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Invited research article

Inter-annual carbon isotope analysis of tree-rings by laser ablation

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ABSTRACT

The stable carbon isotopic analysis of tree-rings for environmental, plant physiological and archaeological applications using conventional methods is occasionally limited by physical constraints (narrow rings) or administrative concerns (requirement for non-destructive sampling) that prevent researcher access to scientifically valuable wood samples. Analysis of such archives by laser-ablation can potentially address these issues and facilitate access to restricted archives. Smaller quantities of wood are required for analysis by laser ablation, hence the approach may be considered less-invasive and is virtually non-destructive compared to standard preparation methods. High levels of intra-annual isotopic variability reported elsewhere mean that a single measurement may not faithfully represent the inter-annual isotopic signal, so before such an approach can be used with confidence it is necessary to compare the stable carbon isotopic data produced using these two methods. This paper presents stable carbon isotope ($\delta^{13}C$) data from the resin-extracted wood of dated Scots Pine (*Pinus sylvestris* L.) tree-rings analysed using a modified Schulze-type laser-ablation system with results obtained using conventional manual sampling and analysis of α-cellulose prepared from the same tree-ring groups. The laser sampling system is found to perform very well against established more invasive methods. High correlations are observed between the methods for both raw and Suess corrected data ($r > 0.90$ $n = 50$). These results highlight the potential for using laser-sampling to support the development of long isotope chronologies, for sampling narrow rings or for pre-screening cores prior to analysis using more detailed or labour intensive methods.

1. Introduction, background & rationale

Since the advent of modern dendroclimatology in the late 19th and early 20th century (Schweingruber, 1988 and references therein) vast archives of increment cores and X-ray densitometry samples have been assembled by tree-ring researchers worldwide in the quest to answer a myriad of cultural, environmental, and archaeological questions. The potential for these samples to provide further environmental information through their chemical analysis remains an attractive possibility and one beyond their original intended use. However, such work has rarely been attempted due to the unique properties of the archive and the destructive nature of the analytical processes typically employed (Loader et al., 2008).

Sampling tree-rings by laser ablation is an establishing technique in isotope dendroclimatology and provides an essentially non-destructive means for accessing elemental and isotopic information from wood samples (Hoffmann et al., 1994; Prohaska et al., 1998; Wieser and Brand, 1999; Schulze et al., 2004; Skomarkova et al., 2006; Vaganov et al., 2009; Schollaen et al., 2014; Rinne et al., 2015; Soudant et al., 2016). The most common application of laser-sampling is to investigate intra-annual isotopic variability across individual tree-rings or the detection of rhythmic trends in ringless trees to elucidate plant physiological processes related to short-term environmental changes. High-resolution (intra-annual) sampling of tree-rings by manual sub-division, microtoming, or micro-milling has previously identified large (*c.* 3‰) quasi-rhythmic intra-annual isotopic variability in tree-rings (Wilson and Grinsted, 1977; Ogle and McCormac, 1994; Loader et al., 1995, Kagawa et al., 2002; Helle and Schleser, 2004; Evans and Schrag, 2004; Poussart et al., 2004, 2006; Poussart and Schrag, 2005; Anchukaitis et al., 2008; Dodd et al., 2008; Ogée et al., 2009; Schubert and Jahren, 2011; Xu et al., 2015; Soudant et al., 2016). The use of laser-sampling has greatly facilitated the production of such series and the potential for improved replication.

A second and less common application of laser sampling is the

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analysis of small-diameter cores, rare or narrow-ringed samples, which are not normally suitable for manual sampling and cellulose isolation techniques due to their small size or high value. Laser sampling of these series can provide a means for developing annually-resolved stable isotope chronologies from such challenging samples or to pre-screen cores for representative or coherent behaviour prior to investing more expensive or labour intensive methods (*e.g.* Waterhouse et al., 2013). As only very small amounts of material are removed for analysis by laser ablation, the sample remains largely intact, and for rare samples and those of archaeological significance, may be returned to the archive after analysis, which represents a significant advantage to both archivists and researcher. However, given the high level of intra-annual carbon isotopic variability preserved within individual tree-rings (*c.* 3‰) it is necessary to evaluate whether simple "one shot" laser sampling at annual resolution is capable of providing a representative annual signal. This paper reports results from a comparison of stable carbon isotopes measured on annual tree-rings using a Schulze-type laser sampling system with series of carbon isotope data from annually-resolved tree-rings prepared and measured using conventional methods.

2. Method, sample preparation and chronology development

To evaluate the potential of the approach for the development of long isotope chronologies and to compare the signal from laser sampling with data from conventional manual preparation and analysis of tree-ring cellulose, four dendrochronologically dated Scots pine (*Pinus sylvestris* L.) trees between 250 and 180 years old from the Torneträsk region of northern Sweden were sampled (68.10–68.30°N, 19.40–20.20°E 350–450 m a.s.l.) (Loader et al., 2013). In preparing samples for laser analysis, an attempt was made to reproduce broadly the sample preparation protocol for X-ray densitometry, as a significant archive of densitometry laths has been developed by laboratories that might prove useful for future isotopic analyses (Grudd, 2008). Radial samples were cut from each tree using a hand-saw and further subdivided into 2 mm thick laths cut using a twin-bladed saw. The resins in each lath were removed by reflux in hot ethanol over a period of 50 h using a Soxhlet apparatus and repeatedly washed with boiling deionised water prior to air drying. The resulting resin-extracted laths were then gently surfaced using abrasive paper to facilitate dating and ring identification in the laser chamber. Similar results to sanding can be obtained using a razor blade to expose the cell walls and ring boundaries. Each lath was then trimmed to a shorter section (max. length 2 cm) to enable it to be fitted into the sample chamber with a sample of IAEA-C3 holocellulose standard. Finer subdivision of samples into thin strips approximately 4 mm wide (similar in dimension to a match stick) is possible and enables multiple core samples to be loaded into the chamber if desired (Fig. 1).

Conventional analyses were performed on radii cut from the same trees, surfaced using abrasive paper and the same group of dated tree-rings (1900–1950 CE) manually subdivided as thin slivers using a scalpel and dissecting microscope. Cellulose was prepared using standard methods (Loader et al., 1997; Rinne et al., 2005) and the sample material (comprising both early- and late-wood) homogenised using a Hielscher-type ultrasonic probe (Laumer et al., 2009). Samples were freeze-dried prior to weighing 0.30–0.35 mg of the dry cellulose into tin foil capsules for carbon isotope analyses by combustion on-line using a Sercon GSL elemental analyser and 20/20 isotope ratio mass spectrometer. Analytical precision is typically 0.10‰ (Boettger et al., 2007; Loader et al., 2013).

The laser ablation system used is a variant on the Schulze-type system and comprises three elements (laser ablation, combustion interface, mass spectrometer). The laser is a 213 nm wavelength UV laser ablation platform (UP-213 New Wave/ESI). The UP-213 system is software controlled enabling the user to select different ablation modes, laser power, sampling resolution (spot size) *etc.* A 50 mm diameter sample chamber is linked to the combustion unit *via* 6 mm outer diameter (OD) and 3 mm internal diameter (ID) Tygon™ tubing through which helium is passed as a carrier gas at 44 ml/min < 2 PSI.

The combustion interface comprises a 500 mm length (6 mm OD, 4 mm ID) quartz tube packed with chromium(III) oxide and quartz fibre wool. The tube is heated to 600 °C and the chrome oxide acts as the oxygen source for conversion of the wood powder to CO_2 and water vapour. The water vapour is removed by a Nafion™ drier and chemical magnesium perchlorate water trap. The remaining CO_2 and non-condensable gases are then passed through a stainless steel capillary tube lowered into liquid nitrogen. This traps the CO_2 cryogenically to permit full transfer and conversion of the wood powder from the sample chamber through the furnace. The helium flow rate is then reduced to 4 ml/min and the cold trap automatically thawed to release the sample CO_2. A single measurement typically takes 8 min.

Samples are measured by isotope ratio mass spectrometry against a reference gas and cellulose standards. A typical batch analysis comprises ten standard analyses located at the start of the run; these provide varying sample sizes for analysis to test for sample size effects in the system introduced through variations in wood density which affect the quantity of wood ablated. Similar to the system described by Schulze et al. (2004), we find good results obtained from slightly larger samples using an 80–100-μm-diameter laser beam moving at 10 μm/s across a 300–400-μm-long sampling track (Laser power 85%, 20 Hz). Multiple passes across the same track advancing the sample in the z-axis yields an ablation pit *c.* 230-μm deep and ensures that sufficient material is available for analysis. At the end of the run and as appropriate, additional cellulose standards are analysed to assess analytical precision and measurement stability. Although the IAEA-C3 holocellulose standard material is not completely homogeneous, measurement precision of 0.11–0.15‰ is routinely obtained.

For inter-annual laser analysis of the tree-ring samples a 50-year period was identified for each series and analysed once per growth ring using a laser beam of 100 μm in diameter sampling three times along the same traverse of 500 μm. For this test, the location of laser sampling within each ring was not positioned systematically, the only constraint being that the laser sampled wood (radially) across the ring and avoided any resin ducts or visible contaminants (*e.g.* abrasive grains embedded in the wood following sample preparation) (Fig. 1). Samples were run in batches of *c.* 60–80 for convenience although larger batches of 150–200 samples have been run successfully.

3. Results

Comparison of the four carbon isotope time series (Fig. 2A–D) developed using conventional methods (manual subdivision, cellulose

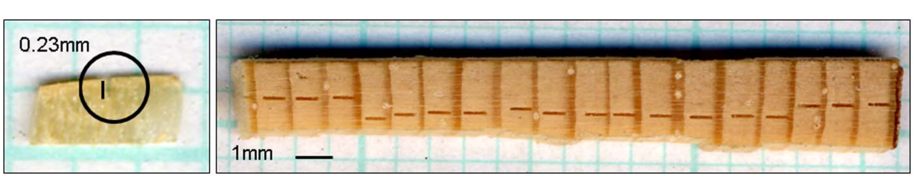

Fig. 1. (Right panel) Example of sub-divided resin-extracted wood lath for annual UV laser ablation carbon isotope analysis. The above lath, from Torneträsk, northern Sweden (68.10–68.30°N, 19.40–20.20°E 350–450 m a.s.l.) is approximately *c.* 20 mm × 2 mm. In this image each dated growth ring is sampled by UV laser ablation as a single traverse (100 μm beam diameter 500 μm traverse length). Each traverse is positioned in a broadly radial direction through the tree-ring avoiding any resin ducts or contamination. Base grid 1 mm × 1 mm. (Left panel) transverse cross-section of lath indicating the position and depth of the ablation pit (*c.* 0.23 mm).

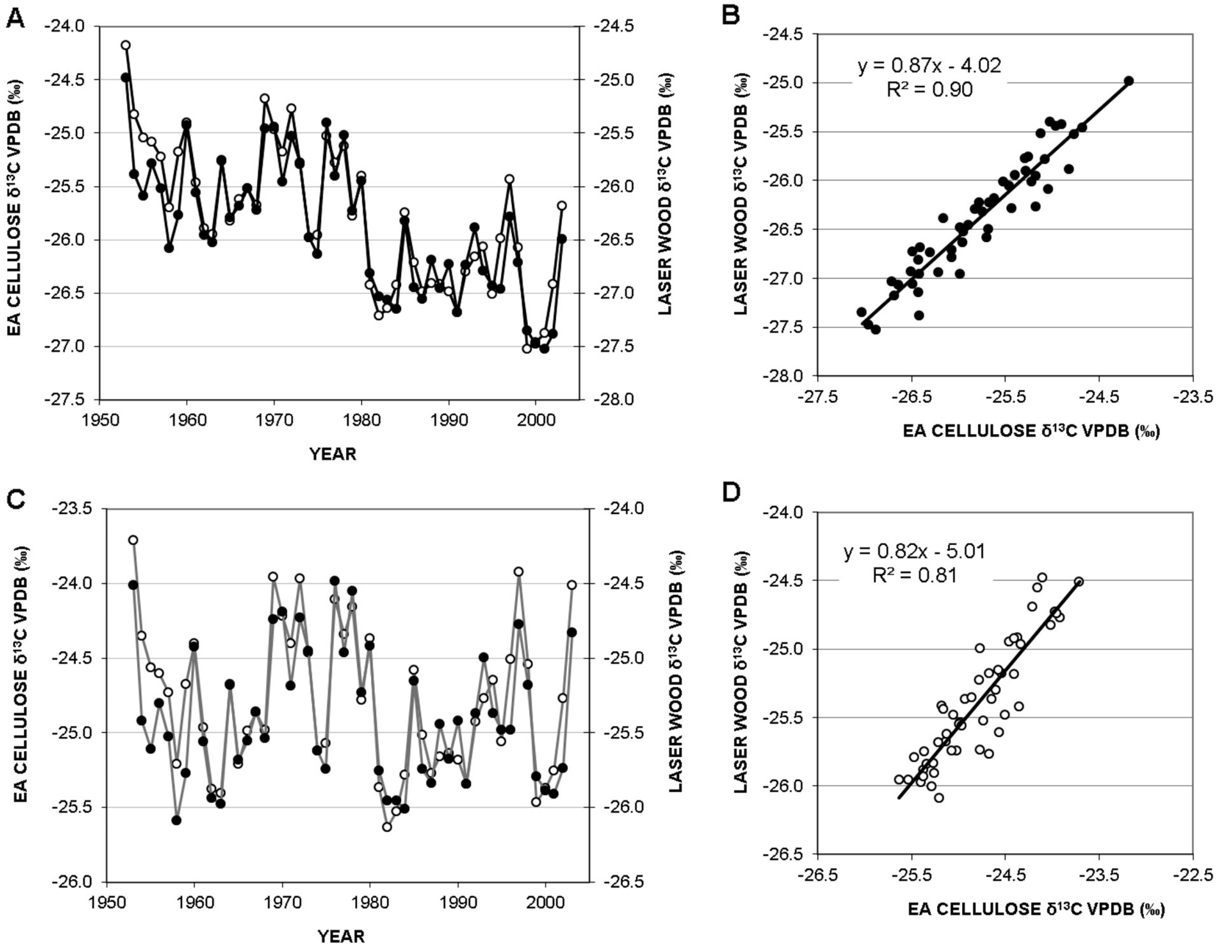

Fig. 2. Comparison between the stable carbon isotope series developed from four northern Swedish *Pinus sylvestris* L. trees analysed using laser ablation and conventional "on-line" methods. A: Comparison of raw laser (filled circles) and conventional data sampled using an elemental analyser (EA) (open circles). B: Bi-plot of raw laser and conventionally-sampled data. C & D: As for A and B but for atmospheric $\delta^{13}C$ (Suess) corrected data (after McCarroll and Loader, 2004).

extraction and mass spectrometry) demonstrate a very high degree of common signal and an expressed population signal (EPS) of 0.95 (0.90) (Wigley et al., 1984) for raw (atmospheric corrected) $\delta^{13}C$ data (McCarroll and Loader, 2004). Similar results are obtained from the resin-extracted wholewood analysed using the laser (0.89 (0.76)). The two series are offset isotopically because of the difference in chemical composition of wholewood and cellulose in Scots pine. The average correlation between the individual series measured on cellulose using the elemental analyser is 0.82 (0.69) and on resin extracted wood sampled annually over 100 μm is 0.66 (0.44) for the raw (atmospheric corrected) $\delta^{13}C$ data. The correlation between the two mean series is 0.95 (0.90). Variability in the laser-ablation data is slightly less than that of the cellulose data, which probably reflects the effect of lignin in the laser analyses, which may temporally "smooth" the environmental signal as it is produced within the tree-ring over a longer period than the cellulose which if formed during a shorter time period. The weaker inter-annual correlation in the laser data *versus* the conventional measurements very likely reflects the failure of the more finely-resolved and somewhat less constrained and more variable sampling strategy (a 400 μm traverse) to capture the mean signal of the entire tree-ring in the same way as the removal and analysis of cellulose from the entire tree-ring.

4. Conclusion and future scope

This paper describes and demonstrates the performance of a laser ablation system for stable carbon isotope analysis in tree-ring research. The system offers potential for developing long tree-ring isotope series, particularly from trees with very narrow rings, as well as the more common application of the method to produce high-resolution intra-annual profiles. The automated cryogenic trap in our modified Schulze-type system widens capability for small volume sampling and also for pooling material from multiple laths. However, factors such as cost of the equipment and practical limitations mean that this technology is unlikely to replace established approaches for measuring (Carbon, Hydrogen, Oxygen) isotopes in tree-rings, at least in the near future.

Apart from resin extraction (for conifers), a standard process in most dendro-laboratories, no special preparation is required for laser sampling other than the preparation of a flat clean surface. The system described runs routinely with a 50 mm diameter sample chamber. The future application of this approach to core samples is therefore constrained by ability to sub-divide core samples and sample chamber dimensions. Recent development of a 200 × 200 mm "large-format" sample cell offers exciting potential to work with larger samples to develop wood (rather than cellulose) isotope chronologies more rapidly, or to pre-screen individual trees prior to their selection for further isotopic analyses or cellulose extraction. Even with such a "large-format" configuration, the sample chamber is currently not able to accommodate large wooden artefacts (*e.g.* violins). To analyse such objects a small sub-sample of wood would first need to be removed for laser-ablation which may not be desirable in many cases. An additional consideration when sampling is that although essentially non-destructive, UV laser ablation still leaves a small "scar" which could affect the aesthetic and/or value of the artefact.

Annually-resolved laser sampling results compare very favourably with conventional manually-sampled methods and demonstrate potential of this approach for pre-screening cores to establish representativeness prior to selection for cellulose extraction or intra-annual analyses.

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