Biogeosciences Discuss., 12, C4704–C4712, 2015
www.biogeosciences-discuss.net/12/C4704/2015/

Biogeosciences Discussions
Open Access

Interactive comment on "Technical Note: An improved guideline for rapid and precise sample preparation of tree-ring stable isotope analysis" *by* K. Schollaen et al.

Anonymous Referee #1

Received and published: 26 August 2015

General remarks

This paper describes further modification of recently developed method for tree-ring cellulose extraction (Li et al. 2011, Kagawa et al. 2015), where cellulose is extracted from tree-ring spline prior to tree-ring separation (cross-section method). Three new major technical advances/findings are reported in this paper:

1) Potential application of UV-laser microdissection microscope to tree-ring cellulose spline. 2) Semi-automated chemical extraction applied to the cross-section method. 3) They evaluated the effects of contaminants (pencil marks, chalk and corn starch) on the oxygen and carbon isotope values for wood samples.

Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

This paper confirms previously reported findings, such as 1) Cellulose spline from various tree species can be extracted without losing its tree-ring structure, (2) stable carbon isotope ratios, and (3) chemical purity of cellulose prepared from teak corresponds to that of the "classical method" (however, such checks were not performed for other nine species).

I found this work still in its preliminary stage and a little more experimental effort would make a better publication, especially if authors checked the validity of the method with other nine species. Another concern is, they tried the method only on one species (teak) and on one element (carbon). For example, checking oxygen isotopes of teak and chemical purity of cellulose for other nine tree species with FTIR does not take too much time (should take about 2 weeks). Previous studies have already confirmed that both oxygen and carbon isotopes, and chemical purity match between the classic and cross-section method for several tree species. The word "guideline" is defined as "a principle put forward to set standards". To be a "guideline", I think at least they should confirm that the method works universally, in terms of chemical purity and stable carbon and oxygen isotope ratios, by testing more than several species.

One of the most original aspects of this study is the (potential) application of UV-laser to tree-ring cellulose splines. UV-laser has a great potential because it may make tree-ring separation process automatic in future. Thanks to recent breakthrough in cellulose extraction, this process became much more efficient and instead, tree-ring separation, weighing and packing have become the major time limiting process now. UV-laser has a potential of automating this bottleneck process, however, the paper does not report about the application of UV-laser to cellulose spline. Contrary to what readers would expect from the title, authors do not state clearly how "improved" their sample preparation method is, compared to previously published cross-section method (Li et al. 2011, Kagawa et al. 2015). Especially, it is not clear how much advances authors have made in terms of how "rapid" (how much improvement does semi-automated extraction make?) and how "precise" (analytical resolution for manual separation is about

BGD
12, C4704–C4712, 2015

0.2mm, what about UV-laser?). Considering the fact that the journal “Biogeosciences” has higher impact factor than “Chemical Geology”, where the two preceding papers (Li et al 2011 and Kagawa et al. 2015) appear, authors should present significant advances from preceding works to warrant publication of their work in this journal. I found this paper focuseing too much on what has already been checked in preceding works and too little on their original advances, such as application of UV-laser microscope on cellulose spline and automation of cellulose extraction. I therefore find this manuscript acceptable with major revision, provided authors can present such significant technical advances from previously published cross-section method and provide additional experimental data to prove that their method is universally applicable to major tree species used in dendrochronology. Otherwise, I think the manuscript should be submitted to other journal, such as “Rapid communications in mass spectrometry” or “Dendrochronologia”, where previous works on cellulose extraction method appear.

Specific comments - scientific:

As I understand, previously reported methods have made following advances: Li et al. (2011) have made (1) direct extraction of cellulose from tree-ring spline possible and (2) confirmed that stable isotope ratios and purity of the cellulose agree well with the “classical” method. This breakthrough has made cellulose extraction process faster by one to two orders of magnitude. Japanese group has further optimized this method, by coming up with methods to prevent disintegration of friable cellulose spline (Teflon case and fixation sheets), (2) confirmed that the method works with five different tree species, and (3) improved analytical accuracy/increased the number of tree-ring cellulose samples processed for oxygen isotope analysis.

Title: Unless authors can specify improvement in rapidity and precision of their method, I think current title is too broad and should be more specific to better reflect the contents of this paper. The title of this paper says “an improved guideline”, however, I think trying the method only on one species and one element (carbon) does not give enough supporting data and is still not universal enough to call it a “guideline”. Authors

BGD
12, C4704–C4712, 2015

should prove that the method is equally applicable to other major tree species used in dendrochronology by providing more experimental data. Authors did confirm cellulose spline can be extracted from nine tree species (Pine, Larch Spruce, Juniper, Fir, Oak, Cedar, Baobab and Beech) with well-preserved tree-ring structure. However, as for chemical purity and carbon isotope ratio of the cellulose prepared, authors checked only with one species (teak). Checking oxygen isotopes of teak tree rings, for example, could have been easily done without taking too much time, and running chemical purity test (FTIR) for the nine tree species can be done within one or two days. Due to the lack of supporting data, I think this work is still in its preliminary stage and it would make a better publication if authors checked the validity of the method with other nine species. Why you did not do these experiments?

Materials and method: P.11593, L.6-7 Here, authors use 10 different tree species for cellulose extraction, but they only measure carbon isotopes of teak. Why you did not measure oxygen isotopes of teak and chemical purity of cellulose from other nine species? It does not take much time to do this experiment.

P11596L3 “3.5 classical cellulose extraction. . .” Did authors compare weight recovery of cellulose (cellulose weight / original wood weight) between classical and cross-section method? Were they similar?

Results P11602L12 “5.2 Classical vs cross-section cellulose extraction method” Add data for oxygen isotopes of teak tree rings. I think it will be d18O analysis of about 200 samples for both classical and cross-section methods and should take too long to do this. L 5.3 “Purity of cellulose cross-sections”: Add FTIR data in this section for other nine species to prove that the cross-section method can universally produce cellulose from sufficient chemical purity.

In conclusion, you use too much space for writing the findings that were already reported in previous studies, or otherwise self-evident. Please delete such description, i.e. cellulose extraction not being time-limiting, or pooling not necessarily required etc.

BGD
12, C4704–C4712, 2015

And use more space for writing your original findings, i.e. what this study clarified for the first time. For example, you can compare your semi-automated extraction method with previous ones (Li et al . 2011, Kagawa et al. 2015) and point out how much improvement you have achieved in terms of time (?? times as many samples processed per man-hour compared to the Teflon-container method?), cost (how much the whole system costs), and user-friendliness (less exposure to toxic gas?, perhaps your method is more successful on thinner cross-sections and fragile species/samples?). I think such information will better meet the readers' interests.

Figure 5. What does the peak around 1630 cm-1 in holocellulose represent? The peak is absent not only in alpha cellulose standards, but also in untreated and resin extracted wood. Is it contamination of chemicals used, such as sodium chlorite, acetic acid or sodium hydroxide?

Specific comments – minor:

Abstract: P11588,L.14-15 "This guideline reduces time and maximizes the tree-ring stable isotope data throughput significantly" This sentence is not clear as to what method it is comparing to. Change the sentence either "This guideline reduces time and maximizes the tree-ring stable isotope data throughput significantly compared to classical method" or "Semi-automation reduces time and maximizes the tree-ring stable isotope data throughput compared to previously reported cross-section method."

The following two parts sound as if chemical purity and both carbon and oxygen isotope values were checked for all ten species. State this clearly at the beginning so that readers will not feel disappointed. L10 Change "The method was applied to ten different tree species . . ." to "Cellulose was extracted from ten different tree species. . ." L13-14 Change the sentence as follows: "FTIR (Fourier transform infrared) spectrometry and the comparison of carbon isotope values with classical method confirm chemical purity of the resultant cellulose prepared from teak." L.14-15: "Sample homogenization is no longer necessary". Later authors state necessity of homogenization for tree rings

BGD
12, C4704–C4712, 2015

wider than 1cm. Change this part to either “no longer necessary for narrow tree rings” or “no longer necessary in most cases”. L16-17 Specify what you are comparing to: “Compared to classical method, cellulose extraction is now faster, cheaper and more user friendly. . .” L18-20 Is it possible to mention the highest achievable resolution with UV-laser microdissection microscopes? (ii) precise tree-ring separation at annual to high-resolution scale utilizing manual devices or UV-laser microdissection microscopes (up to ??? mm).

Introduction: P.11589 L.21 Change “are still required” to “were still required” P.11589, L.28-29 “A breakthrough in terms of high sample throughput was achieved by Nakatsuka et al. (2011), with methodological improvements by Kagawa et al. (2015).” I think the most important breakthrough in terms of sample throughput comes from successful extraction of cellulose spline by Li et al. (2011), rather than from Kagawa et al. (2015). Li et al. (2011) also used perforated U-channel PTFE casing to prevent cellulose spline from breaking apart and this should be mentioned in introduction. P.11589, L.24-29 Change “More, recently, Li et al. (2011). . .cross-sections” to “A breakthrough in terms of high sample throughput was achieved by Li et al. (2011), who came up with a technique to extract cellulose directly from wood cross-sections using perforated U-channel PTFE casing.” P.11589, L.26-L.02 “Here, a container made of teflon (PTFE, polytetrafluoroethylene) punching sheet was designed to prevent disintegration of cellulose laths (see also Xu et al., 2011).” Please change this part as follows. Although not stated in Kagawa et al. (2015), the fixation sheet were designed by Nakatsuka’s group and sealed Teflon case by Kagawa’s group (first presented in Kagawa et al. 2011). “The method was further refined by preparing freeze-dried cellulose lath in a sealed Teflon case (Kagawa et al. 2011) then pasting it on fixation sheet for tree-ring separation (Nakatsuka et al., 2011, Xu et al., 2011, Kagawa et al., 2015). P.11590 L.14-16 Change “Furthermore, we compared FTIR. . ..stable isotope ratios..” to “Using teak cellulose, we compared FTIR...carbon isotope ratios. . .” L.15-16 Change ”as for example lignin or fatty acids” to “such as lignin or fatty acids”. L.P11592L2 Change “may increase up to” to “can increase up to”

BGD
12, C4704–C4712, 2015

Material and methods: P.11593, L18 I could not find explanation for “dnEsample” used in the equation. Maybe it should be changed to “dEsample”? P11595L3 Change “3.3 Stable isotope analysis” to “3.3 Stable carbon isotope analysis” P11597L13-14 “6 single punching sheet holders (Fig.2a)” Add the producing company, parts no., thickness, hole size and pitch of the punching sheet so that readers can easily repeat the same experiment. Alternatively, the information can be inserted after: P11599L9 ”of teflon punching sheets”. P11598L17 ”Very soft woods. . . with high water content should be frozen with liquid nitrogen. . .” This is a very good original idea. Please add more original findings like this, if possible. P11599L1-2 “between well labeled microscopic slides” what do you mean by “well labeled”? Do you mean “indelible” or “permanent” label? What about “between labeled microscopic sliders”? P11601L4 replace “achieved” with “obtained” L7-8 Change “homogenous representatives of the individual tree rings” to “isotope data represents whole tree ring” L13 “carbon or oxygen isotope analysis” L25 “UV-light can facilitate the visualization of tree-ring structures” Did UV-light enhance visibility of tree-ring structures compared to stereomicroscope with transmitted light? If so, please add “compared to stereomicroscope with transmitted light” after this part.

Results P11601L21-22 Perhaps the following part should be deleted because of redundancy: “by manual dissection with a scalpel under a binocular microscope or by UV-laser microscopic dissection (Fig.3)” Same descriptions appear in P11597L21 and Fig.3 caption. P11602L13 Please state clearly that you are referring to teak, not all 10 species studied: “For teak, we found highly significant correlations. . .”

Discussion P11605L2 “. . .by nature and oven-dry cellulose absorbs water very fast” P11605L18-20 Can the glass container of your extraction device (Fig.2C) be completely sealed airtight (or with small ventilation hole)? If so, it would be very nice because the operator can avoid inhaling toxic chloric gas and minimize water evaporation loss. P11606L4-5 “the cross-section cellulose extraction system described facilitates the horizontal position of cross sections. . .” I think this part should be deleted, because Li et al. (2011) have also positions tree-ring cross-section in horizontal way and some

BGD
12, C4704–C4712, 2015

researchers also use larger container and put Teflon cases in horizontal direction. Anyways, does horizontal positioning make a big difference? If so, state the benefit. L7-10 “No time consuming sewing of punching sheets with the wood samples with potentially contaminating cotton threads”: As written in the paper, carbon contamination can be prevented by using Teflon threads. Sewing has an advantage over Teflon screws because sewing prevents the cross-section moving sideways (left or right in Fig. 2A in your paper, cf. Fig. 1b in Kagawa et al. 2015) and prevent broken cellulose pieces from falling apart after chemical treatment and freeze-drying. After testing both Teflon screws and sewing, I preferred sewing. Please rewrite this part, weighing pros and cons of screwing.

P11607L19- “However, oxygen isotopic exchange between sample and water. . .is a known but frequently underestimated risk”. Do you mean exchange of oxygen between cellulose of C-O bonds in six-membered ring? Then I would say it is highly speculative and I suggest either you add reference that proves occurrence of oxygen exchange in C-O bonds with water as a “known but frequently underestimated risk”. Otherwise, please remove L19-27. I think you can check this easily by applying ultrasonic sound to cellulose in heavy water (H2-18O). If you mean oxygen in crystalline water adsorbed between cellulose molecular chains, please mention so.

Conclusions Is the whole system (customized Teflon case and peristatic pump) available from GFZ or other company? If so, how much does it cost? Please write such information, if available. I think the semi-automated method can be useful for some laboratories in the world that analyze tree-ring isotopes extensively. Otherwise I am afraid this automated method does not appeal to wider audience who are involved in tree-ring isotope analysis. P11609L4-6 Add resolution here as in“for highly resolved and very precise intra-annual tree-ring isotope analysis (Shollaen et al. 2014a) at ??? mm”. “Highly resolved” and “very precise” sound redundant. L14-15 Please specify what you are comparing to: “faster, cheaper and user friendly cellulose extraction compared to classical method”

BGD
12, C4704–C4712, 2015

References: Insert following references: P. 11612, L.31-33 “Kagawa, A., Preliminary results of isotope dendrochronology of teak from Southeast Asia by Kagawa et al., 2nd International Asian Dendrochronological Conference, Xian, China, 2011”

Table and Figure captions: Table 1: “(gray shaded boxes)” I cannot see gray shaded boxes in the PDF files. Please check the original uploaded PDF file.

Figure 1C Change “diamant saw” to “diamond saw” Figure 2A and relevant part of the caption. Change “Enclosing of cross-sections in. . .” to “Enclosing cross-sections in. . .” Figure 2(c) caption: “. . .using two silicon tubes connected to a peristaltic pump for chemical treatment” Figure3 Overrepresentation of earlywood or latewood in a tree-ring dissection is caused three dimensionally. In other words, if scalpel incision is not parallel to longitudinal fiber direction at the tree-ring boundary, underrepresentation of earlywood / mixing with the neighboring tree ring can occur. Figure 4. Add data for oxygen isotopes of the same tree rings. Figure 6. Mention whether the cellulose cross-sections presented are alpha cellulose or holocellulose.

Interactive comment on Biogeosciences Discuss., 12, 11587, 2015.