Resolving challenges in the development of a protocol for δ^{18}O determinations on tree-ring cellulose

John du Plessis, Dipayan Paul, Margot Kuitems, Anita Aerts-Bijma, Neil J. Loader, Harro A.J. Meijer & Michael Dee

View supplementary material 🗗

Published online: 31 Jul 2025.

Submit your article to this journal 🗗

Article views: 234

View related articles 🗗

View Crossmark data 🗗

Taylor & Francis
Taylor & Francis Group

Resolving challenges in the development of a protocol for $\delta^{18}O$ determinations on tree-ring cellulose

John du Plessis [a], Dipayan Paul [a], Margot Kuitems [a], Anita Aerts-Bijma [a],
Neil J. Loader [b], Harro A.J. Meijer [a] and Michael Dee [a]

aCentre for Isotope Research, University of Groningen, Groningen, Netherlands; bDepartment of Geography, Swansea University, Swansea, UK

ABSTRACT

Tree rings can provide annual records of environmental and climatic conditions. These records can be obtained through the physical characteristics of tree rings or the isotopic composition of their structural elements. Oxygen isotope chronologies are created by objectively combining data from trees. The diachronic patterns observed in the $\delta^{18}O$ of the tree-ring cellulose represent tree-environment interactions. The abundance of ^{18}O in tree-ring cellulose is closely linked to hydroclimate, and is influenced by source water $\delta^{18}O$ and atmospheric humidity. Long sequences of annually resolved tree-ring $\delta^{18}O$ values have been used, to good effect, in the dating of archaeological timbers and as proxies in the reconstruction of climatic variables. In this research we have established a working methodology for producing and measuring $\delta^{18}O$ in tree-ring α-cellulose at the University of Groningen. We have demonstrated an average precision of approximately 0.2‰ under a variety of conditions, which exceeds the expected performance of continuous flow IRMS techniques. Difficulties were encountered during the calibration of tree-ring cellulose $\delta^{18}O$ determinations using non-cellulose, organic reference materials. A difference in pyrolysis behaviour or one, or more compromised materials, resulted in poor agreement between measured and expected $\delta^{18}O$ values on cellulose standards. This opens the possibility for further study. Analysis of cellulose standards alongside water reference materials and an independent quality control standard proved successful, resulting in a number of cellulose standards being accurately placed on the VSMOW-SLAP scale, including the intercomparison and *de facto* reference material IAEA-C3 holocellulose.

1. Introduction

Trees are capable of providing an annual record of environmental and climatic conditions owing to their seasonal growth rings. Information can be obtained through the

---

Supplemental data for this article can be accessed online at https://doi.org/10.1080/10256016.2025.2529213.

interpretation of the physical or morphological characteristics of tree rings [1], and similarly, through the isotopic composition of their structural constituents (often α-cellulose) [2]. Isotope [3,4] and ring-width chronologies [1,5] can be constructed by combining data from multiple trees of overlapping ages. These chronologies do not contain a simple archive of isotope concentrations in air and water, but a record of interactions between trees and their environment [2]. In recent decades, tree-ring stable isotope analysis has become more widespread, and [18]O is among the isotopes receiving increased attention [6,7]. The hydrogen and oxygen isotope composition of tree-ring cellulose is strongly related to hydroclimate, as it is primarily a function of source water oxygen isotope composition and atmospheric vapour pressure deficit, which itself is tied to relative humidity [8]. Although it is advantageous in many cases to analyse both hydrogen and oxygen isotopes, there are additional challenges that arise due to the propensity for a certain fraction of the hydrogen atoms in cellulose to exchange with those in the environment [2]. Long, annually resolved sequences recording the oxygen isotope composition of tree-ring cellulose have been used to good effect in the dating of timbers [3,9] and reconstructions of climatic variables over long periods [10–12]. Tree-ring cellulose is a chemically resistant and stable biopolymer, making it exceptionally durable. Under favourable conditions, isotope signatures can remain intact in wood for thousands [13], or even millions of years [14,15], after (partial) fossilisation.

The methods employed in tree-ring stable isotope analysis have been in a state of continuous evolution, with considerable diversification of techniques for sampling, sample preparation, and measurement [2,16,17]. As methods have advanced, it has become possible to analyse larger numbers of samples while consuming less starting material [16,17]. However, sampling and sample preparation still tends to be labour intensive, partially due to the high sample purity and homogeneity required for annual resolution, high-precision measurements [16]. Obtaining sufficiently accurate, precise and reproducible measurements of tree-ring cellulose oxygen isotope composition is not trivial. Much research has been done to improve the efficiency and effectiveness of these techniques, and a large range of approaches has been explored [16]. As methods develop, direct intercomparison of measurements [18,19] and showcasing of procedures becomes more important, especially considering the diversity of materials and instruments currently in use.

The concentration of [18]O in a given sample is conventionally expressed relative to the most abundant oxygen isotope, [16]O. This is reported quantitatively as $\delta^{18}O$. In natural substances this value is small, expressed in parts-per-thousand or 'per mille' (‰), and is defined as,

$$\delta^{18}O = \left(\frac{R_{sample}}{R_{RM}}\right) - 1, \tag{1}$$

where R_{sample} and R_{RM} are the ratios of ^{18}O/^{16}O in a sample and a reference material (RM) of known isotopic composition [2]. Measurements of isotope concentrations are always reported relative to a scale which is anchored by one or more RMs [20]. Additionally, many internationally recognised[1] RMs exist for which the isotopic ratios have been determined to an extremely high level of accuracy and precision. Typically these serve as intercomparison materials and are used for calibrating instruments or correcting isotope ratio measurements [20]. In the case of ^{18}O, the VSMOW-SLAP scale is used when reporting values. This scale is defined by the two primary RMs, Vienna Standard Mean Ocean Water (VSMOW, $\delta^{18}O = 0.00$ ‰) and Standard Light Antarctic Precipitation (SLAP, $\delta^{18}O = -55.50$ ‰) [20]. VSMOW and SLAP are exhausted,

the VSMOW-SLAP scale is realised using the RMs VSMOW2 and SLAP2, with the same values (but with a small added uncertainty). Currently no internationally recognised cellulose $\delta^{18}O$ RM exists. Therefore, there is a near universal reliance on locally calibrated cellulose quality control (QC) standards or other organic RMs. Cellulose references are available for carbon isotopes, but have been deemed unsuitable to 'provide a dependable anchor to the VSMOW $\delta^{18}O$ scale' [21]. Despite this fact, cellulose is endorsed as 'a local working reference material' and to be utilised during the analysis of cellulose samples [21]. IAEA-C3 is an internationally recognised cellulose RM for ^{13}C and ^{14}C abundance [22,23]. It has been subject to $\delta^{18}O$ analysis since at least 1993 [24] and has had a wide range of determined $\delta^{18}O$, extending from 31.3 [25] to 33.1 ‰ [26] on the VSMOW-SLAP scale. A commonly quoted assigned $\delta^{18}O$ of IAEA-C3 is 32.6 ‰ [18,26], although this varies between publications [19,27–34]. Whilst some studies provide batch numbers and reference to intercomparison studies, these assigned values are sometimes provided without a clear source or additional details regarding measurement and calibration. As this material is often used to calibrate or correct $\delta^{18}O$ values of tree-ring cellulose, it would be beneficial to discuss the determination of its $\delta^{18}O$ value openly.

The purpose of this research is to establish a working protocol and a performance baseline for $\delta^{18}O$ determination on tree-ring α-cellulose at the Centre for Isotope Research (CIO) of the University of Groningen, and to investigate the efficacy of on-hand RMs to correct cellulose $\delta^{18}O$ values and accurately calibrate cellulose QC standards including IAEA-C3. To achieve this, a variety of tree ring samples, along with QC standards and certified references were selected and analysed for $\delta^{18}O$ via continuous flow Pyrolysis-IRMS in order to determine precision, accuracy and repeatability.

2. Materials and methods

2.1. Samples

The samples selected for this work included different species of wood (both gymnosperm and angiosperm), along with various organic RMs and QC standards. Table 1 contains a list of all samples analysed including some standards kindly provided by Swansea University for the purposes of intercomparison (IAEA-C3 holocellulose and Sigma Aldrich α-cellulose No. C-8002 Lot 92F-0242). The wood samples were split into whole, annual growth rings before α-cellulose extraction, with the exception of the ash (*Fraxinus*) samples, from which only latewood was collected.

2.2. Wood preparation and ring cutting

Wood samples were selected from pieces already on hand with the goal of including both resinous gymnosperm, and angiosperm examples. Wood was received as trunk or branch transverse cross sections, and were sanded or pared with a razor blade to reveal the annual growth rings. After inspecting the quality of the rings, a suitable radial section was selected, and a rectangular lath was carefully fashioned using a crosscut hand saw. The outer surfaces of the lath were shaved off with a razor blade, removing any surface contaminants and rendering the rings clearly visible. With the aid of a binocular microscope, individual tree rings were cut from the laths using a scalpel, and in the case of the ash samples, earlywood and latewood were collected separately. The collected rings were cut into thin slivers and subsequently oven dried (80 °C, 15 min), then left to

Table 1. Summary of all materials analysed in this study. Where applicable, age corresponds to year (CE) of manufacture or collection.

Sample	Measured fraction	Origin	Age
Oak (*Quercus* sp.)	α-cellulose	Lauwersoog, Netherlands	2022 (felled)
Ash (*Fraxinus* sp.)	α-cellulose	Ens, Netherlands	2021 (felled)
Pine (*Pinus* sp.)	α-cellulose	Assen, Netherlands	2023
IAEA-C3	Holocellulose	-	1989
IAEA-C3[a]	Holocellulose	-	1989
IAEA-600	Caffeine	-	2004
IAEA-601	Benzoic acid	-	2004
IAEA-602	Benzoic acid	-	2004
Benzoic acid (in-house standard)	Benzoic acid	-	-
USGS56	S. African red ivorywood powder	-	2016
Sigma Aldrich cellulose 71351 (Lot BCBW8301)	α-cellulose	-	2019
Sigma Aldrich cellulose (Lot 92F-0242)[a]	α-cellulose	-	-

[a]Provided by Swansea University.

rest in a fume hood (RT, 1 h). Each individual ring, as well as any duplicates and standards were registered in the CIO database and 40–50 mg was weighed and transferred into test tubes for cellulose extraction.

2.3. α-cellulose extraction and homogenisation

The procedure followed for α-cellulose extraction is based on the protocol of Dee et al. [35,36]. Wood samples were subjected to the following extraction procedure:

(1) 5.47 % w/vol (1.5 M) HCl (80 °C, 20 min)
(2) Rinse 3 times with reverse osmosis (RO) water
(3) 17.5 % w/vol NaOH (30 °C, 60 min, N_2 atmosphere, ultrasonication)
(4) Rinse 5 times with RO water
(5) 5.47 % w/vol HCl (80 °C, 20 min)
(6) Rinse 3 times with RO water
(7) 1.5 % w/vol $NaClO_2$, acidified with HCl (pH 3) (80 °C, overnight)
(8) Replace $NaClO_2$ solution (80 °C, 3 h)
(9) Rinse 3 times with RO water.

Analytical reagents used were aqueous HCl, 37–38 % (Boom B.V., Meppel, Netherlands, Art. No. 76021889), NaOH pellets, ≥ 99 % (Merck KGaA, Darmstadt, Germany, Art. No. 1.06498), $NaClO_2$ powder, puriss. p.a., 80 % (RT) (Sigma-Aldrich, Germany, Art. No. 71388).

Extraction procedures, with and without organic solvent washes, were tested on the resinous pine rings. These individual rings were split into multiple subsamples, with one subsample receiving a solvent treatment and others not. Organic solvent washed samples were treated with acetone (45 °C, 6 h) and dried in a fume hood (RT, 36 h), then soaked in RO water (45 °C, 6 h) and left to dry in a fume hood (RT, overnight) prior to α-cellulose extraction. After chemical extraction, the resulting tree-ring α-cellulose was left in test tubes, submerged in RO water and ultrasonically homogenised [37] (60 W pulsed, 0.3 s on, 1 s off) for 15–30 s (Sonopuls UW100, 3 mm ∅ sonotrode, Bandelin, Germany) in order to maintain homogeneity within samples. Thereafter, samples were frozen and freeze dried (≥ 24 h). IAEA-C3 holocellulose, which is distributed as compressed sheets, was also homogenised and freeze dried in the same way to greatly ease handling and weighing. Two subsamples of IAEA-C3 (40–50 mg) were also subjected to chemical α-cellulose extraction alongside the tree-ring samples.

2.4. Drying, weighing and storage

Aliquots of tree-ring α-cellulose with masses 0.90–1.10 mg (batch 1 & 2) and 0.45–0.55 mg (subsequent batches) were weighed and placed into 4 × 3 mm silver capsules (Elemtex, Gunnislake, United Kingdom), and manually crushed using a bespoke sample press to remove air and seal the capsules. Subsamples of homogenised IAEA-C3 cellulose with masses around 1.0, 0.5 and 0.1 mg were analysed to determine whether homogeneity, and therefore precision, would be maintained in smaller samples.

In order to minimise the effect of possible absorption of water vapour by the samples at this stage, the weighed, encapsulated samples were placed in a segmented stainless

steel box to be dried in a vacuum oven (50 °C, 1 h) and then, with heating turned off, left under vacuum until pyrolysis and measurement. IAEA-C3 holocellulose was used to evaluate the efficacy of the combined drying processes. This was done by measuring subsamples of IAEA-C3 which had been soaking in two different vials of water with $\delta^{18}O$ equivalent to VSMOW (0 ‰) and SLAP (−55.5‰), for nine days. These were dried identically to all the other cellulose samples before measurement. Additionally, 5 subsamples of IAEA-C3 cellulose were not vacuum dried, and stored at room temperature and atmospheric pressure until measurement. These were analysed to determine the necessity of the auxiliary vacuum drying.

2.5. Isotope measurement

The use of continuous flow Pyrolysis-IRMS systems to measure tree-ring stable isotope concentrations is a well-established technique [18,19,26,38]. Prepared *a*-cellulose, alongside standards and references were pyrolysed at 1450 °C with a vario PYRO cube elemental analyser (EA) (Elementar, Langenselbold, Germany) and the resulting CO was analysed using a precisION isotope ratio mass spectrometer (IRMS) (Elementar, Langenselbold, Germany). Within the EA, pyrolysis occurs in a glassy carbon tube that is filled with a graphite crucible, glassy carbon chips, carbon black, as well as graphite and quartz wool according to the manufacturer's specifications. The system was left running overnight for the temperature to equilibrate and to flush completely with helium, during which blank measurements were performed until the detected CO flow decreased to around 100 arbitrary units on the EA thermal conductivity sensor, and the IRMS background ion current dropped below 1×10^{-11} A. The first measurement in a series is typically erroneous and must be discarded, therefore at the start of each series at least one sacrificial cellulose sample was pyrolysed to precondition the system by flushing it with CO. After relatively ^{18}O enriched samples (i.e. IAEA-602), a similar precaution was taken in order to buffer subsequent samples from potential memory effects. QC standards were placed throughout the series allowing for measurement performance to be evaluated continually and drift to be corrected if present. The masses of samples and RMs within the same batch were selected to ensure a similar quantity of CO was produced during pyrolysis. Using CO reference gas, measurement batches were bracketed with automated tests for amount-dependent effects on $\delta^{18}O$. Mass- or amount-dependent effects were not conclusively observed during analysis, thus, no corrections were applied.

2.5.1. Nitrogen contamination
Within the EA, elution of CO is paused by trapping the gas in a CO-trap and is only released after the contaminants such as Ar and N_2 have been removed completely. The presence of N_2 during CO elution leads to biases in CO measurements due to isobaric interference (molecular mass = 28). Initially, while analysing its isotopic composition, the CO peak, as detected by the IRMS, was bracketed by two monitoring gas pulses (machine reference gas), the average isotopic composition of which was used to determine the isotopic composition of the sample. Upon careful investigation, it was discovered that a very small and broad peak always appeared following the N_2 elution. This was much more pronounced when measuring nitrogen-containing materials such as IAEA-600 caffeine. Due to the variable nature of this peak area, the first monitoring

Table 2. RMs used that are securely placed on the VSMOW-SLAP scale.

Name	Material	$\delta^{18}O$ (VSMOW-SLAP) ‰	
		Assigned value	Uncertainty
IAEA-602	Benzoic acid	71.28	0.36
Sample 3	Water	44.93	0.04
Sample 1	Water	28.53	0.08
USGS56	Wood	27.23	0.03
IAEA-601	Benzoic acid	23.14	0.19
Sample 2	Water	14.67	0.04
VSMOW2	Water	0.00	0.02
IAEA-600	Caffeine	−3.48	0.53

peak was always contaminated. Thus, the release of CO was carefully timed such that the corresponding peak was released only when the baseline showed complete removal of the contaminating peak. Two monitoring pulses were thus positioned following the sample peak and were now isotopically very consistent. Additionally, a custom-built cover was placed on top of the sample carousel with a constant flow of N_2 (~100 ml/min) to displace any air present around the samples in an effort to reduce contamination by atmospheric oxygen and moisture. This issue is discussed further by Aerts-Bijma et al. [39].

2.5.2. Reference materials

Table 2 contains the $\delta^{18}O$ values of the RMs used in varying combinations throughout. Initially, IAEA-600, IAEA-601 and IAEA-602 [21] were used in a three-point linear fit to correct cellulose $\delta^{18}O$ values, but this resulted in poor agreement between determined and assigned values of references. Eventually IAEA-C3 and standard cellulose (Sigma Aldrich) was accurately calibrated using water references, with USGS56 [40] as a control reference. Calibrated IAEA-C3 was then used to correct measurements retroactively. The use of cellulose standards for correction ensures similar pyrolysis behaviour to that of the samples, fulfilling the identical treatment principle. For the purposes of cellulose calibration, VSMOW2 [41] was used alongside three other water references, 'Sample 1', 'Sample 2' and 'Sample 3'. These three are solutions prepared from IAEA-607 [42] and USGS46 [43] that had their $\delta^{18}O$ and relevant uncertainties confirmed by optical spectroscopy over 3 measurement days with 50 measurements each [39]. These water references were prepared and portioned by the United States Geological Survey in sealed silver tubes [44].

3. Results

3.1. Precision and measurement quality

$\delta^{18}O$ determinations on a number of samples and RMs are presented. These include those analysed before and after the changes implemented to mitigate N_2 interference, as outlined in section 2.5.1. They are given as mean $\delta^{18}O$ (‰ with respect to the VSMOW-SLAP scale) and standard deviations of all measured subsamples without added calibration uncertainty of RMs. The collection of QC and sample cellulose was measured with precision better than or equal to 'standard precision' for $\delta^{18}O$ determinations on tree-ring cellulose (± 0.3 ‰ [26]). Average $\delta^{18}O$ standard deviations are ± 0.21 ‰ for the cellulose

measurements acquired before addressing N_2 intrusion (section 3.1.1) and ± 0.20 ‰ for those analysed after changes were made (section 3.1.2).

3.1.1. Before changes to measurement procedure

Determinations of $\delta^{18}O$ on samples and standards in this section were influenced by N_2 interference. Consisting of three batches, these measurements (Table 3) have been single-point corrected using the calibrated value for IAEA-C3 of 32.70 ± 0.19 ‰ (Table 7). Replicate samples of different pine rings, which had been separated before α-cellulose extraction, are named according to which ring they belong (R3, R5 and R8) and whether they were solvent washed (R3 solv), a duplicate (R3 dup) or triplicate (R3 trip) sample.

Mean $\delta^{18}O$ values of tree-ring cellulose show clear annual differences in all three tree species. The measurements on pine rings show that the inter-ring (inter-annual) variations in $\delta^{18}O$ of this sample are smaller than the variations between replicate samples from the same ring. This is visible in Figure 1. The $\delta^{18}O$ of IAEA-C3 with sample masses between 0.1 and 1.0 mg in batch 1 suggests no worsening of measurement precision due to smaller sample masses, meaning that the effect of sample size on homogeneity is not an issue at

Table 3. Calibrated $\delta^{18}O$ as mean (μ) and standard deviation (σ) with number of subsamples measured (n). Target masses of cellulose samples were 1 mg in batches 1 and 2, and 0.5 mg in batch 3. Two sets of IAEA-C3 with target masses of 0.1 and 0.5 mg in batch 2 are the exception. Rx denotes ring number x of a given wood sample (Ash, Oak or Pine). Samples of IAEA-C3 in bold were used for single-point correction in their respective batch.

| Name | Measurement batch | Calibrated $\delta^{18}O$ (VSMOW-SLAP) ‰ | | |
		μ	σ	n
Ash R16	1	31.79	0.08	4
Ash R18	1	32.58	0.12	5
Ash R21	1	32.31	0.21	5
Ash R22	1	32.10	0.28	5
Ash R23	1	32.12	0.32	5
IAEA-C3	**1**	**32.70**	**0.38**	**5**
IAEA-C3 undried	1	32.60	0.19	5
IAEA-C3 0.1 mg	**2**	**30.20**	**0.16**	**4**
IAEA-C3 0.5 mg	2	32.84	0.22	5
IAEA-C3 1.0 mg	2	32.70	0.13	5
Oak R2	2	30.28	0.38	5
Oak R3	2	28.80	0.12	5
Oak R4	2	29.76	0.29	5
Oak R5	2	29.88	0.36	5
Oak R6	2	30.53	0.10	5
Pine R3	3	27.42	0.26	3
Pine R3 dup	3	26.91	0.25	3
Pine R3 trip	3	27.24	0.09	3
Pine R5	3	27.69	0.10	3
Pine R5 dup	3	27.60	0.19	3
Pine R8	3	28.40	0.15	3
Pine R8 dup	3	28.56	0.14	3
Pine SOLV R3	3	27.76	0.46	3
Pine SOLV R5	3	27.66	0.24	3
Pine SOLV R8	3	28.56	0.10	3
IAEA-601	3	26.45	0.22	5
IAEA-602	3	70.37	1.05	5
IAEA-C3	**3**	**32.70**	**0.23**	**6**
IAEA-C3 α-cell	3	31.62	0.25	6
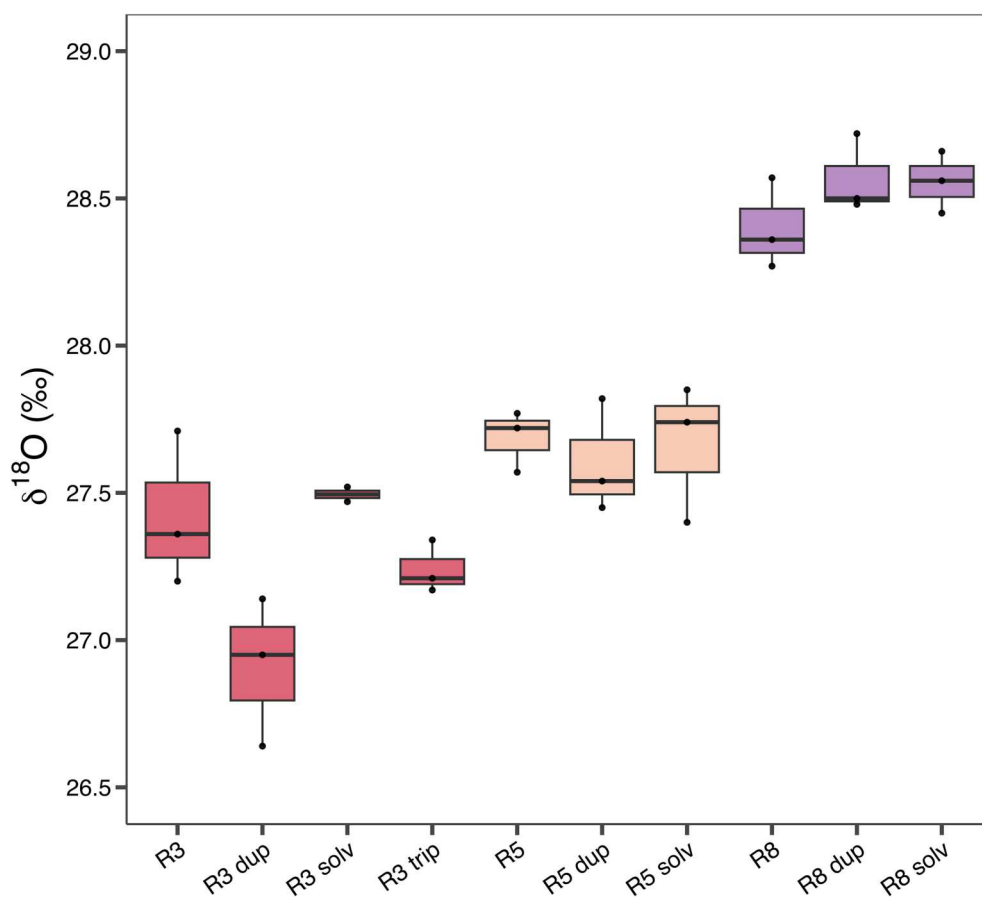
Figure 1. Boxplot of *a*-cellulose $\delta^{18}O$ from solvent washed and unwashed pine tree ring replicates. Black points represent individual subsample measurements.

these masses. *a*-cellulose extracted from IAEA-C3 has a depleted $\delta^{18}O$ (-1.08 ± 0.14 ‰) compared to IAEA-C3 holocellulose.

3.1.2. After changes to measurement procedure

Results from measurements performed after the interference of N_2 had been mitigated and the sample carousel cover had been installed, are shown in Table 4. The results shown in section 3.3 were measured under the same conditions.

A number of measurements affected by N_2 contamination produced outliers, which were identified using the interquartile range (IQR) method. Values 1.5×IQR below the 1st quartile or 1.5×IQR above the 3rd quartile were considered outliers. These contributed to the larger spread of $\delta^{18}O$ values seen in some of the samples, alongside memory effects, which are absent in measurement batches after the change in measurement procedure.

3.2. Effects of organic solvent washes and auxiliary drying

Five subsamples of IAEA-C3, labelled 'undried', were measured alongside five which had been vacuum dried (Table 3), producing a mean $\delta^{18}O$ and standard deviation of 32.6 ±

Table 4. Mean (μ) and standard deviation (σ) of batch 4 $\delta^{18}O$ measurements, along with the number of measured subsamples (n). Measurements were calibrated with IAEA-600, IAEA-601 and IAEA-602.

Sample	Measurement batch	Calibrated $\delta^{18}O$ (VSMOW-SLAP) ‰		n
		μ	σ	
IAEA-600	4	−4.14	0.27	5
IAEA-601	4	24.03	0.29	5
IAEA-602	4	70.94	0.68	5
IAEA-C3	4	31.22	0.27	8
Swansea IAEA-C3	4	31.14	0.24	5
Swansea Sigma cellulose	4	25.67	0.18	5
Sigma cellulose 71351	4	31.29	0.18	11

0.19 ‰ and 32.7 ± 0.38 ‰, respectively. Cellulose samples that were exposed to water of known oxygen isotope composition before drying and measurement show no significant difference in their $\delta^{18}O$. The calibrated $\delta^{18}O$ of the untreated, VSMOW, and SLAP treated IAEA-C3 were 32.70 ± 0.16 ‰, 32.85 ± 0.47 ‰, and 33.01 ± 0.20 ‰, respectively, with an ANOVA yielding a p-value of 0.65.

It can be concluded that the drying protocol is robust enough to remove any water that was present in the samples during α-cellulose extraction, and furthermore, that there was no detectable isotopic exchange.

Median absolute deviation (MAD) was calculated for the measured $\delta^{18}O$ values of solvent washed and unwashed replicate pine samples. Subsample measurements with deviation greater than three MADs were deemed to be outliers and were excluded [45]. An ANOVA performed on replicate pine sample $\delta^{18}O$ values results in p-values of 0.043, 0.855 and 0.297 for R3, R5 and R8, respectively. These suggest that only one sample group, R3 has a potentially significant difference in $\delta^{18}O$ between replicate samples. Figure 1 contains a boxplot of the replicate sample measurements of each group (R3, R5 and R8). The measurements of R3 subsamples have more variability than R5 and R8. These other two sample groups strongly suggest that there is no difference in mean $\delta^{18}O$ between replicates, whether solvent washed or not.

3.3. Correction, calibration and intercomparison

Measurements in batch 4 were calibrated using IAEA Benzoic acid and Caffeine, (Table 1), resulting in the $\delta^{18}O$ values shown in Table 5 and in Figure 2. The three-point linear

Table 5. Calibrated $\delta^{18}O$ of standards and RMs compared to expected values from the literature. Measurements were corrected using IAEA-600, IAEA-601 and IAEA-602.

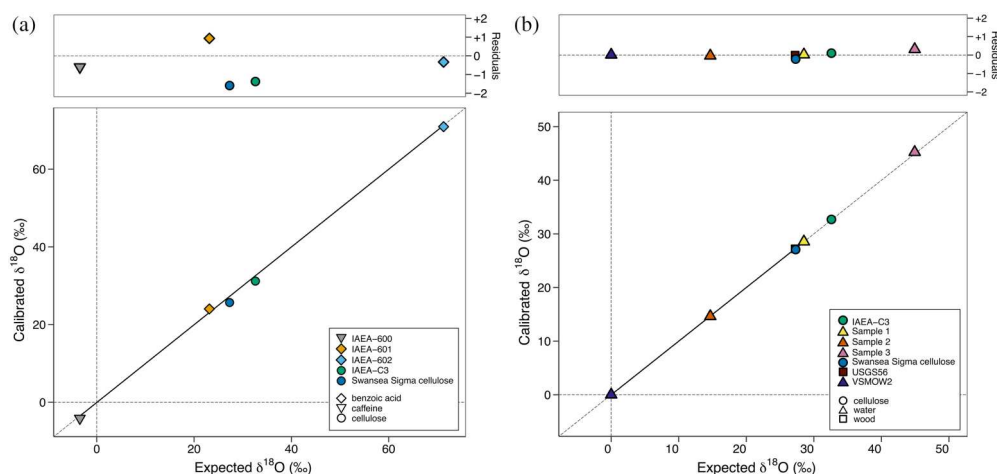| Name	Calibrated $\delta^{18}O$ (VSMOW-SLAP) ‰		Expected $\delta^{18}O$ (VSMOW-SLAP) ‰		n
	μ	σ	Assigned value	Uncertainty	
IAEA-600	−4.14	0.27	−3.48	0.53	5
IAEA-601	24.03	0.29	23.14	0.19	5
IAEA-602	70.94	0.68	71.28	0.36	5
IAEA-C3	31.22	0.27	32.6	-	8
Swansea IAEA-C3	31.14	0.24	32.6	-	5
Swansea Sigma cellulose	25.67	0.18	27.3	-	5
USGS56	25.04	0.26	27.23	0.03	6

Figure 2. Calibrated and expected (reported) $\delta^{18}O$ of samples corrected with (a) IAEA-600, IAEA-601 and IAEA-602, and (b) reference waters. The solid black line shows a linear fit of the RMs used for calibration.

calibration (slope 1.0826, constant −0.7979 and R^2 0.9993) with IAEA-600, 601 and 602 produced a corrected $\delta^{18}O$ of 31.22 ± 0.27 ‰ for IAEA-C3, a full 1.38 ± 0.01 ‰ lower than the expected value of 32.6 ‰ [18,26]. Similarly, the Sigma Aldrich cellulose obtained from Swansea University had a mean $\delta^{18}O$ that was 1.6 ± 0.08 ‰ lower than the expected value of 27.3 ‰. These measurements were repeated with the addition of newly purchased USGS56 ivorywood powder.

When measured and calibrated using IAEA-600, 601 and 602, USGS56 produced a $\delta^{18}O$ of 25.04 ± 0.26 ‰, which is significantly different from the expected value of 27.23 ± 0.03 ‰, indicating that one or more of the IAEA references (600, 601 and 602), and not the cellulose standards were likely responsible for the discrepancies in measured and expected $\delta^{18}O$. When these same samples are single-point corrected using USGS56, the cellulose standards have a calibrated $\delta^{18}O$ much closer to their expected values (Table 6).

A number of cellulose and wood standards were measured alongside water references and calibrated using VSMOW2, Sample 1, and Sample 2 (section 2.5.2) in a three-point linear fit (slope 1.0794, constant −1.4914 and R^2 0.9999). The calibrated and expected $\delta^{18}O$ values are in good agreement, with relatively low uncertainties. This can be seen in the smaller residuals in Figure 2(b) compared to those in Figure 2(a). The resulting calibrated $\delta^{18}O$ value of IAEA-C3 is 32.70 ± 0.19 ‰.

Table 6. Calibrated and expected $\delta^{18}O$ of samples that were single-point corrected with USGS56, presented as a mean (μ) and standard deviation (σ) alongside expected $\delta^{18}O$ and relevant uncertainty.

Name	Calibrated $\delta^{18}O$ (VSMOW-SLAP) ‰		Expected $\delta^{18}O$ (VSMOW-SLAP) ‰		
	μ	σ	Assigned value	Uncertainty	n
IAEA-600	0.92	0.22	−3.48	0.53	3
IAEA-601	25.78	0.59	23.14	0.19	3
IAEA-602	69.48	0.66	71.28	0.36	3
IAEA-C3	32.86	0.44	32.6	-	3
Swansea Sigma cellulose	27.49	0.02	27.3	-	3
USGS56	27.23	0.24	27.23	0.03	6

4. Discussion

4.1. Measurement quality

The precision of measurements performed during this research did not change dramatically as methods developed. Sufficient measurement precision was maintained between tree species, sample masses, as well as measurement and a-cellulose extraction procedures. However, repeatability and accuracy improved. Outliers were greatly diminished after the effects of N_2 contamination were mitigated, and changes to RM selection led to more accurate $\delta^{18}O$ measurements.

It has been argued that it is necessary to rely on true statistical replicates in order to estimate uncertainty accurately [46]. This involves splitting samples directly after sampling, and treating them as separate samples during cellulose extraction and measurement. Mean $\delta^{18}O$ determinations on replicate pine tree rings conducted in this manner in this study results in an average intra-replicate standard deviation of 0.20 ‰, with each replicate result consisting of 3 measured subsamples. When combining the replicate subsamples of R3, R5 and R8, the resulting average, inter-replicate standard deviation of all subsample $\delta^{18}O$ measurements is 0.24 ‰. As inter-replicate and intra-replicate standard deviations both remain around 0.2 ‰, it is then reasonable to forego the subdivision of every individual tree ring before a-cellulose extraction and rather rely on subsamples of the resulting a-cellulose.

Since inter-annual variations in tree-ring $\delta^{18}O$ can be small, it is critical that a representative number of trees are sampled and that high-precision, annually resolved measurements are obtained for applications such as palaeoclimatic reconstruction [2] or $\delta^{18}O$ tree-ring dating [3]. Our measurements satisfy these requirements and are able to capture annual $\delta^{18}O$ variability within tree-ring a-cellulose.

4.2. Evaluation of protocols and methods

The methods employed during sampling and a-cellulose extraction resulted in tree-ring a-cellulose separated into individual fibres which were small and mixed enough for acceptable homogeneity. Tests on resinous pine rings suggest that an organic solvent

Table 7. Corrected results of the calibration of cellulose QC standards using VSMOW2, Sample 1 and Sample 2 water references. Calibrated $\delta^{18}O$ is presented as a mean (μ) and combined measurement and correction uncertainty of n subsample measurements. VSMOW2, which was measured at the beginning and end of the sequence, served also as a drift correction standard.

Name	Calibrated $\delta^{18}O$ (VSMOW-SLAP) ‰		Expected $\delta^{18}O$ (VSMOW-SLAP) ‰		n
	μ	Uncertainty	Assigned value	Uncertainty	
VSMOW2 series 1	0.01	0.03	0	0.02	5
IAEA-C3	32.70	0.19	32.6	-	4
Sample 2	14.63	0.05	14.67	0.04	4
sigma cellulose 71351	32.76	0.15	-	-	4
Sample 1	28.55	0.17	28.53	0.08	4
USGS56	27.22	0.11	27.23	0.03	4
Sample 3	45.24	0.10	44.93	0.04	4
Swansea Sigma cellulose	27.08	0.11	27.3	-	4
VSMOW2 series 2	0.01	0.06	0	0.02	5

wash is not necessary during the preparation of these samples for ^{18}O analysis. A study on purifying α-cellulose from Scots pine (*Pinus sylvestris* L.) by Rinne et al. [47] reached the same conclusions after they observed no difference in $\delta^{18}O$ of solvent washed and unwashed samples. However, the composition and concentration of extractives (such as oils, gums, and resins [48]) differs between species and wood ages [48], and thus, organic solvent treatments may be necessary under different circumstances.

Experiments with two water samples, with a known difference in $\delta^{18}O$ of 55.5 ‰, showed that freeze drying and auxiliary vacuum drying removes any water present during cellulose extraction and homogenisation. Additionally, if there was any meaningful isotopic exchange it would have been detected. Experiments performed by Rinne et al. on oak samples that were pretreated in ^{18}O enriched water similarly showed no effect on final $\delta^{18}O$ measurements [47].

The $\delta^{18}O$ values obtained on vacuum dried IAEA-C3 samples that were stored under vacuum before measurement are not significantly different from those that were not vacuum dried and were stored in plain atmosphere. This indicates that there is either very little absorption of atmospheric water vapour by cellulose during storage or samples are affected equally during the short time between loading of the sample carousel and pyrolysis. The introduction of the nitrogen-flushed carousel cover was aimed at addressing this uncertainty, but these measurements have not yet been repeated since its implementation.

4.2.1. Updated measurement procedure

The relative position of monitoring gas pulses with respect to the elution of sample CO gas affected measurement performance with nitrogen containing RMs, as well as overall accuracy and repeatability. Although the average standard deviation of all $\delta^{18}O$ measurements was very similar before and after changes to monitoring gas pulse timings, the frequency of outliers decreased. The first few batches of measurements were still used for analysis despite N_2 contamination, as only the relative $\delta^{18}O$ between samples in the same batch was of interest. In some cases, the relatively enriched IAEA-602 (71.28 ± 0.36 ‰) caused a consecutive measurement of IAEA-C3 to produce a $\delta^{18}O \approx 1$ ‰ higher than subsequent measurements. This was mitigated by inserting a few sacrificial cellulose samples after each sequence of IAEA-602. Memory effects were no longer seen after the graphite crucible of the pyrolysis tube was emptied more often. A build-up of molten silver caused samples to be pyrolysed higher in the crucible, and thus at a lower temperature, leading to incomplete pyrolysis and a visible memory. This is discussed further by Aerts-Bijma et al. [39].

4.3. Reference materials and cellulose calibration

All of the cellulose standards used demonstrated good repeatability under a variety of conditions. Homogeneity was not a concern, and in the case of IAEA-C3, it proved to be very resistant to possible influences of atmospheric moisture and water from wet preparation stages. $\delta^{18}O$ measurements of the two Sigma Aldrich α-celluloses had standard deviations that never exceeded 0.2 ‰, consistently lower than sample α-cellulose. USGS56 similarly produced satisfactory results. The selection of cellulose and wood materials measured in this study does not span a large enough range of $\delta^{18}O$ values

for an optimal multi-point linear correction or normalisation. Such a correction is preferred as it accounts for offset and scale-factor errors [46,49,50]. Ideally three calibration standards should be used for correction, alongside an independent QC standard to determine accuracy [46]. This was attempted with IAEA-600, IAEA-601 and IAEA-602 for correction and IAEA-C3 serving as the QC standard. This resulted in poor agreement between measured and expected values when corrections were performed with these materials. It is possible that, due to age or storage conditions, the supply used in these measurements has become contaminated or in some other way compromised. Measurements of these materials alongside VSMOW2 and other water references could help determine whether this is the case. More likely, the different pyrolysis behaviours of caffeine, benzoic acid and cellulose could also have contributed to these discrepancies. Further investigation is warranted since these RMs are often used in conjunction. This uncertainty could be eliminated by relying exclusively on cellulose standards for correction. Calibrated α-cellulose with large differences in $\delta^{18}O$ are currently not commercially available. These could be produced, for instance, by sampling trees in equatorial and arctic regions, and would allow for multi-point linear calibrations of tree ring α-cellulose using RMs of the same material. The calibration of cellulose standards with water references in this work (Table 7) opens up the possibility of creating the necessary calibrated, local cellulose standards for future use.

4.3.1. Intercomparison and experience with IAEA-C3

The $\delta^{18}O$ obtained on the Sigma Aldrich cellulose provided by Swansea University is close to its assigned value, and assuming an uncertainty of ± 0.15‰ based on relevant measurements from their laboratory [26], within the margin of error. Mean $\delta^{18}O$ values of IAEA-C3 cellulose from Swansea University and that from the CIO are nearly identical (Table 5). These portions of IAEA-C3 were no doubt separated and stored under different conditions for many years but still maintain a very similar $\delta^{18}O$. IAEA-C3 is likely still a useful and appropriate QC and intercomparison standard.

The quoted $\delta^{18}O$ value of IAEA-C3 varies by publication. A table of measured and quoted $\delta^{18}O$ values of IAEA-C3 from literature is provided in the Supplementary Materials. These measurements were performed using a variety of techniques, including older, off-line nickel or mercuric chloride pyrolysis methods. The mean of these reported $\delta^{18}O$ measurements is 32.15‰ with a mean quoted uncertainty of 0.28‰. Using our Pyrolysis-IRMS system with VSMOW2 and the IAEA-607 and USGS46 solutions, we place the $\delta^{18}O$ of our IAEA-C3 at 32.70 ± 0.19‰ with respect to the VSMOW-SLAP scale. Additional intercomparison exercises would be beneficial for clearly defining a $\delta^{18}O$ value for widely used materials such as IAEA-C3.

5. Conclusion

We have established a working methodology for producing and measuring $\delta^{18}O$ in tree-ring α-cellulose at the CIO. We have demonstrated an average precision of < 0.3‰ standard deviation under a variety of conditions, which is considered typical for continuous flow IRMS techniques [26]. Certain considerations that should be made when attempting oxygen isotope analysis were highlighted in this study. These included potentially redundant solvent treatments of pine tree rings, drying of cellulose before pyrolysis

and the intrusion of N_2 during IRMS measurements. Attempts to correct $\delta^{18}O$ results on cellulose using IAEA-600, IAEA-601 and IAEA-602 were unsuccessful. The cause could be related to one or more references becoming compromised, or disparity in the pyrolysis behaviour of the different materials. It is suggested that further study be done to address this issue. Measurements of cellulose standards with water references proved more successful, with an independent QC standard (USGS56) producing a mean $\delta^{18}O$ $-0.01 \pm 0.14\,‰$ from its assigned value. This also resulted in a calibrated $\delta^{18}O$ for IAEA-C3 of $32.70 \pm 0.19\,‰$ with respect to the VSMOW-SLAP scale. These results have highlighted the need for cellulose RMs that span a relatively large range of $\delta^{18}O$ in order to calibrate measurements of tree-ring cellulose optimally.

Note

1. Certified by the International Atomic Energy Agency (IAEA) or another recognized body.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

ORCID

John du Plessis 🆔 http://orcid.org/0009-0003-6849-7324
Dipayan Paul 🆔 http://orcid.org/0000-0002-2640-8122
Margot Kuitems 🆔 http://orcid.org/0000-0002-8803-2650
Anita Aerts-Bijma 🆔 http://orcid.org/0000-0002-7227-791X
Neil J. Loader 🆔 http://orcid.org/0000-0002-6841-1813
Harro A.J. Meijer 🆔 http://orcid.org/0000-0001-8744-5632
Michael Dee 🆔 http://orcid.org/0000-0002-3116-453X

References

[1] Fritts H. Tree rings and climate. London (UK): Academic Press; 1976.
[2] McCarroll D, Loader NJ. Stable isotopes in tree rings. Quat Sci Rev. 2004;23(7–8):771–801. doi:10.1016/j.quascirev.2003.06.017
[3] Loader NJ, McCarroll D, Miles D, et al. Tree ring dating using oxygen isotopes: a master chronology for central England. J Quat Sci. 2019;34(6):475–490. doi:10.1002/jqs.3115
[4] Nakatsuka T, Sano M, Li Z, et al. A 2600-year summer climate reconstruction in central Japan by integrating tree-ring stable oxygen and hydrogen isotopes. Clim Past. 2020;16(6):2153–2172. doi:10.5194/cp-16-2153-2020

[5] Briffa KR. Annual climate variability in the Holocene: interpreting the message of ancient trees. Quat Sci Rev. 2000;19((1–5):87–105. doi:10.1016/S0277-3791(99)00056-6

[6] Leavitt SW, Roden J. Isotope dendrochronology: historical perspective. In: Siegwolf RTW, Brooks JR, Roden J, editors. Stable isotopes in tree rings: inferring physiological, climatic and environmental responses. Cham: Springer International Publishing; 2022. p. 3–20.

[7] Treydte K, Liu L, Padrón RS, et al. Recent human-induced atmospheric drying across Europe unprecedented in the last 400 years. Nat Geosci. 2024;17(1):58–65. doi:10.1038/s41561-023-01335-8

[8] Roden JS, Lin G, Ehleringer JR. A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. Geochim Cosmochim Acta. 2000;64(1):21–35. doi:10.1016/S0016-7037(99)00195-7

[9] Sano M, Li Z, Murakami Y, et al. Tree ring oxygen isotope dating of wood recovered from a canal in the ancient capital of Japan. J Archaeol Sci Rep. 2022;45:103626. doi:10.1016/j.jasrep.2022.103626

[10] Iizuka H, Sho K, Li Z, et al. Reconstruction of drought and long-rain chronologies since the 17th century in central Japan using intra-annual tree-ring oxygen isotope ratios and documentary records. Clim Past. 2025;21(1):133–144. doi:10.5194/cp-21-133-2025

[11] Rinne KT, Loader NJ, Switsur VR, et al. 400-year may–August precipitation reconstruction for southern England using oxygen isotopes in tree rings. Quat Sci Rev. 2013;60:13–25. doi:10.1016/j.quascirev.2012.10.048

[12] Loader NJ, Young GH, McCarroll D, et al. Summer precipitation for the England and Wales region, 1201–2000 CE, from stable oxygen isotopes in oak tree rings. J Quat Sci. 2020;35(6):731–736. doi:10.1002/jqs.3226

[13] Boettger T, Hiller A, Kremenetski K. Mid-Holocene warming in the northwest Kola Peninsula, Russia: northern pine-limit movement and stable isotope evidence. Holocene. 2003;13(3):403–410. doi:10.1191/0959683603hl633rp

[14] Ballantyne A, Rybczynski N, Baker P, et al. Pliocene Arctic temperature constraints from the growth rings and isotopic composition of fossil larch. Palaeogeogr Palaeoclimatol Palaeoecol. 2006;242(3–4):188–200. doi:10.1016/j.palaeo.2006.05.016

[15] Jahren AH, Sternberg LS. Annual patterns within tree rings of the Arctic middle eocene (ca. 45 Ma): isotopic signatures of precipitation, relative humidity, and deciduousness. Geology. 2008;36(2):99–102. doi:10.1130/G23876A.1

[16] Schollaen K, Baschek H, Heinrich I, et al. A guideline for sample preparation in modern tree-ring stable isotope research. Dendrochronologia. 2017;44:133–145. doi:10.1016/j.dendro.2017.05.002

[17] Badea SL, Botoran OR, Ionete RE. Recent progresses in stable isotope analysis of cellulose extracted from tree rings. Plants. 2021;10(12):2743. doi:10.3390/plants10122743

[18] Boettger T, Haupt M, Knöller K, et al. Wood cellulose preparation methods and mass spectrometric analyses of δ^{13}C, δ^{18}O, and nonexchangeable δ^2H values in cellulose, sugar, and starch: an interlaboratory comparison. Anal Chem. 2007;79(12):4603–4612. doi:10.1021/ac0700023

[19] Saurer M, Robertson I, Siegwolf R, et al. Oxygen isotope analysis of cellulose: an interlaboratory comparison. Anal Chem. 1998;70(10):2074–2080. doi:10.1021/ac971022f

[20] Gröning M. International stable isotope reference materials. In: de Groot PA, editors. Handbook of stable isotope analytical techniques. Amsterdam: Elsevier; 2004. p. 874–906.

[21] Brand WA, Coplen TB, Aerts-Bijma AT, et al. Comprehensive inter-laboratory calibration of reference materials for δ^{18}O versus VSMOW using various on-line high-temperature conversion techniques. Rapid Commun Mass Spectrom. 2009;23(7):999–1019. doi:10.1002/rcm.3958

[22] Rozanski K. International Atomic Energy Agency consultants' group meeting on C-14 reference materials for radiocarbon laboratories. Vienna: International Atomic Energy Agency; 1991. (IAEA Report; INIS-XA-421).

[23] Le Clercq M, Van Der Plicht J, Gröning M. New ^{14}C reference materials with activities of 15 and 50 pMC. Radiocarbon. 1997;40(1):295–297. doi:10.1017/S0033822200018178

[24] Buhay WM, Wolfe BB, Elgood RJ, et al. A report on the δ^{18}O measurements of the IAEA cellulose intercomparison material IAEA-C3. In: Reference and intercomparison materials for stable isotopes of light elements. Proceedings of a consultants meeting held in Vienna, 1–3 December 1993. Vienna, Austria: International Atomic Energy Agency; 1995. p. 85–86. (IAEA TECDOC; 825).

[25] Saurer M, Siegwolf R. Pyrolysis techniques for oxygen isotope analysis of cellulose. In: de Groot PA, editors. Handbook of stable isotope analytical techniques. Amsterdam: Elsevier; 2004. p. 497–506.

[26] Loader N, Street-Perrott F, Daley T, et al. Simultaneous determination of stable carbon, oxygen, and hydrogen isotopes in cellulose. Anal Chem. 2015;87(1):376–380. doi:10.1021/ac502557x

[27] Borella S, Leuenberger M, Saurer M. Analysis of δ^{18}O in tree rings: wood-cellulose comparison and method dependent sensitivity. J Geophys Res Atmos. 1999;104(D16):19267–19273. doi:10.1029/1999JD900298

[28] Raffalli-Delerce G, Masson-Delmotte V, Dupouey JL, et al. Reconstruction of summer droughts using tree-ring cellulose isotopes: a calibration study with living oaks from Brittany (western France). Tellus B. 2004;56(2):160–174. doi:10.3402/tellusb.v56i2.16405

[29] Berkelhammer M, Stott LD. Modeled and observed intra-ring δ^{18}O cycles within late holocene bristlecone pine tree samples. Chem Geol. 2009;264(1–4):13–23. doi:10.1016/j.chemgeo.2009.02.010

[30] Hunsinger GB, Hagopian WM, Jahren AH. Offline oxygen isotope analysis of organic compounds with high N:O. Rapid Commun Mass Spectrom. 2010;24(21):3182–3186. doi:10.1002/rcm.4752

[31] Kanner L, Buenning N, Stott L, et al. Climatologic and hydrologic influences on the oxygen isotope ratio of tree cellulose in coastal southern California during the late 20th century. Geochem Geophys Geosyst. 2013;14(10):4488–4503. doi:10.1002/ggge.20256

[32] Zhu Z, Chen J, Zeng Y. Paleotemperature variations at lake caohai, southwestern China, during the past 500 years: evidence from combined δ^{18}O analysis of cellulose and carbonates. Sci China Earth Sci. 2014;57:1245–1253. doi:10.1007/s11430-014-4831-6

[33] Xu G, Liu X, Wu G, et al. Tree ring δ^{18}O's indication of a shift to a wetter climate since the 1880s in the western Tianshan Mountains of northwestern China. J Geophys Res Atmos. 2015;120(13):6409–6425. doi:10.1002/2014JD023027

[34] Granath G, Rydin H, Baltzer JL, et al. Environmental and taxonomic controls of carbon and oxygen stable isotope composition in *Sphagnum* across broad climatic and geographic ranges. Biogeosciences. 2018;15(16):5189–5202. doi:10.5194/bg-15-5189-2018

[35] Dee MW, Palstra S, Aerts-Bijma AT, et al. Radiocarbon dating at Groningen: new and updated chemical pretreatment procedures. Radiocarbon. 2020;62(1):63–74. doi:10.1017/RDC.2019.101

[36] Wacker L, Scott EM, Bayliss A, et al. Findings from an in-depth annual tree-ring radiocarbon intercomparison. Radiocarbon. 2020;62(4):873–882. doi:10.1017/RDC.2020.49

[37] Laumer W, Andreu L, Helle G, et al. A novel approach for the homogenization of cellulose to use micro-amounts for stable isotope analyses. Rapid Commun Mass Spectrom. 2009;23(13):1934–1940. doi:10.1002/rcm.4105

[38] Kornexl BE, Gehre M, Höfling R, et al. On-line δ^{18}O measurement of organic and inorganic substances. Rapid Commun Mass Spectrom. 1999;13(16):1685–1693. doi:10.1002/(SICI)1097-0231(19990830)13:16<1685::AID-RCM699>3.0.CO;2-9

[39] Aerts-Bijma AT, Paul D, van Buuren AC, et al. Carbonate and water pyrolysis measurements suggest minor adjustment to the VPDB and VSMOW-SLAP δ^{18}O scale relation. Rapid Commun Mass Spectrom. 2025;39(19):e10093. doi:10.1002/rcm.10093

[40] Qi H, Coplen TB, Jordan JA. Three whole-wood isotopic reference materials, USGS54, USGS55, and USGS56, for δ^2H, δ^{18}O, δ^{13}C, and δ^{15}N measurements. Chem Geol. 2016;442:47–53. doi:10.1016/j.chemgeo.2016.07.017

[41] Gröning M, van Duren M, Andreescu L. Metrological characteristics of the conventional measurement scales for hydrogen and oxygen stable isotope amount ratios: The δ-scales. In: Fajgelji A, Belli M, Sansone U, editors. Combining and reporting analytical results. Cambridge: The Royal Society of Chemistry; 2006. p. 62–72.

[42] Faghihi V, Verstappen-Dumoulin B, Jansen H, et al. A new high-quality set of singly (^{2}H) and doubly (^{2}H and ^{18}O) stable isotope labeled reference waters for biomedical and other isotope-labeled research. Rapid Commun Mass Spectrom. 2015;29(4):311–321. doi:10.1002/rcm.7108

[43] Coplen TB, Qi H, Tarbox L, et al. USGS46 Greenland ice core water–a new isotopic reference material for δ^2H and δ^{18}O measurements of water. Geostds Geoanal Res. 2014;38(2):153–157. doi:10.1111/j.1751-908X.2013.00267.x

[44] Qi H, Gröning M, Coplen TB, et al. Novel silver-tubing method for quantitative introduction of water into high-temperature conversion systems for stable hydrogen and oxygen isotopic measurements. Rapid Commun Mass Spectrom. 2010;24(13):1821–1827. doi:10.1002/rcm.4559

[45] Rousseeuw PJ, Verboven S. Robust estimation in very small samples. Comput Stat Data Anal. 2002;40(4):741–758. doi:10.1016/S0167-9473(02)00078-6

[46] Brooks JR, Rugh WD, Werner RA. Tree-ring stable isotope measurements: the role of quality assurance and quality control to ensure high quality data. In: Siegwolf RTW, Brooks JR, Roden J, editors. Stable isotopes in tree rings: inferring physiological, climatic and environmental responses. Cham: Springer International Publishing; 2022. p. 191–213.

[47] Rinne KT, Boettger T, Loader NJ, et al. On the purification of α-cellulose from resinous wood for stable isotope (H, C and O) analysis. Chem Geol. 2005;222(1–2):75–82. doi:10.1016/j.chemgeo.2005.06.010

[48] Evert RF. Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development. Hoboken, NJ: John Wiley & Sons; 2006.

[49] Coplen TB. Normalization of oxygen and hydrogen isotope data. Chem Geol. 1988;72(4):293–297.

[50] Meijer H, Neubert R, Visser G. Cross contamination in dual inlet isotope ratio mass spectrometers. Int J Mass Spectrom. 2000;198(1–2):45–61. doi:10.1016/S1387-3806(99)00266-3