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RESEARCH ARTICLE

Long-term performance of simultaneous measurement of stable isotopes of oxygen and carbon in cellulose with a high-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry system at the Institute for Geological and Geochemical Research

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ABSTRACT

A high-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry system was established at the Institute for Geological and Geochemical Research in 2013. A dedicated field of application of the system is the simultaneous measurement of stable carbon and oxygen isotope ratios in the cellulose of modern, relict and subfossil plant tissues and sediments. The measurement protocol was fine-tuned during the first year of operation and documented in detail in this report. To quantify the long-term reproducibility of the simultaneous measurement of stable isotopes of oxygen and carbon in cellulose, a 2σ range inferred from repeated measurements of a Quality Assurance standard can be recommended: 0.16 and 0.20‰, for δ^{13}C and δ^{18}O, respectively. An extensive set of samples with known pyrolysis-based δ^{13}C data was analyzed in combustion mode and the paired results were used to assess the necessity of adjustment of the pyrolysis-based δ^{13}C measurements. The variances of the two datasets were not significantly different; the slope (intercept) of the regression was indistinguishable from unity (zero), suggesting that probably owing to the relatively frequent cleaning of the pyrolysis furnace, pyrolysis-based δ^{13}C data neither suffer from a variance bias nor require a specific adjustment.

KEYWORDS

dendroisotopes, δ^{13}C, δ^{18}O, precision, pyrolysis adjustment

INTRODUCTION

Stable isotopes can serve as valuable non-radioactive tracers and non-destructive integrators of how plants today and in the past have interacted with and adapted to their abiotic and biotic environment (Dawson et al., 2002; Badea et al., 2021; Siegwolf et al., 2022). The use of stable isotope techniques in plant ecological and paleoecological research has grown steadily during the past decades (Dawson et al., 2002; Badea et al., 2021), owing to remarkable advances in the theoretical understanding of discrimination processes, as well as technical developments in isotope analytical techniques (Maguas & Griffiths, 2003; Siegwolf et al., 2022). Arguably, stable isotope methods are now among the most important empirical tools

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in modern plant ecological and paleoecological research, and their provided information has yielded some of the newest and most important insights about plants in the natural environment. Cellulose can be separated from a wide range of living, relict and subfossil plant tissues e.g., xylem (McCarrol & Loader, 2004; Badea et al., 2021; Siegwolf et al., 2022) or foliage (Kimak et al., 2015), peat material (Moschen et al., 2009; Daley et al., 2010; Csank et al., 2011) and even from sediments (Wolfe et al., 2007; Wissel et al., 2008), and stable isotopic composition of oxygen and carbon ($\delta^{18}O$ and $\delta^{13}C$) of cellulose are among the most important proxy indicators of paleoenvironmental and paleoecological changes.

A wide range of analytical techniques has been developed for the separate analysis of stable carbon and oxygen isotopic composition of plant material (e.g., Boettger et al., 2007; Badea et al., 2021); however, on-line pyrolysis was found to allow determination of $\delta^{13}C$ and $\delta^{18}O$ values of a cellulose sample in a single run (Gehre & Strauch, 2003; Knöller et al., 2005). High-temperature pyrolysis of cellulose results in the complete conversion of oxygen into CO (Gehre & Strauch, 2003; Leuenberger & Filot, 2007). However, there is not enough oxygen present in α-cellulose ($C_6H_{10}O_5$) for the total conversion of the sample carbon into CO (Knöller et al., 2005), so it is inevitable that a sixth of sample carbon is deposited in the furnace during pyrolysis, with the reservoir experiencing a net increase until the furnace is cleaned or replaced (Woodley et al., 2012). The potential reason for the difference between the $\delta^{13}C$ results obtained by pyrolysis and combustion is likely the presence of CO produced from carbon present/deposited in the reaction tube (Young et al., 2011). Given the risk of large and time-dependent bias in $\delta^{13}C$ results, it is clear that $\delta^{13}C$ chronologies obtained by high-temperature pyrolysis must check whether they require some adjustment before they are to be used for paleoclimate research or to monitor changes in ecophysiology (Woodley et al., 2012). Combustion results in the complete conversion of sample carbon into CO_2, so it can be considered a 'true' reflection of the $\delta^{13}C$ composition of cellulose and provide a suitable approach to test potential bias of pyrolysis-based $\delta^{13}C$ data (Knöller et al., 2005; Woodley et al., 2012).

To compare stable isotope data from different locations or different sampling times at a high level of precision, a measurement strategy must include reliable traceability to an international stable isotope-scale via reference materials (Brand, 2009). Quality assurance and long-term precision are used to control the long-term performance of the δ-value assignments (Brand, 2009; Spötl, 2011). While usage of international calibration standards helps placing the measured values on a common scale (Coplen, 1994), facilitating the comparability of the results produced by different labs, the precision, another crucial aspect of quality assurance, of each laboratory must be assessed via copious repeated measurements of laboratory working reference material (Spötl, 2011).

A high-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry system (Gehre & Strauch, 2003; Knöller et al., 2005) was established at the Institute for Geological and Geochemical Research, Research Centre for Astronomy and Earth Sciences (IGGR RCAES) at the end of 2013 (Fig. 1). The main dedicated field of application is the simultaneous measurement of stable carbon and oxygen isotope ratios in cellulose. Following the installation of the system it has been in operation since the 12th of February, 2014.

Here we provide documentation of the analytical details of simultaneous measurement of stable carbon and oxygen isotope ratios in cellulose by this high-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry system and report long-term precision, following nine years of practically continuous operation in the Stable Isotope Lab at the IGGR RCAES. This information quantifies the quality of the provided data for reporting in publications (Brooks et al., 2022). In addition, results of testing the potential variance bias of pyrolysis on the $\delta^{13}C$ measurements are also reported.

METHODS

For stable isotope analyses α-cellulose was separated from the plant materials using the modified Jayme-Wise method (Boettger et al., 2007; Loader et al., 1997). Afterward it was homogenized by a standard ultrasonic protocol (Laumer et al., 2009) using a VCX130 (Sonics & Materials Inc/USA) device and dried at 60 °C in an oven.

Carbon and oxygen isotope values are reported in permille (‰) using the traditional δ (delta) notation (Coplen,

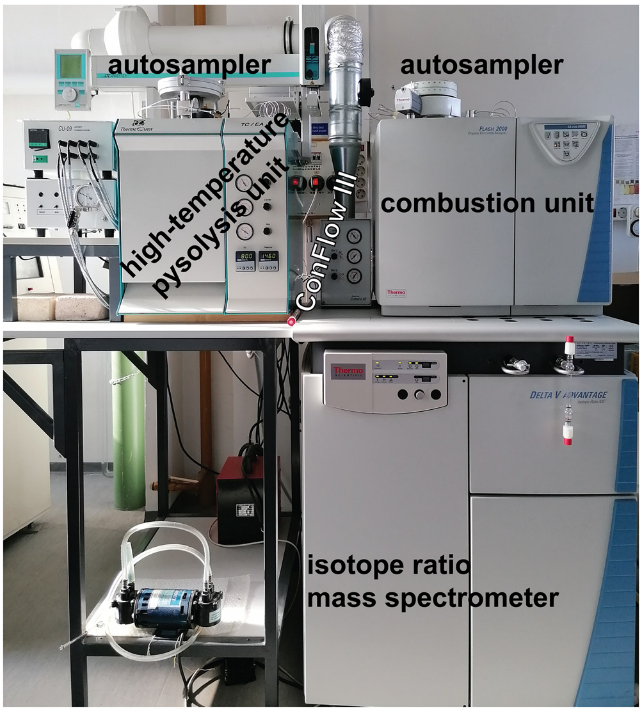

Fig. 1. A stable isotope ratio mass spectrometry system with coupled pyrolysis and combustion peripheries operating at the Institute for Geological and Geochemical Research, Research Centre for Astronomy and Earth Sciences

1994) δX = $R_{sample}/R_{standard}$ −1, where X is ^{13}C or ^{18}O, R_{sample} and $R_{standard}$ are either $^{13}C/^{12}C$ or $^{18}O/^{16}O$ ratios of the sample and the standard, respectively. The stable carbon isotope composition was converted to VPDB (Vienna Pee-Dee Belemnite) scale (Coplen et al., 2006) and stable oxygen isotope composition was converted to VSMOW-SLAP (Vienna Standard Mean Ocean-Standard Antarctic Light Precipitation) scale (IAEA 2017).

High-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry system

For the simultaneous measurements of carbon and oxygen isotope ratios (Loader et al., 2015), 0.2 mg (±10%) of α-cellulose was packed in silver foil and dried at 60 °C for 24 h. The dried samples were placed into a 50-position sealed-carousel autosampler (Zero Blank, Costech) and purged in a stream of high purity He (Messer 4.6) for 10 min during the first 4 months of operation and increased to 15 min since June 2014. The rotating autosampler dropped the samples to a thermal conversion elemental analyzer (TCEA, ThermoQuest Finnigan). The pyrolysis furnace consisted of a ceramic outer tube and a glassy carbon inner tube and the samples were dropped into a graphite crucible, positioned on glassy carbon chips within the pyrolysis tube (Knöller et al., 2005). Pyrolysis efficiency for oxygen yield is 100% above 1,400 °C (Gehre & Strauch, 2003; Leuenberger & Filot, 2007); hence the pyrolysis temperature was set to 1,450 °C until 2016 and to 1,460 °C afterwards. The pyrolysis products (CO and H_2) were flushed continuously in a He stream (~100 mL min^{-1}) and separated using a gas chromatography (GC) column (5Å molecular sieve, 80–100 mesh) heated to 75 °C, before CO is transferred to a Delta V Advantage isotope ratio mass spectrometer (IRMS) via a Conflo III open-split (Werner et al., 1999). The $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios were derived from measurements of CO ions taking mass 30 to 28 and 29 to 28 ratios, respectively (Sauer et al., 1998; Gehre & Strauch, 2003). The relative isotopic ratio of the sample was measured against a reference gas pulse of CO (Messer 4.7) introduced via the same gas injector system (ConFlow III). The operation protocol included cleaning the system following the measurement of 4–5 complete sequences (c. 200 to 250 samples) and heating the GC column to 300 °C overnight as needed for reactivation.

Considering the suitability for the simultaneous measurement of oxygen and carbon isotope ratios (Knöller et al., 2005) and a relatively good matrix match with regard to chemical nature and relative elemental composition of the cellulose samples (Boettger et al., 2007; Skrzypek, 2013; Meier-Augenstein & Schimmelmann, 2019) IAEA-CH-3 and IAEA-CH-6 were used (Table 1) from the available secondary light stable isotope reference materials (https://ciaaw.org/reference-materials.htm) and commercial Merck cellulose was used as an internal standard. IAEA-CH-3 lies within the δ-range of the expected cellulose samples; however, considering the lack of other available reference material (Boettger et al., 2007), the fact that some samples may lie outside the calibration range has to be cautiously tolerated (Knöller et al., 2005). Calibration reference materials were measured in triplicates both at the beginning and end of the sequences to reduce calibration error (Boettger et al., 2007; Skrzypek, 2013) and QA Merck standards were measured as unknown after measurements of every five real samples. We note that initially the certified value of the δ^{13}C Merck cellulose was considered as −24.25‰ (Knöller et al., 2005); however, δ^{13}C value was determined by multiple (n = 20) combustion measurements (see below) providing very constantly (1 STD: 0.04%) a value of −24.70‰, so this value was used in all subsequent evaluations and former sequences have been re-evaluated considering the mean value (Table 1).

Combustion – pyrolysis adjustment

Stratified sub-samples of the pyrolysis results were selected from a dendroisotope dataset consisting of annual increments of three living and two relict Swiss stone pine (*Pinus cembra* L.) individuals covering the 1622 to 2012 CE time interval. A subset (n = 30) was selected from the living samples in November 2014, complemented with an additional subset (n = 13) selected from the relict samples in October 2015, to cover the full range of carbon isotope ratios (from −25.70 to −20.24‰) available at the time of the experiment. The samples were included in studies on age-trend assessment (Kern et al., 2023) and dendroclimatological studies (Nagavciuc et al., 2020, 2022). Stable carbon isotope ratios of the selected samples were analyzed in combustion and the paired results were used to compare variances and to define a linear regression to test the necessity of adjustment of the pyrolysis-based δ^{13}C measurements (Woodley et al., 2012).

Combustion was performed using a Flash 2000 Elemental Analyzer (Thermo Scientific, Rhodano, Italy) interfaced with the same Delta V Advantage IRMS (Fig. 1) operating in continuous flow mode with a carrier gas ($He+O_2$, Messer 4.6) at a flow rate of ~90 mL min^{-1}. The samples were combusted (1,000 °C) over a chromium(III) oxide catalyst in

Table 1. The assigned values of the standard materials used in simultaneous measurement of stable isotopes of oxygen and carbon in cellulose at the Institute for Geological and Geochemical Research between 2014 and 2022

	δ^{13}C (‰)	Reference	δ^{18}O (‰)	Reference
IAEA-CH-3	−24.72	Coplen et al. (2006)	32.52	Sauer et al. (1998)
IAEA-CH-6	−10.45	Coplen et al. (2006)	36.40	Farquhar et al. (1997), Kornexl et al. (1999)
Merck	−24.70	this study	28.67	Sauer et al. (1998)

the presence of excess oxygen. Silvered cobaltous oxide and copper(II) oxide, positioned lower in a quartz combustion tube, ensured the complete conversion of sample carbon into CO_2 and the removal of residual halogens or sulfur. Following the removal of any nitrogen oxides by passage over copper wire (640 °C), traces of water were removed using a magnesium perchlorate trap. The CO_2 peak for each sample was separated using a GC column (75 °C), before analysis by IRMS. The $^{13}C/^{12}C$ ratios were derived from measurements of CO_2 ions (mass 45 to 44) (Knöller et al., 2005).

In the case of combustion, similarly IAEA-CH-3 and IAEA-CH-6 were used as standard reference material considering the same certified values for calibration (Table 1), following a two-point linear normalization scheme (Paul et al., 2007; Meier-Augenstein & Schimmelmann, 2019). Merck cellulose were run together with batches of samples as an internal QA standard similarly to the pyrolysis sequences.

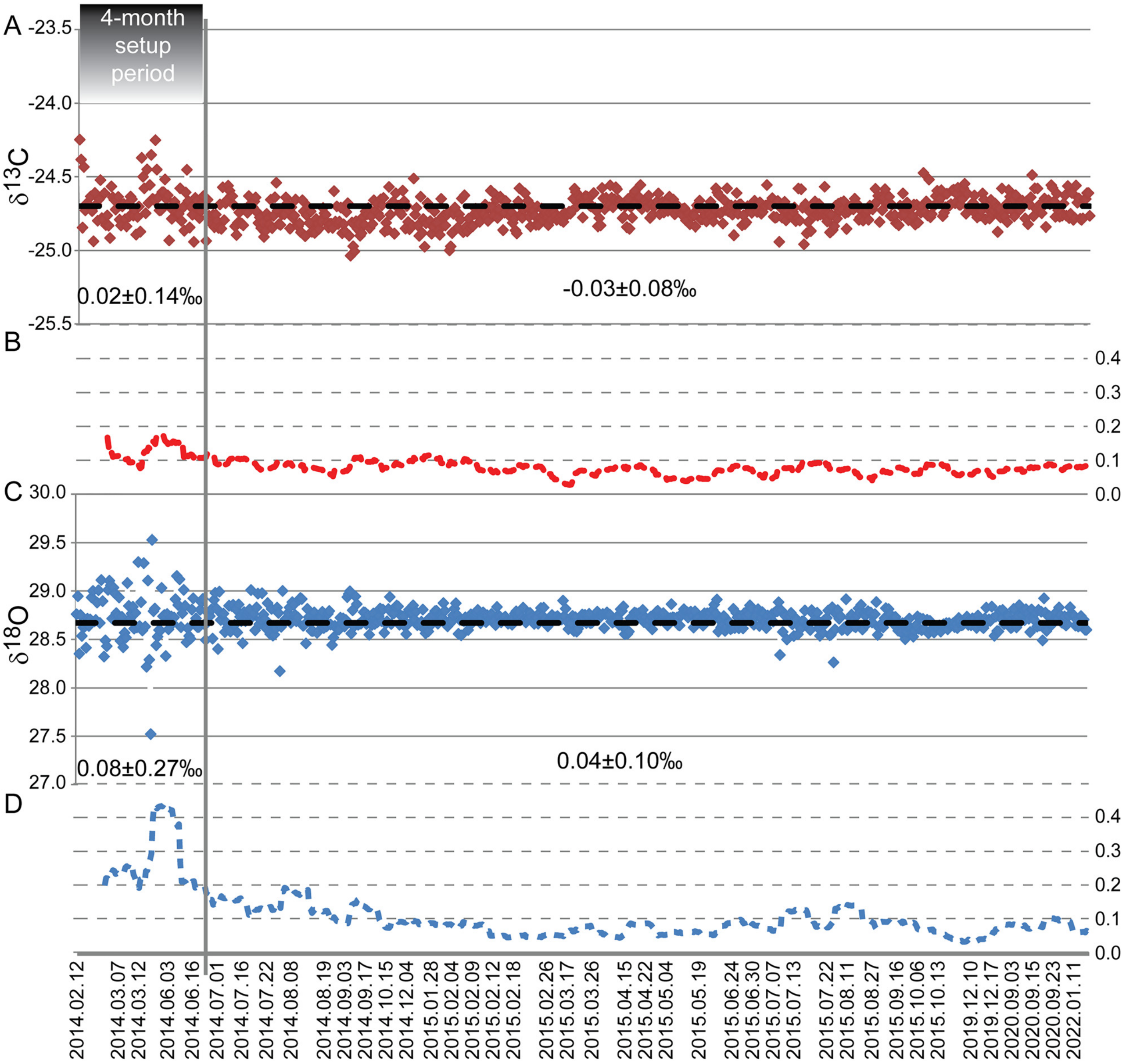

Fig. 2. Performance charts of a QA material (Merck cellulose) in simultaneous measurement of stable isotopes of carbon (A, B) and oxygen (C, D) in cellulose using the high-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry system at the Institute for Geological and Geochemical Research over c. nine-years of operation. The individual measurements of $\delta^{13}C$ and $\delta^{18}O$ with the assigned value (indicated by the horizontal black dashed line, see Table 1) are shown in A and C, while the standard deviation calculated for 20 consecutive data points are shown below (B, D). The termination of the initial period (mid-June of 2014), due to some modification of the measurement protocol, show the precision of the simultaneous measurements of $\delta^{13}C$ and $\delta^{18}O$ of the cellulose samples remarkably improved, marked by a vertical line. The accuracy (mean bias ± standard deviation) calculated from the values obtained before and after 26 June, 2014 are annotated both for $\delta^{13}C$ (A) and $\delta^{18}O$ (C) performance charts

RESULTS AND DISCUSSION

Long-term performance of the high-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry system for the stable isotope analysis of cellulose

During this 9-yr-long operation a total of ca. 4,500 individual natural cellulose samples (chiefly xylem ~93%, less commonly foliage ~3% and peat ~6%) were processed. Among the actual samples ~800 measurements were performed on the QA working standard (commercial Merck cellulose) processed as unknown. The copious number of repeated measurements of the same material allows the construction of quality assurance performance charts (Brand, 2009; Spötl, 2011).

The standard deviation was relatively larger (STD-δ^{13}C: 0.14‰ and STD-δ^{18}O: 0.27‰) for an initial 4-month period, but owing to a 5-min increase in the pre-flushing of the autosampler and a more prudent operation by the personnel, remarkably reduced (STD-δ^{13}C: 0.08‰ and STD-δ^{18}O: 0.10‰) the scatter of the values since mid-June of 2014 (Fig. 2). These values conform with the precision reported from an interlaboratory comparison on IRMS measurements of cellulose involving nine European stable isotope laboratories (Boettger et al., 2007). For carbon isotope values the standard deviation agrees (or is slightly lower) with the ones reported for synthetic cellulose (Knöller et al., 2005) or homogenized internal laboratory cellulose standards (Brooks et al., 2022). The latter values are essentially constant over 8-yrs of operation of the facility, so we suggest considering the long-term precision as 2σ inferred based on these QA standard as 0.16 and 0.20‰, for δ^{13}C and δ^{18}O, respectively. The higher deviations for oxygen compared to carbon could reflect a noise due to hygroscopically-bounded water of different isotopic ratios (retained from the preparation procedure or adsorbed from the lab atmosphere) remaining after incomplete drying (see Boettger et al., 2007).

Beside reproducibility, trueness of the QA working standard has also improved after mid-June of 2014, mainly for δ^{18}O (Fig. 2 A, B). The 10 °C increase in pyrolysis temperature since 2016 has not changed the long-term precision remarkably.

Comparing pyrolysis-based and combustion-based δ13C measurements

As expected, the comparison of paired pyrolysis-based δ^{13}C measurements with combustion-based counterparts demonstrates a very strong correlation (Fig. 3A). The variances of the two datasets are almost equal (VAR (pyrolysis-δ^{13}C) = 2.11, VAR (combustion-δ^{13}C) = 2.10) and an *F*-test also confirms that the variances of the two datasets are not significantly different (F (1,42) = 0.99, *P* = 0.49). The slope of the regression is indistinguishable from unity (95% CI: 0.95, 1.03) and the intercept does not differ significantly from zero (95% CI: −1.15, 0.82) (Fig. 3A). It was suggested that the composition closer to the margin of the range of the δ^{13}C data are more prone to bias (Woodley et al., 2012). However, the difference values computed between pyrolysis-based and combustion-based δ^{13}C data-pairs do not show an increased rate of occurrence at the extremes of the range (Fig. 3B).

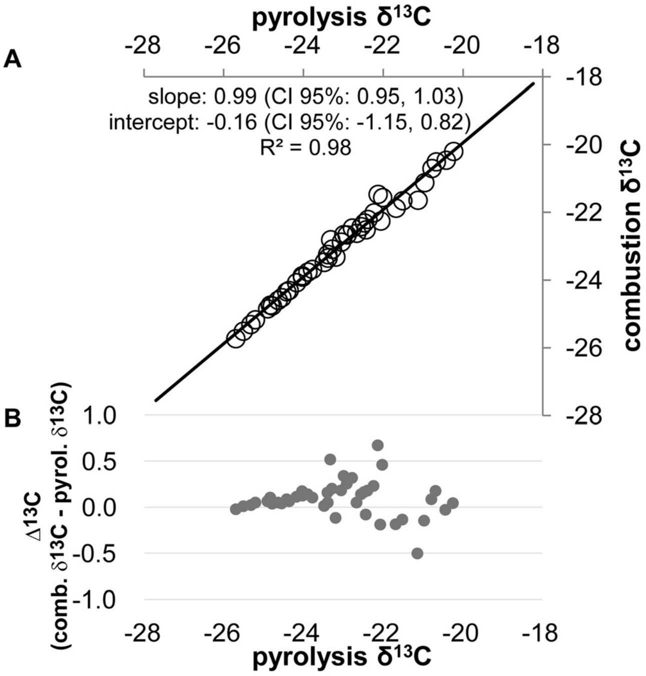

Fig. 3. Comparison of paired pyrolysis-based and combustion-based δ^{13}C measurements. The linear regression is fitted to the paired data (A). The estimated regression parameters are displayed in the plot. The distribution of difference values computed between pyrolysis-based and combustion-based δ^{13}C data-pairs is plotted against the pyrolysis-based δ^{13}C counterparts (B)

These peculiarities suggest that pyrolysis-based δ^{13}C data obtained by the considered system and analytical protocol neither suffer from a variance bias nor require any specific adjustment. Beside the appropriate normalization and the application of the principles of identical treatment for reference materials and samples (Knöller et al., 2005; Brand, 2009) the explanation is probably the relatively frequent cleaning of the pyrolysis furnace which prevents the accumulation of the residual carbon which is thought to be the critical source of bias in pyrolysis-based δ^{13}C measurements (Young et al., 2011; Woodley et al., 2012).

CONCLUSIONS

The analytical details of simultaneous measurement of stable oxygen and carbon isotope composition (δ^{18}O and δ^{13}C) in cellulose by the high-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry system operating in the Stable Isotope Lab at the IGGR RCAES have been documented. The long-term reproducibility, essential for reporting in paleoenvironmental and paleoecological studies, recommended for δ^{13}C and δ^{18}O data, derived by this facility, are 0.16 and 0.20‰, respectively. A systematic comparison between pyrolysis-based and combustion-based δ^{13}C measurements indicated that pyrolysis-based δ^{13}C data, derived by the described facility and analytical protocol, neither suffer from a variance bias nor require any specific adjustment.

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REFERENCES

Badea, S.-L., Botoran, O.R., and Ionete, R.E. (2021). Recent progresses in stable isotope analysis of cellulose extracted from tree rings. *Plants*, 10: 2743. https://doi.org/10.3390/plants10122743.

Boettger, T., Haupt, M., Knöller, K., Weise, S.M., Waterhouse, J.S., Rinne, K.T., Loader, N.J., Sonninen, E., Jungner, H., Masson-Delmotte, V., Stievenard, M., Guillemin, M.-T., Pierre, M., Pazdur, A., Leuenberger, M., Filot, M., Saurer, M., Reynolds-Henne, C.E., Helle, G., and Schleser. G.H. (2007). Wood cellulose preparation methods and mass spectrometric analyses of δ^{13}C, δ^{18}O, and nonexchangeable δ^{2}H values in cellulose, sugar, and starch: an interlaboratory comparison. *Analytical Chemistry*, 79: 4603.

Brand, W. (2009). Maintaining high precision of isotope ratio analysis over extended periods of time. *Isotopes in Environmental and Health Studies*, 45(2): 135–149. https://doi.org/10.1080/10256010902869097.

Brooks, J.R., Rugh, W.D., and Werner, R.A. (2022). Tree-Ring stable isotope measurements: the role of quality assurance and quality control to ensure high quality data. In: Siegwolf, R.T.W., Brooks, J.R., Roden, J., and Saurer, M. (Eds.), *Stable isotopes in tree rings. Tree physiology*, Vol. 8. Springer, Cham. pp. 191–213. https://doi.org/10.1007/978-3-030-92698-4_6.

Coplen, T.B. (1994). Reporting of stable hydrogen, carbon, and oxygen isotopic abundances (technical report). *Pure and Applied Chemistry*, 66(2): 273–276.

Coplen, T.B., Brand, W.A., Gehre, M., Gröning, M., Meijer, H.A., Toman, B., and Verkouteren, R.M. (2006). New guidelines for δ^{13}C measurements. *Analytical Chemistry*, 78(7): 2439–2441.

Csank, A.Z., Tripati, A.K., Patterson, W.P., Eagle, R.A., Rybczynski, N., Ballantyne, A.P., and Eiler, J.M. (2011). Estimates of Arctic land surface temperatures during the early Pliocene from two novel proxies. *Earth and Planetary Science Letters*, 304(3–4): 291–299.

Daley, T.J., Barber, K.E., Street-Perrott, F.A., Loader, N.J., Marshall, J.D., Crowley, S.F., and Fisher, E.H. (2010). Holocene climate variability revealed by oxygen isotope analysis of Sphagnum cellulose from Walton Moss northern England. *Quaternary Science Reviews*, 29: 1590.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., and Tu, K.P. (2002). Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, 33: 507–559.

Farquhar, G.D., Henry, B.K., and Styles, J.M. (1997). A rapid on-line technique for determination of oxygen isotope composition of nitrogen-containing organic matter and water. *Rapid Communications in Mass Spectrometry*, 11: 1554–1560.

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

IAEA. (2017). *Reference sheet for VSMOW2 and SLAP2 international measurement standards*. IAEA, Vienna, 8 pp. (Rev 1 dated 2017-07-11).

Kern, Z., Nagavciuc, V., Hatvani, I.G., Hegyi, I.N., Loader, N.J., and Popa, I. (2023). Evaluation of the non-climatic (age-related) trends of stable oxygen and carbon isotopes in Swiss stone pine (*Pinus cembra* L.) tree-rings from the Eastern Carpathians, Romania. *Dendrochronologia*, 78: 126061. https://doi.org/10.1016/j.dendro.2023.126061.

Kimák, Á., Kern, Z., and Leuenberger, M. (2015). Qualitative distinction of autotrophic and heterotrophic processes at the leaf level by means of triple stable isotope (C-O-H) patterns. *Frontiers in Plant Science*, 6: 1008. https://doi.org/10.3389/fpls.2015.01008.

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

Kornexl, B.E., Gehre, M., Höfling, R., and Werner, R.A. (1999). On-line δ^{18}O measurement of organic and inorganic substances. *Rapid Communications in Mass Spectrometry*, 13: 1685–1693.

Laumer, W., Andreu, L., Helle, G., Schleser, G.H., Wieloch, T., and Wissel, H. (2009). A novel approach for the homogenization of cellulose to use micro-amounts for stable isotope analyses. *Rapid Communications in Mass Spectrometry*, 23: 1934.

Leuenberger, M.C. and Filot, M.S. (2007). Temperature dependencies of high-temperature reduction on conversion products and their isotopic signatures. *Rapid Communications in Mass Spectrometry*, 21: 1587.

Loader, N.J., Robertson, I., Barker, A.C., Switsur, V.R., and Waterhouse, J.S. (1997). An improved technique for the batch processing of small wholewood samples to a-cellulose. *Chemical Geology*, 136: 313.

Loader, N.J., Street-Perrott, F.A., Daley, T.J., Hughes, P.D.M., Kimak, A., LevanicT., Mauquoy, G.M.D., Robertson, I., Roland, T.P., van Bellen, S., Ziehmer, M.M., and Leuenberger, M. (2015). Simultaneous determination of stable carbon, oxygen, and hydrogen isotopes in cellulose. *Analytical Chemistry*, 87(1): 376–380.

Máguas, C. and Griffiths, H. (2003). Applications of stable isotopes in plant ecology. *Progress in Botany*: 472–505.

McCarroll, D. and Loader, N.J. (2004). Stable isotopes in tree rings. *Quaternary Science Reviews*, 23: 771—881.

Meier-Augenstein, W. and Schimmelmann, A. (2019). A guide for proper utilisation of stable isotope reference materials. *Isotopes* *in Environmental and Health Studies*, 55: 113−128, https://doi.org/10.1080/10256016.2018.1538137.

Moschen, R., Kühl, N., Rehberger, I., and Lücke, A. (2009). Stable carbon and oxygen isotopes in sub-fossil Sphagnum: assessment of their applicability for palaeoclimatology. *Chemical Geology*, 259: 262−272.

Nagavciuc, V., Kern, Z., Ionita, M., Hartl, C., Konter, O., Esper, J., and Popa, I. (2020). Climate signals in carbon and oxygen isotope ratios of Pinus cembra tree-ring cellulose from Călimani Mountains, Romania. *International Journal of Climatology*, 40: 2539–2556. https://doi.org/10.1002/joc.6349.

Nagavciuc, V., Ionita, M., Kern, Z., McCarroll, D., and Popa, I. (2022). A ~700 years perspective on the 21st century drying in the eastern part of Europe based on δ^{18}O in tree ring cellulose. *Communications Earth & Environment*, 3: 277. https://doi.org/10.1038/s43247-022-00605-4.

Paul, D., Skrzypek, G., and Fórizs, I. (2007). Normalization of measured stable isotope composition to isotope reference scale – a review. *Rapid Communications in Mass Spectrometry*, 21: 3006–3014. http://doi.org/10.1002/rcm.3185.

Saurer, M., Robertson, I., Siegwolf, R., and Leuenberger, M. (1998). Oxygen isotope analysis of cellulose: an interlaboratory comparison. *Analytical Chemistry*, 70: 2074.

Siegwolf, R.T.W., Brooks, J.R., Roden, J., and Saurer, M. (Eds.), (2022). *Stable isotopes in tree rings. Tree physiology*, Vol. 8. Springer, Cham. https://doi.org/10.1007/978-3-030-92698-4.

Skrzypek, G. (2013). Normalization procedures and reference material selection in stable HCNOS isotope analyses: an overview. *Analytical and Bioanalytical Chemistry*, 405(9): 2815–2823.

Spötl, C. (2011). Long-term performance of the Gasbench isotope ratio mass spectrometry system for the stable isotope analysis of carbonate microsamples. *Rapid Communications in Mass Spectrometry*, 25: 1683–1685. https://doi.org/10.1002/rcm.5037.

Werner, R.A., Bruch, B.A., and Brand, W.A. (1999). ConFlo III – an interface for high precision δ^{13}C and δ^{15}N analysis with an extended dynamic range. *Rapid Communications in Mass Spectrometry*, 13: 1237.

Wissel, H., Mayr, C., and Lücke, A. (2008). A new approach for the isolation of cellulose from aquatic plant tissue and freshwater sediments for stable isotope analysis. *Organic Geochemistry*, 39(11): 1545–1561.

Wolfe, B.B., Falcone, M.D., Clogg-Wright, K.P., Mongeon, C.L., Yi, Y., Brock, B.E., St. Amour, N.A., Mark W.A., and Edwards, T.W. (2007). Progress in isotope paleohydrology using lake sediment cellulose. *Journal of Paleolimnology*, 37(2): 221–231.

Woodley, E.J., Loader, N.J., McCarroll, D., Young, G.H., Robertson, I., Heaton, T. H., Gagen M.H., and Warham, J.O. (2012). High-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry: simultaneous measurement of the stable isotopes of oxygen and carbon in cellulose. *Rapid Communications in Mass Spectrometry*, 26(2): 109–114.

Young, G.H.F., Loader, N.J., and McCarroll, D. (2011). A large-scale comparative study of stable carbon isotope ratios determined using on-line combustion and low-temperature pyrolysis techniques. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 300: 23–28. https://doi.org/10.1016/j.palaeo.2010.11.018.