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Demystifying the tropics: FTIR characterization of pantropical woods and their α-cellulose extracts for past atmospheric ^{14}C reconstructions

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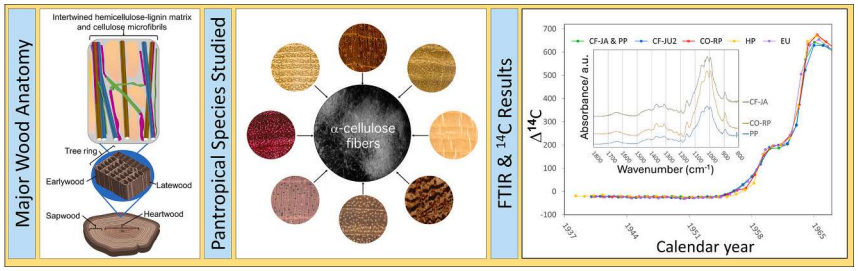
HIGHLIGHTS

- Eight tropical tree species and their α-cellulose were assessed by 56 FTIR spectra.
- FTIR analysis revealed the main wood compounds removed by chemical treatment.
- A simplified chemical protocol with no organic solvents yielded pure α-cellulose.
- ^{14}C measurements of pantropical α-cellulose revealed reliable results.
- Simpler wood chemistry is efficient/effective, can bolster global ^{14}C studies.

GRAPHICAL ABSTRACT



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ABSTRACT

To ensure unbiased tree-ring radiocarbon (^{14}C) results, traditional pretreatments carefully isolate wood cellulose from extractives using organic solvents, among other chemicals. The addition of solvents is laborious, time-consuming, and can increase the risk of carbon contamination. Tropical woods show a high diversity in wood-anatomical and extractive composition, but the necessity of organic-solvent extraction for the ^{14}C dating of these diverse woods remains untested. We applied a chemical treatment that excludes the solvent step on the wood of 8 tropical tree species sampled in South-America and Africa, with different wood-anatomical and extractive properties. We analyzed the success of the extractive removal along with several steps of the α-cellulose extraction procedure using Fourier Transform Infrared (FTIR) spectroscopy and further confirmed the quality of ^{14}C measurements after extraction. The α-cellulose extracts obtained here showed FTIR-spectra free of signals from various extractives and the ^{14}C results on these samples showed reliable results. The chemical method evaluated reduces the technical complexity required to prepare α-cellulose samples for ^{14}C dating, and therefore can bolster global atmospheric ^{14}C applications, especially in the tropics.

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1. Introduction

Past atmospheric radiocarbon (^{14}C) reconstructions describe the history of the earth's climate and carbon cycle (Heaton et al., 2021). Specifically, ^{14}C datasets can serve as a baseline for accessing air-mass mixing and dynamic climate changes, helping estimate the air-sea flux of CO_2, measuring soil carbon stocks and turnover times, and determining ground-based fossil fuel CO_2 emissions and sources of carbonaceous aerosols (Kutschera, 2022). Because ^{14}C levels in the atmosphere have fluctuated over time, reference materials of securely dated samples (such as speleothems, and organisms with consistent growth patterns, i.e., trees) are used for calibration reconstructions.

Trees fix carbon directly from the atmosphere through photosynthesis. Therefore, trees that form annual rings can be used to link ^{14}C signatures in a wood-ring to a specific growing season within a calendar year (Farquhar et al., 1982). This, combined with the global abundance of trees, makes dendrochronologically-dated tree rings an excellent material for reconstructing past atmospheric ^{14}C. The first published ^{14}C sequence was based on tree-ring samples of bristlecone pine (Ferguson et al., 1966). These samples were used to build the first calibration curve for radiocarbon dating in 1967 (Suess, 1970) and nowadays, several ^{14}C calibration curves from the common era up to ~55 kyrs ago have been produced using tree-ring data (Hogg et al., 2020; Hua et al., 2022; Reimer et al., 2020).

Dendrochronologically-dated tree ring data, which the ^{14}C calibration curve is reliant on, is sparser in pantropical regions (Pearl et al., 2020). The scarcity of pantropical tree ring data prevails despite 45 % of global forest cover being concentrated in the tropics ("Global Forest Resources Assessment", 2020). This tropical data shortage is the result of a variety of issues. The misconception that tropical trees do not produce annually-resolved rings has undermined tropical dendrochronological studies for decades (Worbes, 2002). The high diversity in the anatomy of ring boundaries and the complex climatic signals registered in tropical wood make it challenging to assign a ring's exact year of formation (Baker et al., 2017; Linares et al., 2017; Santos et al., 2021). Tropical forests can be remote and difficult to reach, making tropical dendrochronological studies more expensive and demanding (Quesada-Román et al., 2022). Reconstructions over multiple centuries are hampered by the quicker decomposition of fallen trees in the warm and humid tropical climate (Schöngart et al., 2017). Finally, inaccurate cutting of wood material and/or shortcomings in extraction procedures can mislead interpretations of material assigned for ^{14}C reconstructions (Santos et al., 2024a). When combined, these obstacles explain the slow development of tropical and subtropical dendrochronology (Brienen et al., 2016; Silva et al., 2019) and how tropical wood came to be understudied in the atmospheric ^{14}C reconstruction field (Santos et al., 2015, 2020; Reimer et al., 2020).

The major components of wood are cellulose, hemicellulose, and lignin (Browning, 1967), followed by extractives (i.e., a whole range of metabolites, such as phenolics, resins, waxes, and oils), that in tropical species is evident by their "distinctive wood coloration" (N'Guessan et al., 2023). While cellulose is known to be mainly structural, the extractives mentioned above, as well as some carbohydrates, sugars, and starch are nonstructural, and therefore retain some mobility across woody tissues (Hartmann and Trumbore, 2016). Nonstructural carbon may vary in isotopic signatures, due to their different biological pathways, carbon pools, and/or fluxes over time (Gessler and Treydte, 2016). Healthy trees under standard climatic conditions (not stressed) tend to build up structural carbon (cellulose), mainly from recent CO_2 assimilates (Carbone et al., 2013). Also, cellulose provides accurate ^{14}C readings, making holocellulose and/or α-cellulose, the desired product for ^{14}C analysis (Hua et al., 2022).

Extractives, conversely, can interfere with ^{14}C dating (Cain and Suess, 1976; Tans et al., 1978; Worbes and Junk, 1989; Westbrook et al., 2006), as they can move across several tree rings (Hartmann and Trumbore, 2016). Soluble compounds can affect the color, smell, and mechanical properties of wood (Willför et al., 2003; Pandey, 2005; Mayer et al., 2006; Baar et al., 2014; Pinto et al., 2018) and tend to be more abundant in heartwood than sapwood, in outer-heartwood than inner-heartwood, and near injuries and knots (Hillis, 1972; Wilson and Grinsted, 1977; Belt et al., 2017). Tropical wood is known to have more extractives, being composed of up to 18–22 % extractives compared to only 2–8 % in extratropical woods (Pettersen, 1984; Mosedale et al., 1998; Hon and Shiraishi, 2000). The abundant content of extractives in tropical wood has been associated with the up-to-threefold higher proportion of parenchyma tissue when compared to temperate woods (Morris et al., 2016). Moreover, extractive quantities in trees and their attributes can vary between and within tree species (N'Guessan et al., 2023), due to defense mechanisms (Langenheim, 1990; Schollaen et al., 2017), growth stages (Funda et al., 2020), and location (Traoré et al., 2018).

Because organic solvents can remove wood extractives, researchers have theorized that a blend of solvents should be used in wood treatments for isotopic analysis (Olsson, 1980; Hoper et al., 1997). Chemical treatments on woods to isolate cellulose have been developed over decades and evaluated by means of best-analysis results for $\delta^{13}C$, ^{14}C, or both (Linick et al., 1986; Olsson and Possnert, 1992; Leavitt and Danzer, 1993; Gaudinski et al., 2005; Richard et al., 2014; Schollaen et al., 2017). Over time, in aiming for more accurate ^{14}C measurements, the preferred chemical process of isolating structural carbon from wood came to be known as α-cellulose extraction.

The α-cellulose extraction procedure follows a general framework: (1) organic solvent(s) for removal of soluble extractives (Hoper et al., 1997; Wal, 2021), (2) cycles of acids and base solutions with heat, which induces wood swelling and structural disintegration, as well as lignin and hemicellulose removal (Jungnikl et al., 2008; Beg et al., 2023), (3) warm acidified bleach (with acetic acid to help weaken lignin bonds) for lignin removal (Southon and Magana, 2010; Beg et al., 2023), and (4) further delignification with a strong alkaline solution (10–17 % NaOH) at room temperature (Goh et al., 1972; Gaudinski et al., 2005; Wal, 2021). For ^{14}C analysis specifically, chemical treatments are completed by a weak acid rinse to remove atmospheric CO_2 adsorbed during alkaline treatments (Staff et al., 2014; Santos et al., 2020, 2023).

Several variations have emerged for the pretreatment of woods to streamline extraction for ^{14}C analysis (e.g., Southon and Magana, 2010; Němec et al., 2010; Gillespie, 2019). Products from such procedures have been termed cellulose in the literature (Capano et al., 2018; Cercatillo et al., 2021), whether or not they are preceded by solvent extraction, or make use of the final delignification step. Nonetheless, many extraction protocol evaluations have been limited to extratropical tree species and thus do not capture the complexity inherent to tropical wood. Moreover, variations in chemical treatments depend on expected wood extractive abundances, available sample amount, and/or expected ^{14}C accuracy and precision based on the age ranges of interest. Turney et al. (2021) and Hogg et al. (2022) advanced the need for a solvent coupled with α-cellulose extractions for subfossil trees found in wetlands and/or bogs. Dee et al. (2020) proposed applying solvents to tree species known to or expected to be resinous, while Hua et al. (2000 and 2004) and Steinhof et al. (2017) produced α-cellulose using blended solvent mixtures, regardless of wood types or their origins.

Several works on tropical woods have already excluded the organic solvent step in their studies (e.g., Hadad et al., 2015; Santos et al., 2015, 2020, 2021; Linares et al., 2017; Ancapichún et al., 2021), with no apparent impact on the resulting ^{14}C. Santos et al. (2022, 2024b) produced complete atmospheric post-1950 ^{14}C records of tree species from the Amazon Basin using a streamlined α-cellulose treatment that completely cuts out the organic solvent step as well as the acetic acid buffer. To validate the effects of this novel chemical approach on ^{14}C results, Santos et al. (2024b) also applied an organic extraction solvent protocol (Hua et al., 2004) on a small portion of their samples for intercomparison purposes ($n = 10$ tree-ring sample duplicates). Though sporadic validations exist, a thorough appraisal of Santos et al. (2023)

protocol with a larger set of tropical tree species is still lacking. This evaluation is timely (Reimer et al., 2020), given the much-needed increases in ^{14}C reconstructions in the tropics that will need to deal with the high levels of extractives in tropical wood (Morris et al., 2016).

The primary purpose of this paper is to verify by an independent method – Fourier transform infrared spectroscopy (FTIR) analysis – that a simplified wood α-cellulose extraction procedure (cf. Santos et al., 2023) can effectively produce pure α-cellulose from a variety of pantropical tree species (Fig. 1). Secondly, we aimed to assess each chemical step of the α-cellulose extraction procedure by comparing their subproducts, starting from untreated woods. We also evaluated if there are detectable differences in extractive composition within the same tropical tree species sampled over varying distances. All analyses were conducted by comparing FTIR spectra in order to observe changes in the chemical compounds in woods alongside the extraction steps. Finally, we showed ^{14}C results on a selected number of samples in this study to benchmark the chemical method and to support the FTIR results. For the latter, we make use of specific fractions of tree ring ^{14}C datasets (the sequence between 1938 and 1959 calendar years) from independent studies.

2. Materials and methods

2.1. Sample selection

Sample selection targeted pantropical tree species from different families with varying wood anatomical properties (porosity of vessels, axial and radial parenchyma distributions – Fig. 1) that were sampled from different locations in South America and Africa.

We evaluated 11 wood samples from 8 distinct tree species (Table 1). Age ranges for the wood sections used in this collection are variable, but mostly cover the last few decades to centuries. Therefore, the material was not degraded. To further evaluate differences in soluble extractives between individual trees from the same species, comparisons were made between the untreated woods of *Cedrela fissilis* pairs taken in close proximity of each other, as well as *Cedrela fissilis* and *Cedrela odorata* pairs taken from distant locations (selected samples of sites #1 and 2; Table 1). This evaluation intended to qualitatively characterize the impacts of individuality and environmental factors on the presence of extractives. Some of the woods in this study are part of full chronologies deposited in the International Tree-Ring Data Bank (ITRDB, https://www.ncei.noaa.gov/products/paleoclimatology/tree-ring, Table S1 - supplementary material). Finally, three reference materials were also investigated (Table 1), totaling 14 chemically untreated samples. IAEA-C3 cellulose was added as a control for α-cellulose FTIR quality. Samples underwent the same chemical extraction procedure (Fig. 2), except the subsamples termed untreated and the reference material C3.

2.2. Wood handling for chemical treatments and FTIR spectroscopy analysis

FTIR spectroscopy is a pragmatic and economical method for determining the chemical composition of wood (Alonso-Simón et al., 2011), and has successfully identified hemicellulose, cellulose, holocellulose and lignin in trees and other plant material (Rinne et al., 2005; Ling et al., 2019; Javier-Astete et al., 2021; Manian et al., 2022). This technique requires pulverized material for the most accurate readings. Multiple tree rings (i.e., calendar years with distinct ^{14}C signatures) were merged per sample via pulverization to ensure a high enough starting mass. All samples were reduced to ≤250 μm by grinding or sawing methods (Figs. S1 and S2). Pulverized samples were kept in capped culture tubes (Fisherbrand 13x100mm) until further steps took place (Section 2.3, Fig. S3).

A total of 56 FTIR spectra were obtained at the Laser Spectroscopy Labs, University of California Irvine, with a JASCO FT/IR-4700 spectrometer (Fig. S4) set to a spectral range of 4000 to 400 cm^{-1} (wavelengths 2.5–25 μm, 20 scans, a resolution of 2.0 cm^{-1}, and a gain of 8). For evaluations of wavenumber ranges in the FTIR spectrum produced, we made use of information provided by others, i.e., Richard et al. (2014), Rinne et al. (2005) and Wal (2021), all of whom drew from

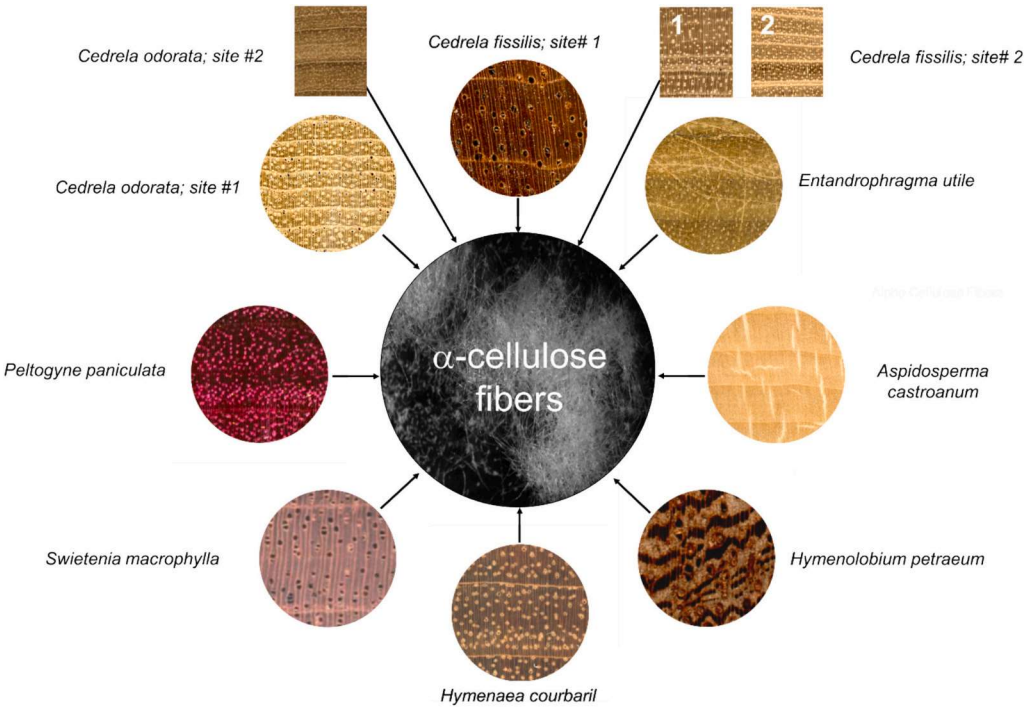

Fig. 1. Images of the pantropical tree species explored in this study (Table 1). Differences in wood coloring and wood anatomy features (diffuse- and semi ring-porous species, different parenchyma tissue conformations) are discerned. Homogenized α-cellulose fibers, achieved by the protocol evaluated here, are shown at the center (40× magnification).

Table 1
Pantropical tree samples and typical ^{14}C reference materials selected in this work are described below. Abbreviations were used in Figs. 3–6 and S5.

Abbr.	Sample Name	Location	Coordinates	Citations
Primary Set				
CF-JA	*Cedrela fissilis* Vell*; site# 1*	Jamari, Rondônia, Brazil	09°17′47.3" S, 62°58′40.7" W	Santos et al. (2021)
CO-RP	*Cedrela odorata* L.*; site# 1*	Rio Paru State Forest, Pará, Brazil	0° 58′ 44.4" S, 53° 19′ 33.6" W	Granato-Souza et al. (2019); Santos et al. (2020, 2024b)
PP	*Peltogyne paniculata* Benth	Jamari, Rondônia, Brazil	09°17′41.8" S, 62°58′34.7" W	Santos et al. (2021)
SM	*Swietenia macrophylla King*	Manoel Urbano, Acre, Brazil	8°29′40.4" S, 69°04′45.1" W	Unpublished
HC	*Hymenaea courbaril* L.	Manoel Urbano, Acre, Brazil	8° 28′23.57" S, 69° 1′30.60" W	Unpublished
HP	*Hymenolobium petraeum* Ducke	Porto Trombetas, Pará, Brazil	1°27′59"S, 56°22′45"W	Santos et al. (2022)
AP	*Aspidosperma castroanum* A.C.D. Castello	Ubajara, Ceará, Brazil	3°51′22.99" S, 70°57′44.0" W	Aragão et al. (2022)
EU	*Entandrophragma utile* (Dawe & Sprague) Sprague	Cameroon	5°23′N, 9°09′E to 9°12′E	Groenendijk et al. (2014); van der Sleen et al. (2015)
Secondary Set				
CF-JU1	*Cedrela fissilis* Vell*; site# 2*	Juvenília, Minas Gerais, Brazil MTV114B*	14°30′10.08"S, 44°10′4.08"W	Pereira et al. (2018)
CF-JU2	*Cedrela fissilis* Vell*; site# 2*	Juvenília, Minas Gerais, Brazil MTV001D**	14°30′10.08"S, 44°10′4.08"W	Pereira et al. (2018)
CO-MU	*Cedrela odorata* L*; site# 2*	Manoel Urbano, Acre, Brazil	8°28′44.23" S, 69°02′29.25" W	Unpublished
Reference Materials		^{14}C age and/or calendar year		
C3	IAEA-C3 cellulose	40-year-old trees harvested in 1989		Rozanski et al. (1992)
FIRI-F	*Scots pine (Pinus sylvestris* L.*)*	4508 ± 3 yrs. BP (3239–3200 BCE)		Scott et al. (2019) Santos et al. (2023) Spurk et al. (1998)
FIRI-H	*Oak (Quercus robur* L. *or Q. petraea* M.*)*	2232 ± 5 yrs. BP (313–294 BCE)		Scott et al. (2004, 2019) Santos et al. (2023)

*Increment Core, **Cross-section, IAEA refers to International Atomic Energy Agency, and FIRI to the Fourth International Radiocarbon Intercomparison.

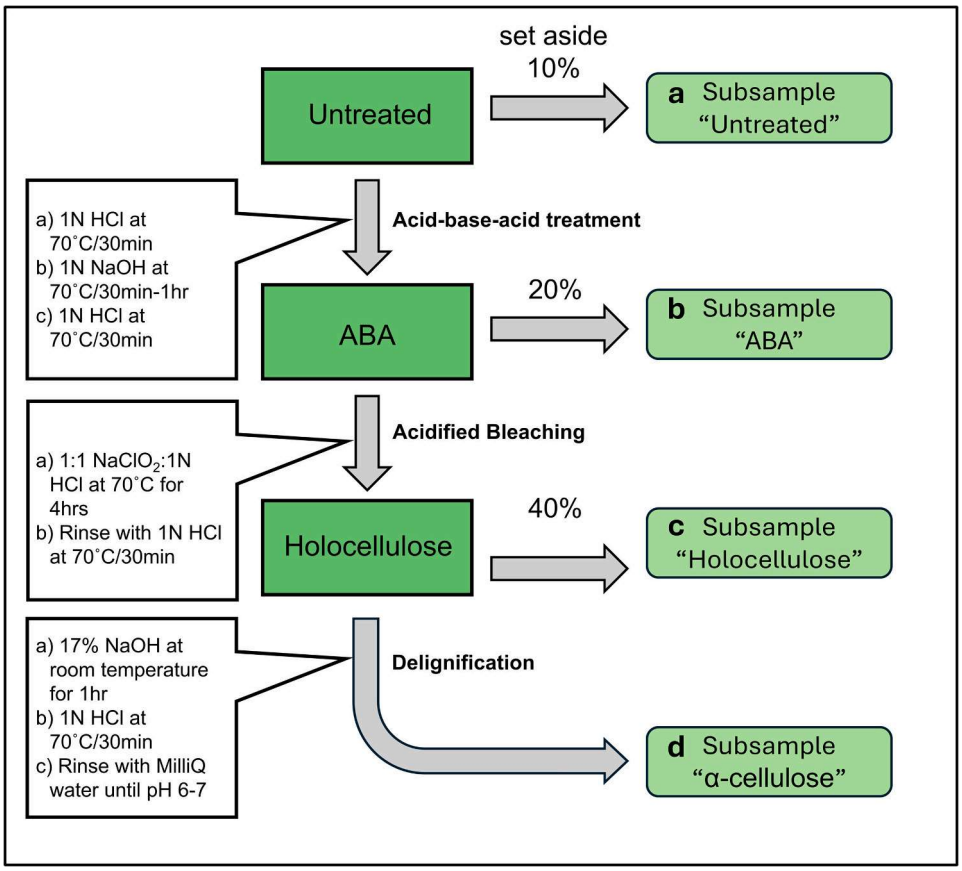

Fig. 2. Chemical steps to reach α-cellulose extracts (cf. Santos et al., 2023). At each stage, a percentage of the sample (termed subsample) was taken and set aside for FTIR spectroscopy analysis. Subsamples (a, b, c, and d) are used as panels in Figs. 3 and S5.

multiple citations therein (Table S2). Finally, to enhance the qualitative interpretation of the FTIR spectra displaying the same species pairs at different sites (Emmanuel et al., 2015), we also took the second order derivative of the infrared spectra (Jackson and Mantsch, 1995). This allowed us to improve the spectral resolution in the wavenumber range generally associated with νC=O resins (1694–1732 cm^{-1}). The presence of a peak within a compound's wavenumber range indicates that compound is present.

2.3. Chemical treatment

Pulverized wood was extracted to α-cellulose (Fig. 2). Four subsamples were produced from each wood sample, i.e., untreated, acid-base-acid (ABA), holocellulose, and α-cellulose. The proportion of the subsample set aside was sufficient to cover the circle lens of the JASCO FT/IR-4700 spectrometer three or more times (Fig. S4). The subsample amount was determined by previous knowledge of the recovery yield of each chemical step, e.g., ~ 69 % for ABA, ~ 30 % for bleaching, and ~ 27 % for α-cellulose (Santos et al., 2023). Subsamples were transferred using a wide-bore pipette, and solids were rinsed with Milli-Q® water until the solution pH rose to 6–7, then dried overnight on a heat block before FTIR analysis.

3. Results and discussion

3.1. FTIR spectra evaluations of untreated and chemically treated pantropical tree species

FTIR spectroscopy has been instrumental in comparing the

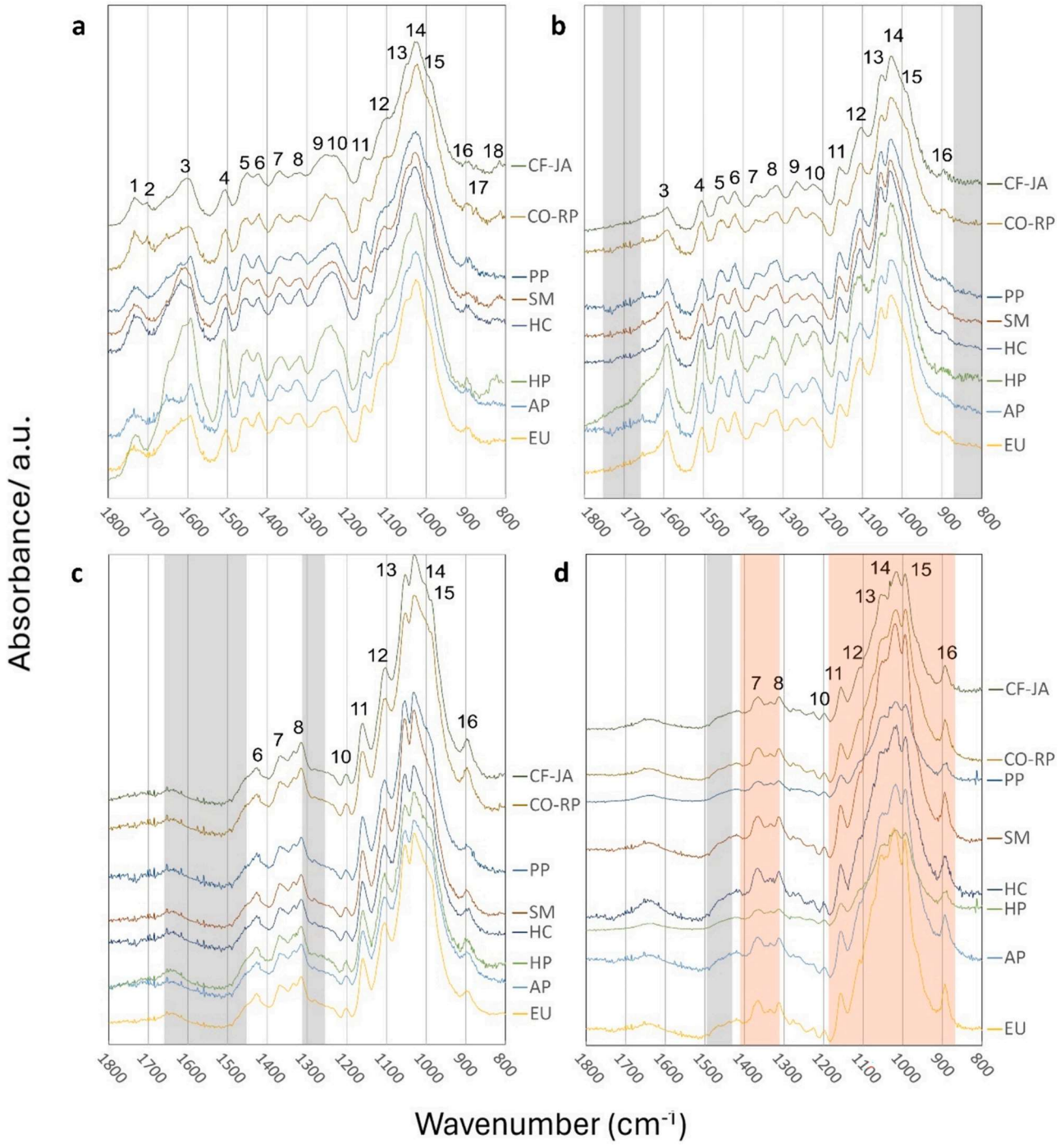

Fig. 3. FTIR spectra following chemical steps from Fig. 2: (a) untreated woods, (b) ABA, (c) holocellulose, and (d) α-cellulose extracts of 8 pantropical tree species (for the species list, see Table 1). The numbers above spectra peaks indicate wavenumber ranges and associated compounds (Table S2). Spectra were shifted vertically to enhance visual clarity, and narrowed to 1800–800 cm^{-1} range to focus on the typical cellulose "fingerprint" spectral region. Grey-shaded areas represent regions where peaks were removed after treatment, while rosy areas are associated with enhanced cellulose readings.

compound composition of different tree species as well as in determining differences in compound composition between wood chemical treatments for isotopic analysis (Michczyńska et al., 2018; Wal, 2021). All 8 untreated woods have rather similar FTIR spectra with the exception of peak (2) νC=O resin band (associated with gums or natural oils), which is discernible in the *Cedrela* samples of CF-JA and CO-RP (Fig. 3a, Table S2). This may indicate that tropical tree species have similar compounds, although proportions may vary based on their strikingly different visual appearances (Fig. 1). Funda et al. (2020) showed that the FTIR spectral regions associated with extractives, including the resin band, can be slightly shadowed by lignin when studying mature, natural Scots pine. Therefore, the presence of νC=O gums or natural oils cannot be ruled out from other tree species. FTIR spectrum of untreated woods also show overlapping bands of extractives, lignin, hemicelluloses, and cellulose from peaks 2–17 (Fig. 3a, Table S2). The emergence of new peaks or differences in the magnitude of peaks can be attributed to the phenomenon of remaining peaks (cellulose) becoming more enhanced as extractives, mobile lignin, and hemicelluloses are removed (Santos et al., 2023). Differences will be more closely analyzed below.

3.1.1. Acid-base-acid (ABA) treatment

We compared which FTIR peaks disappeared from the untreated spectra to the ABA spectra, after ABA treatment was finished (Fig. 3a-b). Peaks (1) νC=O hemicelluloses, (2) νC=O resins, (17) lignins G, and (18) mannan were removed (Table S2). Hoper et al. (1997) and Olsson (1980) found that the organic solvent step removed waxes, fats, oils, and other non-structural carbon. Here, the removal of some extractives, including resin (or gum) by ABA alone, may mimic the organic solvent's function. This seems to be supported by the work of Tenorio et al. (2016), which shows that, akin to some organic solvents, hot water as well as 1 % NaOH are independently effective in removing a variety of extractives when studying Costa-Rican trees (e.g., tannins, gum, phenolics, sugars and carbohydrates). The replacement/removal of the organic solvent step in favor of ABA treatment has been supported by Southon and Magana (2010). Nonetheless, solvent treatment may be required for wetland subfossil trees (Turney et al., 2021; Hogg et al., 2022). Further advantages of ABA treatment as an early step in cellulose extractions includes saving time and reducing instrumentation (i.e., Soxhlet apparatus).

3.1.2. Acidified bleach treatment

We compared which FTIR peaks disappeared from the ABA spectra to the holocellulose spectra, between which acidified bleaching takes place (Fig. 3b-c). Peaks (3) and (4) aromatic ring lignin - S & G, (5) δC-H lignin, carbohydrates, and hemicellulose, and (9) aromatic ring and νC-O lignin – G, and hemicellulose were removed (Table S2). The emergence of a sloped peak at approximately 1650 cm^{-1} is attributed to the H_2O peak being revealed and magnified as other peaks are removed (Richard et al., 2014; Santos et al., 2023). These results are corroborated by Hoper et al. (1997), which found that most lignin was removed by the acidified bleach step in the production of holocellulose. Lovell (1945) also mentioned that holocellulose should be relatively lignin-free. Because peak (6) residual lignin and carbohydrates are still present (Fig. 3c), α-cellulose extraction is encouraged.

3.1.3. Delignification treatment

Upon delignification (Fig. 3c-d), peak (6) δC-H lignins and carbohydrates and hemicellulose were removed from all 8 tree species (Table S2), advancing enhanced cellulose peaks (7, 8 and 11–16) as described in Aguayo et al. (2018). Studies on NaOH high-concentration solutions and temperatures coupled with FTIR analysis (Oh et al., 2005; Jungnikl et al., 2008) give further detail on cellulose molecular transformation. Our findings are similar to that of Hoper et al. (1997) and Wal (2021), which reported the removal of lignin and hemicellulose, respectively, as the primary delineation between holocellulose and α-cellulose for isotopic analysis. Hence, it appears that our α-cellulose extract (Fig. 3d) is in fact more refined than holocellulose (Fig. 3c) and may be more suitable for ^{14}C measurements. Nonetheless, neither Wal (2021) nor Santos et al. (2023) detected substantial differences between the cellulose products by FTIR alone, when evaluating a reduced set of samples.

3.2. Evaluations of same tree species from different sites

In Fig. 4, we show the FTIR spectra comparisons of *Cedrela* tree pairs apart by some distance, and the second order derivative analysis of the original IR. For the latter, we explored the spectra surrounding peak (2) νC=O resins with a 2-point moving average procedure. Plots were placed alongside their respective original FTIR spectra pairs.

The rationale behind these comparisons is that when the distance between trees is relatively short (several kms), one may expect near-identical environmental conditions affecting trees and their compounds. However, longer distances (hundreds of kms) may trigger different responses to distinct environment regimes on same tree species. *Cedrela* trees from Juvenília were apart by approximately 20 km, while other tree pairs were apart by 1500 to 2000 km, i.e., between Juvenília and Jamari Forests, or the Rio Paru State Forest and Manoel Urbano. Moreover, *Cedrela* spp. of Juvenília and Manoel Urbano experience drier climate conditions than those of Jamari and Rio Paru. Detailed sites coordinates are shown in Table 1.

At the Juvenília site, the original FTIR spectra of the untreated *C. fissilis* subsamples appear to have similar peak placement (Fig. 4a). A similar picture emerged from the original FTIR spectra comparisons between trees found at significant distances, i.e., 2000 km (Fig. 4c and e), except for peak 2 (νC=O resins). Specifically, the *C. fissilis* of the wet forest of Jamari (CF-JA) appears to contain a more recognizable gum peak, while the sample from the drier forest of Juvenilia (CF-JU1) does not. Similarly, small differences were detected between the original FTIR spectra of *C. fissilis* of Jamari (CF-JA) and those from Juvenilia (CF-JU1 or CF-JU2), as well as between the *C. odorata* trees between Rio Paru (CO-RP) and Manoel Urbano (CO-MU). We recognize that there are other small differences in the aromatic's region, between 800 and 1600 cm^{-1}, however, we have chosen to focus on the resin band due to its close relationship with extractives in hardwoods such as gums or natural oils.

Regarding the detrended and superimposed second order derivative analysis for each original IR pair, we observed discernible differences in the magnitudes of overlapping peaks between pairs, which is typically related to wood chemistry compound concentrations (Movasaghi et al., 2008). Second-derivative spectra can help to separate any overlapping component peaks in the original IR (Rieppo et al., 2012). Therefore, we aimed to show differences in wood compound composition at critical ranges of interest. According to the descriptions in Table S2, the wavelengths of resins and hemicellulose slightly overlap, making it difficult to differentiate them. However, based on peaks separated in the second-derivative spectra, Traoré et al. (2018) defines 1690 cm^{-1} as specific to resin acid compounds and 1730 cm^{-1} as hemicellulose. Thus here, it is likely that the occurrence of multiple peaks in the wavenumber range 1725-1740 cm^{-1} for hemicellulose (Table S2) may in fact indicate the presence of mild variations of this compound, or even other extractives, such as fats, wax compounds or in esterified resin acids (Zhou et al., 2015). Differences were more evident between the *C. fissilis* pair of the drier forest of Juvenilia (CF-JU1 versus CF-JU2; Fig. 4b), and the *C. fissilis* pair of Juvenilia and Jamari forests (CF-JU1 versus CF-JA; drier versus wet, respectively, Fig. 4d). Peculiarly, overlapping peak intensities were more analogous between the *C. odorata* trees of Rio Paru (CO-RP) and Manoel Urbano (CO-MU) (Fig. 4f). Differences can be observed as peak-offsets in specific areas, e.g., 1720 and 1730 cm^{-1} (Fig. 4b, d), 1730 and 1739 cm^{-1} (Fig. 4f), which may be associated to differences in fatty acids and carboxyl groups. Despite such differences, all species discussed in Fig. 4 were effectively reduced to α-cellulose

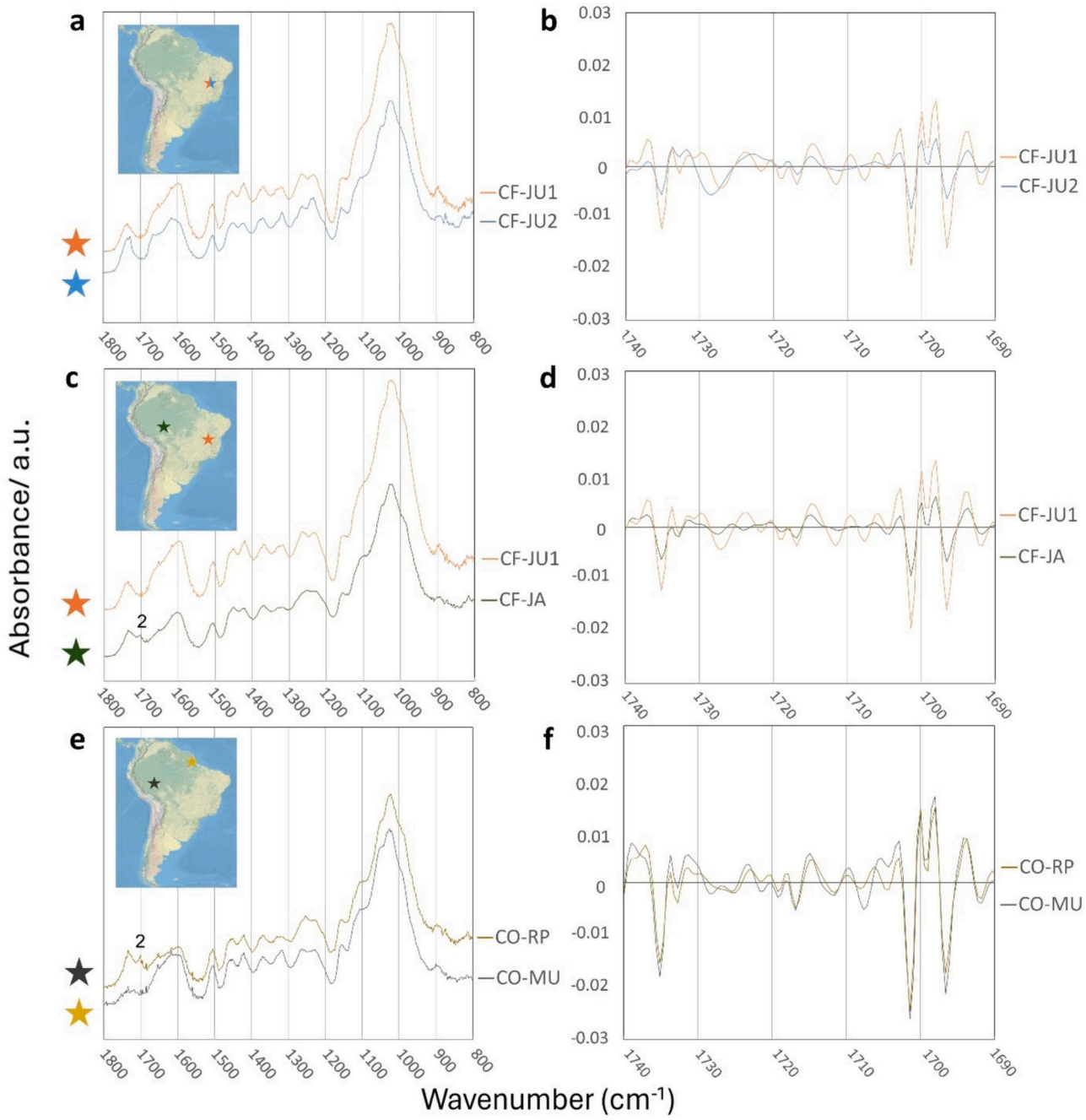

Fig. 4. FTIR spectra and second order derivative analysis of untreated woods of *C. fissilis* of Juvenília, collected 20 km apart (a, b), *C. fissilis* of Juvenília and Jamari, apart by ~2100 km (c, d) and *C. odorata* between Rio Paru and Manoel Urbano, apart by ~2000 km (e, f). Second order derivative analysis allowed us to superimpose the same tree spectral bands of *Cedrelas* collected at different sites in order to compare fine spectral details within a short range of 50 cm^{-1} wavenumbers. Precise tree locations are described in Table 1, while wood appearances are displayed in Fig. 1.

(Fig. 3d), indicating that even enhanced differences in compound composition can be overcome by a single extraction procedure.

The small yet discernable differences observed in Fig. 4 may be due to distinct microclimates and their complex effects on trees. Resin production in trees can increase in response to herbivore attacks (Langenheim, 1990; Schollaen et al., 2017), which tend to increase with higher humidity and heat (Neis et al., 2018; Zobel and van Buijtenen, 1989). Our results suggest that resin production among trees under distinct moisture regimes may be different. Alongside other analysis tools, visual observations of wood color and shades have been attributed to resin content as well (Pandey, 2005; Mayer et al., 2006; Baar et al., 2014). The differences in wood coloration between *Cedrelas* (Fig. 1) at different locations, and the distinctions shown in Fig. 4 may be correlated. Nonetheless, we call for caution with respect to our interpretations, as the comparisons we performed were based on a small sample size.

3.3. FTIR spectra of reference materials as α-cellulose extracts

Here, we present the α-cellulose FTIR spectrum of the subfossil woods of FIRI-F and FIRI-H, as well as the commercial C3 together with one of our tropical tree species (i.e., EU; Fig. 3). This should help the reader accurately assess the quality of the α-cellulose extracts isolated here, as FIRI-F, FIRI-H, and EU were purified via the same chemical procedure as the tropical tree species in Fig. 3, and C3 is a well-

established cellulose reference material used in isotopic laboratories (Table 1). Regarding ^{14}C results on subfossil and archeological samples, this chemical method has been already tested to a large array of ^{14}C-aged woods with precision and accuracy better than 0.3 ‰ (110 results in total - see details in Santos et al., 2023).

The tree species of Scots pine (FIRI-F) and Oak (FIRI-H) are known to contain significant amounts of νC=O resin (Dorado et al., 2000; Rinne et al., 2005). However, these early-Holocene woods (Table 1) may retain lesser amounts of hemicellulose and carbohydrates than their contemporary counterparts due to losses to the environment (Guo et al., 2022). Indeed, here, FTIR spectra profiles indicate that a fraction of the mobile compounds have been weathered from those subfossil woods (Fig. S5a – supplementary material). Upon chemical extraction completion, the FTIR spectra of their α-cellulose extracts (Fig. 5) were similar to those of tropical woods (Fig. 3d or S5) as well as to that of the reference material C3, illustrating once again that the removal of extractives was handled appropriately.

The FTIR spectra of C3, a cellulose produced in the late 1980s and redistributed by the IAEA, still displays peaks (6) δC-H lignins and carbohydrates and hemicellulose, and (18) mannan (Fig. 5). While these residues are clearly minimal, this illustrated that the chemical treatment we are evaluating is equally efficient, or even superior, at producing high-quality α-cellulose extracts. For complete FTIR view of the 14 samples in this study, refer to Fig. S5 (supplementary material).

In sum, the α-cellulose extracts of all eight tropical tree species have qualitatively identical peak placements as those of reference materials (Fig. S5). Moreover, the peaks of (1) νC=O hemicelluloses; (2) νC=O resins; (3) aromatic ring lignins (S & G); (4) aromatic ring lignins (S & G) again; (5) δC-H lignins and carbohydrates and hemicellulose; (6) δC-H lignins and carbohydrates and hemicellulose; (9) aromatic ring and νC-O lignins (G) and hemicellulose; (17) lignins (G); and (18) mannan (described in Table S2, and shown in Fig. 3) have been successfully removed from untreated woods. Our FTIR results combined with the ^{14}C results of subfossil and archeological woods in Santos et al. (2023) corroborate that the chemical treatment described in Fig. 2 consistently produces similar α-cellulose products for a variety of woods, regardless of extractive compounds or preservation status.

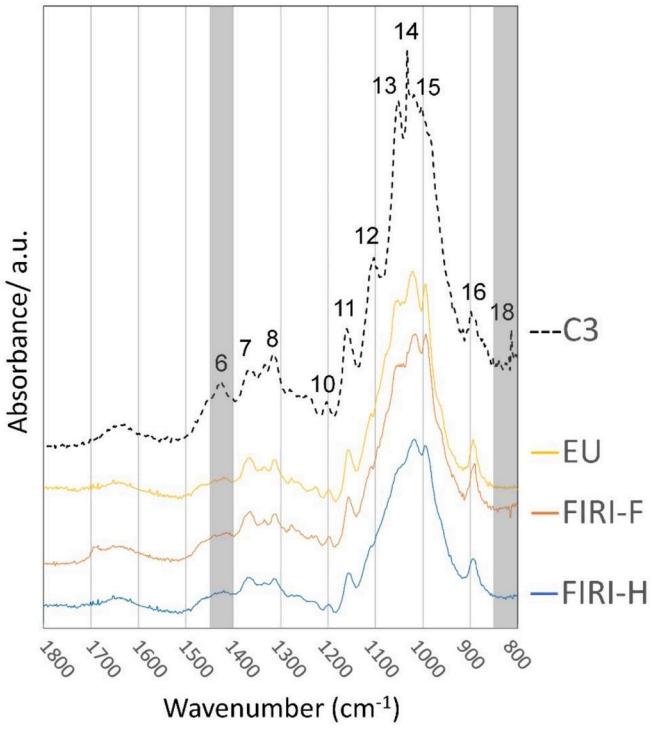

Fig. 5. FTIR spectra of α-cellulose extracts of reference materials described in Table 1, and the tropical tree species *E. utile* (EU), as a reference. Numbers above peaks indicate wavenumber ranges and associated compounds (Table S2). Grey-shaded areas represent regions where peaks were removed.

3.4. Implications for wood-based ^{14}C studies

The presence of mobile carbon in wood samples can create false readings, most notably an unexpected increase in ^{14}C levels detected at the pre-1950 calibration curve portion (Cain and Suess, 1976; Worbes and Junk, 1989), yet unexpected or erroneous post-1950 results are not uncommon (Tans et al., 1978; Westbrook et al., 2006). These false ^{14}C readings are attributed to secondary metabolites translocated within tree rings (Hartmann and Trumbore, 2016), which can happen to any calendar year (past to present). Thus, wood chemical extractions that remove such metabolites are required before ^{14}C analyses take place.

As gums, oils, lignin, carbohydrates, some sugars and starch are forms of nonstructural carbon (Carbone et al., 2013), it is to the benefit of ^{14}C dating that these compounds are effectively excluded. Early instances of wood chemical treatments record the need for organic solvent steps when treating woods for isotopic analysis (Olsson and Possnert, 1992). These chemical treatments and recommendations still persist today. It is not within the scope of this paper to suggest that organic solvents should never be employed, as we are aware of that some ancient Kauri woods require pre-solvent steps before α-cellulose extractions (Southon and Magana, 2010; Hogg et al., 2022). Several authors have already excluded the organic solvent step in their studies, further bolstering the claim that solvent usage is not always necessary. Regardless of their specifics, all wood chemical treatments intended for atmospheric ^{14}C reconstructions must be rigorously evaluated.

In Fig. 6, we show ^{14}C results of α-cellulose extracts from several of the pantropical trees evaluated here. Samples were produced from 15 to 30 mg of absolute-dated single tree rings, and later measured by high-precision accelerator mass spectrometry (AMS) to produce atmospheric pre- to post-1950 ^{14}C values. Radiocarbon results are from a west-east gradient (62°W to 9°E) with the goal of evaluating year-to-year intra-hemispheric variations in association with atmospheric circulation (Levin et al., 2022; Hua et al., 2022; Santos et al., 2022, 2024a, 2024b) across the Tropical Low-Pressure Belt (TLPB; conventionally termed the Inter-Tropical Convergence Zone). While those ^{14}C results have independent objectives, the details are beyond the scope of this article; here we are taking advantage of them to reinforce that all non-cellulosic compounds have been effectively removed. The latter can be derived from the ^{14}C data displayed in the dashed border-area shown in Fig. 6 (compiled in Table S3), as ^{14}C anomalies are clearly not present. Thus, we are confident that the high quality of α-cellulose extracts from a variety of tropical tree species (Fig. 1), as shown by the FTIR spectra (Fig. 3), is not incidental.

The removal of the organic solvent step in α-cellulose extractions has additional practical benefits as well. It remains effective, becomes time and cost-efficient, and reduces the consumption of potential environmental chemical contaminants (such as benzene, toluene, acetone, etc.). Note that the final step of the chemical procedure evaluated here, recommends the homogenization of α-cellulose fibers by an ultra-sonic bath (cf. Santos et al., 2023; Fig. 1) to evenly distribute the early and late-wood of a single tree ring and decrease the likelihood of skewed ^{14}C dating results. This procedure is critical and pragmatic, and easily accomplished after α-cellulose extractions, as the delignification step with 17 % NaOH facilitates defibrillation of cellulose fibril bundles (Beg et al., 2023). The FTIR spectra and ^{14}C results of several α-cellulose subsamples support that the chemical treatment proposed in Fig. 2 is robust and effective for treating pantropical woods, temperate oak, resinous Scots pine, as well as subfossil samples. We anticipate that this method will therefore be widely applied to derive ^{14}C levels from a variety of wood types across temperate and tropical studies.

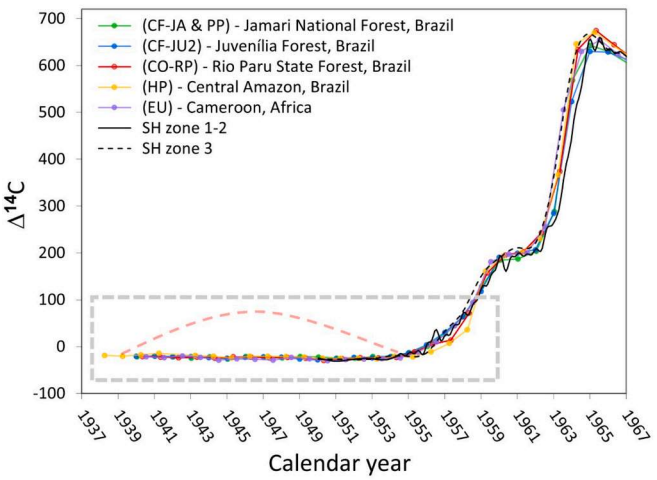

Fig. 6. Atmospheric $\Delta^{14}C$ of α-cellulose extracts from multiple tropical tree species and sites, i.e., *Cedrela odorata* (CO-RP) from Pará State (Santos et al., 2024b), *Hymenolobium petraeum* (HP) from Central Amazon (Santos et al., 2022), *Cedrela fissilis* (CF-JA) and *Peltogyne paniculate* (PP) from Jamari, *Cedrela fissilis* (CF-JU1) from Juvenília, and *Entandrophragma utile* (EU) from Cameroon (Table 1). Importantly, atmospheric $\Delta^{14}C$ results of α-cellulose extracts are in good to excellent agreement with expected atmospheric ^{14}C values of zonal curves (SH zone 1–2 and zone 3), as described in Hua et al. (2022), and Santos et al. (2022, 2024b). Moreover, ^{14}C values are mostly based on averaged results of duplicated data with a weighed-mean standard deviation error below 0.3 % (Santos et al., 2024a). The dashed-grey rectangle in the plot shows the calendar year range (1938–1959; Table S3) where abnormally high ^{14}C values tend to be detected due to the translocation of extractives from outer to inner-wood (after Cain and Suess, 1976; Worbes and Junk, 1989), if extractives are not properly removed. The dashed red bump in this rectangle is an example (not real data) of how a biased reading could present should mobile carbon remain.

3.5. Future recommendations

In a recent work, Khumalo et al. (2024) performed ^{14}C measurements on subsamples associated with different steps of the chemical protocols evaluated. The extent of the compounds removed was evaluated by ^{14}C differences of individual pine tree rings (*Pinus sylvestris L.*) between 1933 and 1937. Overall, the exercise showed that bleaching and delignification after acid and base cycling steps worked best. Such ^{14}C readings were not pursued here, as the wood material we used for our 56 FTIR spectra comprised of multiple calendar years, each with distinct ^{14}C signatures. Nonetheless, the findings in Khumalo et al. (2024) are not significantly different from ours (Figs. 3 and 6). To more precisely evaluate the ^{14}C response to distinct stages of chemical procedures on tropical trees, we posit several considerations:

- To better assess the ^{14}C signatures of chemical fractions, single rings should be sampled in large quantities (i.e., several milligrams), so that sufficient amounts of insoluble fractions from each chemical step can be used to derive graphite targets to undergo ^{14}C-AMS measurements.
- A large array of tropical tree species should be included in the exercise, due to their high diversity in wood extractive compositions (Pettersen, 1984; Baar et al., 2014; N'Guessan et al., 2023).
- We also assume that the calendar years range tested by ^{14}C should be larger than that tested by Khumalo et al. (2024). For instance, while Cain and Suess (1976) and Worbes and Junk (1989) detected abnormal ^{14}C data at the late pre-bomb era, Tans et al. (1978) and Westbrook et al. (2006) detected it at the rising of the radiocarbon bomb-peak slope. It should be noted that both Worbes and Junk (1989) and Westbrook et al. (2006) made use of tropical species in their studies.

4. Conclusions

Researchers have already stressed the importance of removing mobile carbon during wood chemical pretreatments to avoid biasing ^{14}C results. Over the years, several wood chemical protocols have been proposed. In this study, we addressed a long-overlooked issue, the effective removal of extractives when tropical trees are treated to α-cellulose. FTIR analysis was employed in this project to analyze the compound composition of a large suit of pantropical tree species and the purity of their α-cellulose extracts produced with a recently published chemical protocol that excludes organic solvents and acetic acid.

We determined that pure α-cellulose for reliable ^{14}C analysis can be attained with a straightforward chemical protocol, even when working with tropical tree species known to contain vast amounts of extractives or products such as gums and oils. This conclusion was drawn based on the presence or absence of compounds in FTIR spectra throughout the progress of the chemical treatment. Results imply that pure cellulose fibers can be produced by the combined approach of using only acid, alkaline, and bleaching treatments at the correct concentrations, temperatures, and time ranges. The use of a simplified chemical treatment streamlines the α-cellulose procedure while being cost-effective. The extraction protocol evaluated here implies that researchers will be able to improve tree ring ^{14}C-based studies and atmospheric ^{14}C datasets without compromising data quality.

CRediT authorship contribution statement

June Nakachi Griffin: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis. **Guaciara M. Santos:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Lucas Duy Nguyen:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Daigard R.O. Rodriguez:** Writing – review & editing, Resources. **Lucas G. Pereira:** Writing – review & editing, Resources. **Nelson Jaén-Barrios:** Writing – review & editing, Resources. **Gabriel Assis-Pereira:** Writing – review & editing, Resources. **Nathan de Oliveira Barreto:** Writing – review & editing, Resources. **Arno F.N. Brandes:** Writing – review & editing, Resources. **Ana Carolina Barbosa:** Writing – review & editing, Resources. **Peter Groenendijk:** Writing – review & editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.175010.

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