



Method to measure tree-ring width, density, elemental composition, and stable carbon and oxygen isotopes using one sample

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Abstract Tree-ring width (RW), density, elemental composition, and stable carbon and oxygen isotope ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) are widely used as proxies to assess climate change, ecology, and environmental pollution; however, a specific pretreatment has been needed for each proxy. Here, we developed a method by which each proxy can be measured in the same sample. First, the sample is polished for ring width measurement. After obtaining the ring width data, the sample is cut to form a 1-mm-thick wood plate. The sample is then mounted in a vertical sample holder, and gradually scanned by an X-ray beam. Simultaneously, the count rates of the fluorescent photons of elements (for chemical characterization) and a radiographic grayscale image (for wood density)

are obtained, i.e. the density and the element content are obtained. Then, cellulose is isolated from the 1-mm wood plate by removal of lignin, and hemicellulose. After producing this cellulose plate, cellulose subsamples are separated by knife under the microscope for inter-annual and intra-annual stable carbon and oxygen isotope ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) analysis. Based on this method, RW, density, elemental composition, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ can be measured from the same sample, which reduces sample amount and treatment time, and is helpful for multi-proxy comparison and combination research.

Keywords Tree-ring width · Tree-ring density · Tree-ring elemental composition · Tree-ring stable carbon and oxygen isotopes

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Introduction

Over the past century, tree rings have been widely used for climatic reconstructions and studies of environmental change because they have the important advantages of annual resolution and being precise dated (Fritts 1976; Cook et al. 2010; Büntgen et al. 2021). The proxies typically derived from tree rings include the ring width and ring density, as well as the stable isotopes ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) and elemental composition of the rings. Tree-ring width is the widely used proxy for temperature, precipitation and streamflow reconstruction (Gou et al. 2007; Cook et al. 2012; Wang et al. 2017; Yan et al. 2020; Singh et al. 2021). Tree-ring density is also an important tool in tree ring research, with latewood density being considered one of the best proxies for reconstructing past historical temperatures (Fan et al. 2009; Liang et al. 2016; Björklund et al. 2019). In addition, by detecting changes in the element composition contained in tree rings,

we can track the pollution history of polluted sites (Rocha et al. 2020). Stable carbon and oxygen isotopes can be used to reconstruct temperature and precipitation variations, and calculating intrinsic water-use efficiency (iWUE) (Gagen et al. 2007; Xu et al. 2011, 2023; Frank et al. 2015).

Multi-proxy analysis of tree rings can assist with efforts to obtain a comprehensive understanding of different aspects of natural and human history (McCarroll and Loader 2004; Binda et al. 2021; Nguyen et al. 2021). For example, combining tree-ring width and maximum late wood density (MXD) data can achieve a more effective summer temperature reconstruction than using only ring width data (Chen et al. 2019; George and Esper 2019). In addition, the combination of ring width and $\delta^{13}\text{C}$ can well reconstruct the past temperature change (Liu et al. 2007), and may be an effective method to reconstruct the regional snow cover change (Liu et al. 2011). The combination of tree-ring width and $\delta^{18}\text{O}$ explore the physiological response of tree growth to climate change, and reconstruct monthly streamflow (Fang et al. 2020; Nguyen et al. 2022; Zhao et al. 2023). The combination of tree-ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ not only helps to separate the effect of stomatal conductance or photosynthesis on water use efficiency (Grießinger et al. 2019; Guerrieri et al. 2019; Mathias and Thomas 2021), but also strengthens the climate reconstruction signal whilst dampening the noise (Freund et al. 2023).

Usually, each proxy is analyzed separately due to the different pre-treatment methods, the limited number of samples, or different research goals. For example, 5-mm cores are collected for tree-ring width analysis, but 10-mm

or 12-mm cores may be needed to produce enough sample material for density and isotopic analysis. The measurement of tree-ring width results in no sample loss except for polishing. For the analysis of density and elemental composition, X-ray technology is now a widely used measurement technique, but the samples must be cut into laths and treated with alcohol to remove resins and other extractable compounds in the wood prior to X-raying (Schweingruber et al. 1978). Moreover, the analysis of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ requires further chemical treatment to obtain α -cellulose for isotopic measurement (Loader et al. 1997). The process of cutting and chemical treatment causes large and permanent losses from the tree-ring samples. Hence, in situations where the amount of sample material is limited, a method that uses the minimum sample amount but makes it possible to obtain all these tree-ring proxies, and simultaneously reduces the time required and the sample loss, would be beneficial.

In this study, we present a method that can be used to measure the tree-ring width and density, as well as the elemental composition and the stable carbon and oxygen isotope ratios, from a single sample. After a single tree-ring core is dated, the sample is then cut to make a 1-mm wood plate for measurement of the density and elemental composition, and then this plate is treated to extract cellulose and cut into subsamples at annual or seasonal resolution to obtain the tree-ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values. This method is described with respect to the instrumentation and equipment we use in our laboratory and makes maximum use of one sample to obtain the tree-ring width, density, elemental composition, and $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ data (Fig. 1).

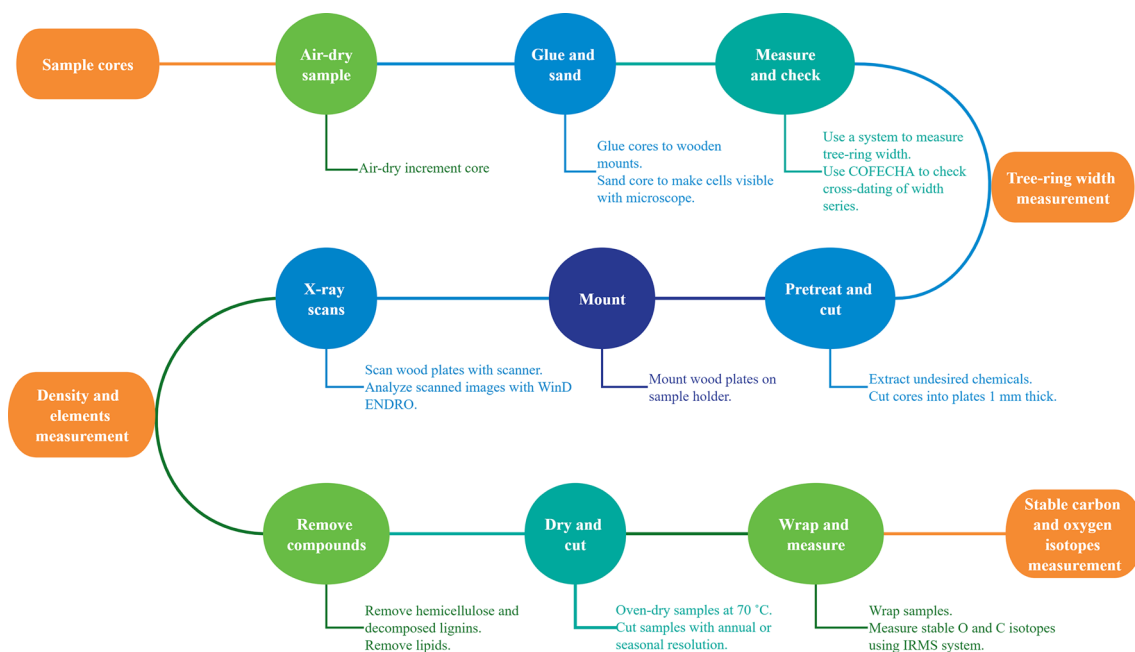


Fig. 1 Processing flow chart

Materials and method

Tree core sampling

The sample example in this text was collected from sub-alpine *Abies fargesii* in Shennongjia, central China (SNJ, 31.45 °N, 110.25 °E, 2800 m a.s.l.). Tree ring cores were extracted from healthy trees using 10 mm diameter increment borers at chest height. After each tree core was collected, its quality was visually assessed. If problems such as decay are found, the sampling site of the tree should be replaced and resampled. The cores collected from each tree were wrapped in dry paper tubes and numbered, and finally brought back to the laboratory.

Tree-ring width measurement

After the cores were air-dried, sample cores were removed from the paper tubes and placed with the fiber direction perpendicular to the horizontal into a groove on a slat. Each core was then sanded with a series of finer-grit sandpaper (400, 600, 800, 1000–2000). Sanding did not affect values for tree ring oxygen and carbon isotopes. After sanding, ring structure is then examined using a microscope (Fig. 2a) and coarsely dated to 10, 50, and 100 years using visual dating, and very narrow or suspected rings are marked.

We used a ring width measurement system (e.g., LINTAB measuring table (Rinntech, Heidelberg, Germany; the precision of 0.01 mm) and TSAP-Win software, the WinD-ENDRO tree-ring analysis system (Regent Instruments, Canada), or Velmex measuring system (Velmex, Inc., Bloomfield, USA)) to measure the tree-ring widths, and then cross-date the cores (Fig. 2b). Finally, COFECHA (Holmes 1983) was used to check the quality of the cross-dating results.

Tree-ring density and elemental composition measurement

Sample cores were soaked in a water bath at 80 °C for 48 h to remove water-soluble materials. The water was replaced with hot water every 8 h to ensure that water-soluble compounds are fully removed. The sample cores were then placed in a glass vessel with 99% alcohol for 48 h to remove resins and other soluble extracts.

Each core was glued to a wooden mount, and then cut into 1-mm-thick wood plates using a high-precision double-edged saw (Fig. 3a) with the cuts made perpendicular to the fiber direction. Any glue at the edge of the wood plates was removed with a carving knife, and the rest of the glue was removed with a solvent such as acetone or ethanol in the cellulose extraction step, so the glue does not affect the extracted cellulose. A comparison of cellulose oxygen

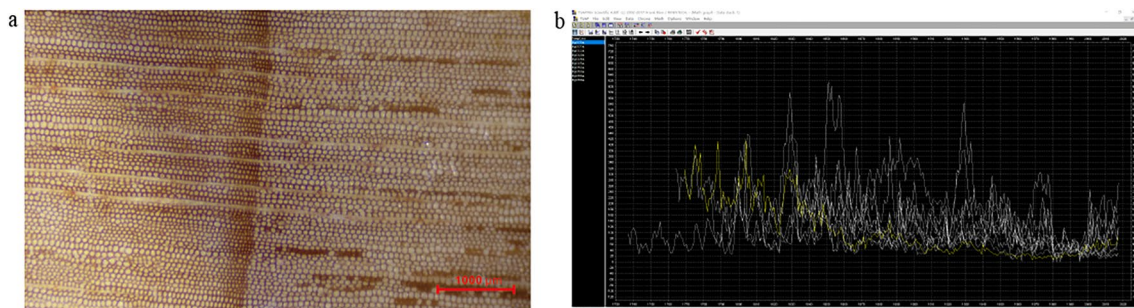
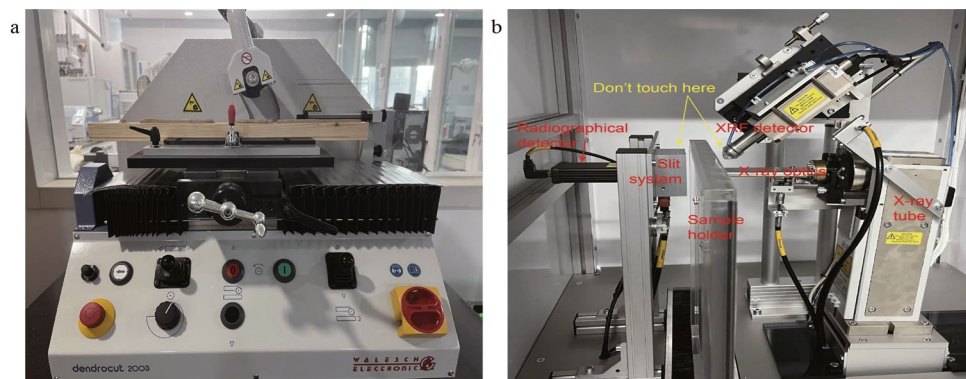


Fig. 2 Tree-ring width measurement. **a** Sanded samples. **b** Tree-ring widths measured using LINTAB

Fig. 3 Equipment for measuring tree-ring density and elemental composition. **a** Double-edged DendroCut saw (Walesch Electronic GmbH, Effretikon, Switzerland). **b** Itrax Multiscanner (Cox Analytical Systems, Sweden)



isotopes in samples prepared by the wood-plate method with glue and the traditional method without glue showed that there was no significant difference in the oxygen isotopes values (Xu et al. 2013b).

Laths 1 mm thick were cut from the tree cores, then scanned with X-rays in an Itrax Multiscanner (Cox Analytical Systems, Mölndal, Sweden; Fig. 3b) that measures wood density and quantifies chemical elements using X-ray fluorescence (XRF) (Jacquin et al. 2017; Björklund et al. 2019). The Itrax was operated at 30 kV and 35 mA with a Cr-tube, and the sample was exposed to the X-ray beam for 100 s at each measurement point and advanced in the radial direction in 20- μ m steps. Simultaneously, count rates of fluorescent photons for chemical elements (for chemical characterization) and a radiographic greyscale image (for wood density) were generated. The scanner has a resolution of 50 μ m, which is thus the minimum ring width that can be analyzed in the tree-ring samples.

Peaks in the continuous XRF spectrum were assigned to specific elements using the Q-spec software (Cox Analytical Systems), producing relative concentrations (counts of fluorescent photons) of those elements detected and pre-defined within the wood structure for each analyzed point. Tree-ring

boundaries were defined on the radiographic image using WinDENDRO (Regent Instruments, Canada) and the pixel-based output was used to transfer these boundaries to the elemental counts. In addition, maximum latewood densities were extracted from the radiographic images by calibrating the greyscale intensities to wood densities using a light calibration curve derived from a calibration wedge.

Cellulose extraction for oxygen and carbon isotope measurement

After the density and elemental analyses, each wood plate was cut into several sections (e.g., 7–8 cm for each section) with a knife designed to fit into glass tubes (Fig. 4a). Then, we scanned these wood plates with the multiscanner to record the original ring structure information. Finally, we sandwiched the wood plate between two Teflon punch sheets that were then tied together with cotton string (Fig. 4b) and inserted the sample into a glass test tube.

The samples in test tubes were then chemically treated to extract cellulose (Table 1). An acidified NaClO_2 solution was used to remove the lignin (Fig. 4c) with successive extractions until the wood plate turned white or light yellow,

Fig. 4 Wood plates after different extraction steps before oxygen and carbon isotope measurements. **a** Before cellulose extraction. **b** Packed between Teflon punch sheets. After removal of **c** lignin, then **d** hemicellulose and decomposed lignin, then **e** lipids. **f** Dried 1-mm-thick cellulose plate. **g** Wrapped in silver foil to measure oxygen isotope (roll shape, left) and in tin foil for carbon isotope (cuboid shape, right)



Table 1 Chemical extraction to analyze cellulose in tree-ring cores

Step	Solution	Duration	No. of times	Bath temperature
Step 1: removal of lignin	Acidified NaClO ₂	1 h	4 or more	70 °C
Wash	Hot distilled water (98~100 °C)	15 min	3	
Step 2: removal of hemicellulose and decomposed lignin	NaOH (17 wt %)	1 h	3	80 °C
Wash	distilled water	10 min	Until pH < 10	
Neutralization	0.01N HCl	10 min	1	
Wash	distilled water	10 min	Until pH 5–7	
Step 3: removal of lipids	Solvent (“acetone”; 1:1 “toluene–ethanol”)	Acetone: 10 min; 1:1 toluene–ethanol: over night	1	

indicating that the lignin has been removed. Samples require vary in the number of extractions, but generally require at least four times. Samples are then soaked in an NaOH (17 wt%) solution to remove hemicellulose and decomposed lignin (Xu et al. 2011, 2013a) (Fig. 4d), then washed gently and thoroughly in distilled water until pH < 10, the solution is then neutralized with diluted HCl. Samples were then wash a few times with distilled water until pH was 5–7. After organic solvents removed lipids (Fig. 4e), samples were oven-dried at 70 °C to yield cellulose plates for further analysis.

The dried 1-mm-thick cellulose plate was then placed on a photo-binder with an adherent black surface and transparent plastic film (Fig. 4f). The samples were then viewed with a microscope. Subsamples were cut and weighed (± 0.001 mg) to measure oxygen isotopes (120–200 μg) and carbon isotopes (60–120 μg) and rolled in a 7 mm \times 7 mm piece of silver foil for oxygen isotopes or a 7 mm \times 7 mm piece of tin foil in a cuboid shape for carbon isotopes (Fig. 4g).

Stable isotope ratios of the cellulose samples were measured with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Germany) coupled to a pyrolysis-type, high-temperature conversion elemental analyzer (Flash 2000-HT, Thermo Scientific, Germany). We measured oxygen isotopes using the pyrolysis method and carbon isotopes using the combustion method. Merck’s cellulose microcrystalline was used as the authentic standard ($\delta^{18}\text{O}$ value: 29‰, $\delta^{13}\text{C}$ value: –24.58‰). The Merck standard was used for each of eight cellulose samples to calibrate $\delta^{18}\text{O}/^{16}\text{O}$ and $\delta^{13}\text{C}/^{12}\text{C}$ ratios for the sample. The standard deviation for the Merck sample in one batch was less than 0.2‰ for the oxygen isotope and 0.1‰ for the carbon isotope.

Results

The width, density, elemental composition and stable oxygen isotope levels for sample SNJ-526A from Shennongjia

in Hubei Province, China are shown in Fig. 5. The ring-width time series in Fig. 5a shows the interannual variations. In particular, the ring width in 1971–1973, 1989, 2000 and 2014 was extremely narrow.

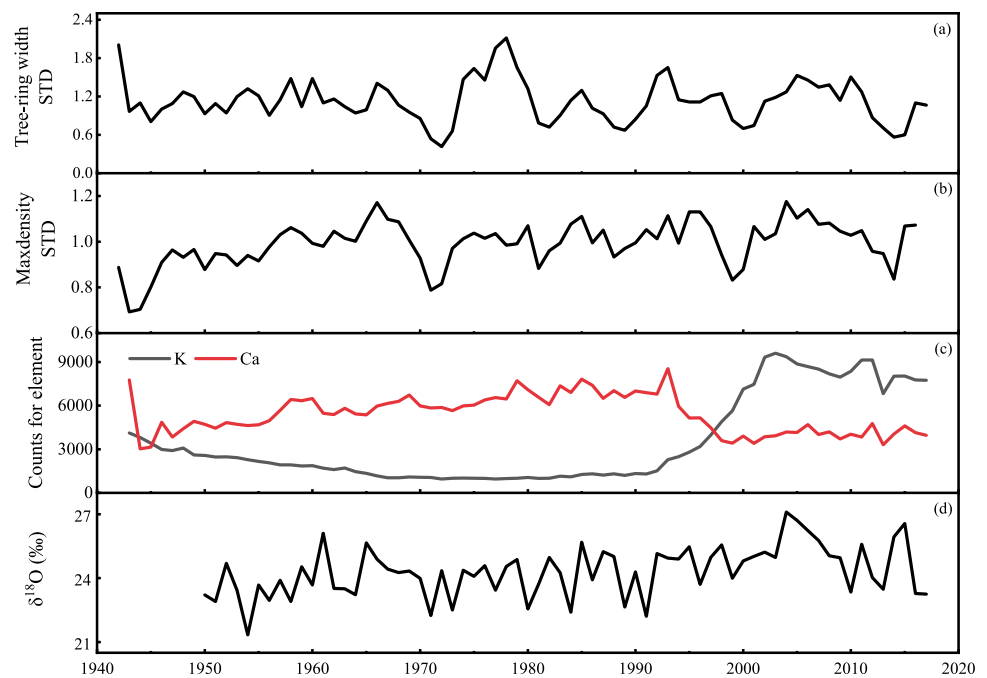
Comparing Fig. 5a and 5b, the maximum latewood density is consistent with the tree-ring width, and the density decreases obviously in years with very narrow tree rings. In addition, the maximum latewood density has an increasing trend in the whole period. Using K and Ca as an example of elemental analysis (Fig. 5c), we found sudden changes in their content around 1995, which may be influenced by sapwood.

After the chemical extractions of the samples, the final cellulose plates are white (Fig. 4f), with no hemicellulose or lignin. The purity of the resulting cellulose obtained from this extraction process was verified in a previous study (Xu et al. 2013b). The tree-ring boundaries can be clearly identified, allowing binocular-aided tree-ring dissection with a suitable knife. In Fig. 5d, the $\delta^{18}\text{O}$ ratio of the sample seems to increase slowly from 1950 to 2014. In addition, previous study showed stable oxygen isotope levels were fairly consistent between different trees at this sampling site (Zhao et al. 2023).

Discussion

The proposed method can be used to obtain tree-ring width, density, elemental composition, and stable carbon and oxygen isotope data from the same ring in one core from a tree. Therefore, the accurate separation of each ring is very important. For the width, density, and elemental composition measurements, it is easy to accurately define each ring because the original ring structure and dating information can be seen. For the carbon and oxygen isotope measurements, individual cellulose rings should be separated from the cellulose plate, but matching the cellulose plate to the original wood plate is sometimes difficult. Shrinkage of the samples during the cellulose extraction

Fig. 5 Tree-ring (between 1942 and 2017) multiproxy analytical data obtained from sample SNJ-526A from central China using the proposed method. **a** Tree-ring width. **b** Maximum latewood density. **c** K and Ca contents. **d** Stable oxygen isotope levels. STD=standard deviation



(Xu et al. 2011, 2013b; Kagawa et al. 2015) may cause the cellulose plate to break into several places, and several rings on the edge of the wood plate may be lost. Therefore, matching the broken cellulose plate with the original wood plate is a critical step and can be achieved by carefully comparing the cellulose plate to the original wood plate scanned image. The high inter-tree correlation (0.8–0.9) that was previously found for cellulose oxygen isotope levels from different trees indicates the robustness of this method (Xu et al. 2019).

This method combines procedures for measuring ring width, wood density and elemental composition, and stable carbon and oxygen isotopes. However, it is of limited use for samples with extremely narrow rings (< 50 μm, the resolution of the scanner). In addition, for stable isotope analysis, very narrow rings (< 0.1 mm) do not produce enough material to measure (Xu et al. 2013a).

One advantage of this method is that more data are generated from a single sample. Only one wood plate is used, and the remaining sample material can be used for other analyses. If the core is collected with a 10 or 12 mm increment borer, then two or three 1-mm wood plates would be available for replicate analyses or for measuring other variables such as hydrogen, nitrogen, sulfur, or radioisotopes. In addition, the scanned high-resolution image that is produced includes anatomical information such as frost rings and resin ducts.

This method can be expanded when the sample material is limited and can help to maximize the paleo-information extracted from a limited number of samples. It may also be useful for other paleoclimate proxies such as stalagmites

and coral, for measuring the thickness of the laminae, then density and chemical elements, and finally carbon and oxygen isotopes.

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