al., 2005. Comparative analysis of cellulose preparation techniques for use with C-13, C-14, and O-18 isotopic measurements. Anal. Chem. 77, 7212–7224. https://doi.org/10.1021/ac050548u.

Gehre, M., Strauch, G., 2003. High-temperature elemental analysis and pyrolysis techniques for stable isotope analysis. Rapid Commun. Mass Spectrom. 17, 1497–1503. https://doi.org/10.1002/rcm.1076.

Green, J.W., 1963. Wood cellulose. In: Whistler, R.L., Green, J.W., BeMiller, J.N., Wolfrom, M.L. (Eds.), Methods in Carbohydrate Chemistry: Cellulose. 3. Academic Press, New York, pp. 9–21.

Harada, M., Watanabe, Y., Nakatsuka, T., Tazuru-Mizuno, S., Horikawa, Y., Sugiyama, J., Tsuda, T., Tagami, T., 2014. Alpha-cellulose extraction procedure for the tropical tree sungkai (*Peronema canescens* Jack) by using an improved vessel for reliable paleoclimate reconstruction. Geochem. J. 48, 299–307. https://doi.org/10.2343/geochemj.2.0306.

Kagawa, A., Sano, M., Nakatsuka, T., Ikeda, T., Kubo, S., 2015. An optimized method for stable isotope analysis of tree rings by extracting cellulose directly from cross-sectional laths. Chem. Geol. 393–394, 16–25. https://doi.org/10.1016/j.chemgeo.2014.11.019.

Knoller, K., Boettger, T., Weise, S.M., Gehre, M., 2005. Carbon isotope analyses of cellulose using two different on-line techniques (elemental analysis and high-temperature pyrolysis) - a comparison. Rapid Commun. Mass Spectrom. 19, 343–348. https://doi.org/10.1002/rcm.1793.

Kornexl, B.E., Gehre, M., Höfling, R., Werner, R.A., 1999. On-line δ18O measurement of organic and inorganic substances. Rapid Commun. Mass Spectrom. 13, 1685–1693. https://doi.org/10.1002/(sici)1097-0231(19990830)13:16<1685::aid-rcm699>3.0.co;2-9.

Kress, A., Saurer, M., Büntgen, U., Treydte, K.S., Bugmann, H., Siegwolf, R.T.W., 2009. Summer temperature dependency of larch budmoth outbreaks revealed by alpine tree-ring isotope chronologies. Oecologia 160, 353–365.

Kürschner, K., Popik, M.G., 1962. Zur Analyse von Hölzern. In: Holzchemie, Mitteilungen zur Chemie, Physik, Biologie, und Technologie des Holzes. vol 16/1. Technischer Verlag H. Cram, Berlin, pp. 1–11.

Laumer, W., Andreu, L., Helle, G., Schleser, G.H., Wieloch, T., Wissel, H., 2009. A novel approach for the homogenization of cellulose to use micro-amounts for stable isotope analyses. Rapid Commun. Mass Spectrom. 23, 1934–1940.

Leavitt, S.W., Danzer, S.R., 1993. Method for batch processing small wood samples to holocellulose for stable-carbon isotope analysis. Anal. Chem. 65, 87–89. https://doi.org/10.1021/ac00049a017.

Li, Z.H., Labbe, N., Driese, S.G., Grissino-Mayer, H.D., 2011. Micro-scale analysis of tree-ring delta O-18 and delta C-13 on alpha-cellulose spline reveals high-resolution intra-annual climate variability and tropical cyclone activity. Chem. Geol. 284, 138–147. https://doi.org/10.1016/j.chemgeo.2011.02.015.

Lin, W., Noormets, A., King, J.S., Sun, G., McNulty, S., Domec, J.-C., 2017. An extractive removal step optimized for a high-throughput alpha-cellulose extraction method for delta C-13 and delta O-18 stable isotope ratio analysis in conifer tree rings. Tree Physiol. 37, 142–150. https://doi.org/10.1093/treephys/tpw084.

Loader, N.J., Robertson, I., Barker, A.C., Switsur, V.R., Waterhouse, J.S., 1997. An improved technique for the batch processing of small wholewood samples to α-cellulose. Chem. Geol. 136, 313–317.

Loader, N.J., Robertson, I., McCarroll, D., 2003. Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings. Palaeogeogr. Palaeoclimatol. Palaeoecol. 196, 395–407.

Loader, N.J., Street-Perrott, F.A., Daley, T.J., Hughes, P.D.M., Kimak, A., Levanič, T., Mallon, G., Mauquoy, D., et al., 2015. Simultaneous determination of stable carbon, oxygen, and hydrogen isotopes in cellulose. Anal. Chem. 87, 376–380. https://doi.org/10.1021/ac502557x.

Macfarlane, C., Warren, C.R., White, D.A., Adams, M.A., 1999. A rapid and simple method for processing wood to crude cellulose for analysis of stable carbon isotopes in tree rings. Tree Physiol. 19, 831–835.

Martin-Benito, D., Anchukaitis, J.K., Evans, N.M., del Río, M., Beeckman, H., Cañellas, I., 2017. Effects of drought on xylem anatomy and water-use efficiency of two co-occurring pine species. Forests 8. https://doi.org/10.3390/f8090332.

McCarroll, D., Loader, N.J., 2004. Stable isotopes in tree rings. Quat. Res. Rev. 23, 771–801.

Paul, D., Skrzypek, G., Fórizs, I., 2007. Normalization of measured stable isotopic compositions to isotope reference scales – a review. Rapid Commun. Mass Spectrom. 21, 3006–3014. https://doi.org/10.1002/rcm.3185.

Pichlmayer, F., Blochberger, K., 1988. Isotopic abundance analysis of carbon, nitrogen and sulfur with a combined elemental analyzer-mass spectrometer system. Fresenius J. Anal. Chem. 331, 196–201.

Richard, B., Quilès, F., Carteret, C., Brendel, O., 2014. Infrared spectroscopy and multivariate analysis to appraise α-cellulose extracted from wood for stable carbon isotope measurements. Chem. Geol. 381, 168–179. https://doi.org/10.1016/j.chemgeo.2014.05.010.

Rinne, K.T., Boettger, T., Loader, N.J., Robertson, I., Switsur, V.R., Waterhouse, J.S., 2005. On the purification of α-cellulose from resinous wood for stable isotope (H, C and O) analysis. Chem. Geol. 222, 75–82. https://doi.org/10.1016/j.chemgeo.2005.06.010.

Saurer, M., Siegwolf, R.T.W., Schweingruber, F.H., 2004. Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. Glob. Chang. Biol. 10, 2109–2120.

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

Treydte, K.S., Schleser, G.H., Helle, G., Frank, D.C., Winiger, M., Haug, G.H., Esper, J., 2006. The twentieth century was the wettest period in northern Pakistan over the past millennium. Nature 440, 1179–1182.

Wieloch, T., Helle, G., Heinrich, I., Voigt, M., Schyma, P., 2011. A novel device for batch-wise isolation of α-cellulose from small-amount wholewood samples. Dendrochronologia 29, 115–117.

Woodley, E.J., Loader, N.J., McCarroll, D., Young, G.H.F., Robertson, I., Heaton, T.H.E., Gagen, M.H., 2012. Estimating uncertainty in pooled stable isotope time-series from tree-rings. Chem. Geol. 294–295, 243–248. https://doi.org/10.1016/j.chemgeo.2011.12.008.