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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.

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General response:

Dear Referees, dear Editors,

We appreciate very much the opportunity to respond to the concerns and thoughtful comments raised by the referees of our manuscript. We gratefully acknowledge that they indicate our paper may be published after minor to major revisions. In this response – prior to the editor's decision - we provide additional input and perspective

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that make some of the referees' concerns less pressing and may dispel others altogether. In the revised MS minor comments will be considered, grammar and spelling mistakes corrected. Adequate statements of the articles put forward by the reviewers will be regarded.

Here, we detail key changes and additional data we intend to incorporate into the final manuscript to consider the major points raised by the referees. The most critical comments of the referees can be summarized in two points: 1) A certain lack of novelty is brought forward. 2) Our methods performance should have been tested on more than one species.

General response to the first issue:

Referee #1 criticized that our MS describes just "...a further modification of recently developed method...", #2 comments that "...the manuscript is somewhat limited with respect to the contribution of new ideas, data, or methods..." and #3 points out that "...this method may be overselling a bit...". However and in fact, despite these apparently pressing statements, all three referees have acknowledged that we are introducing "...three new major technical advances/findings" (#1), "a newly designed apparatus" (#2) and "...two novel additions..." (#3). In light of these somewhat contradictory statements of the referees, we see a major weakness in the presentation of our improved guideline. Independent from the different numbers of innovations identified by the referees they, unfortunately, have not recognized our MS as a "guideline". Hence, we will improve and rewrite the MS to be hopefully accepted by BG as "an improved guideline for rapid and precise sample preparation of tree ring stable isotope analysis".

Why is this a guideline? Our MS fully describes, for the first time, how tree rings shall be prepared for stable isotope analysis. The guideline starts with guidance to testing and calculating for the potential impact of contaminants like chalk and pencil marks and it ends with a description on how tree-ring cellulose samples can be weighed and packed into tin or silver cups avoiding the laborious step of sample homogenization.

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For the intermediate step of tree-ring cellulose extraction we introduce a new device that improves the extraction process while allowing well established chemical protocols and published procedures to be utilized. In order to test for the completeness of the cellulose extraction process we suggest utilizing FTIR analyses proving the purity of the cellulose extracted from wood. Altogether, to our knowledge, such overall procedure, or guideline, has not been published before in entirety. If someone follows this guideline (or approach or procedure), we claim that, the isotope ratios measured on tree-ring cellulose samples processed and controlled (FTIR) this way are reliable and representative, i.e. homogenous, for any investigated tree ring. We do hope the editor will give us the chance to modify our MS accordingly. We would like to stick to the term "An improved guideline", however, we do not insist and may change the title of the MS to "An improved approach to. . ." or "An improved procedure to. . ."

All Referees pointed out that one of the most original advances is the application of a UV-laser microscope on cellulose spline and criticized that the use of the UV laser is not discussed in further detail. Indeed, UV-laser microdissection of tree-rings or parts thereof is predestined for use within our guideline. However, details of this particular application have been published earlier (Schollaen et al. 2014) and we want to stress that our guideline can be combined with traditional methods and one does not require such expensive equipment for preparing tree-ring cellulose samples for IRMS analyses. Nonetheless, we happily for the referees' advice. We will add a paragraph illustrating and explaining the application of the tree-ring dissection technique utilizing a UV-laser microscope for obtaining stable isotope ratios from tree rings of the African baobab (Adansonia digitata). In doing so, we will be also addressing the 2nd major issue raised by the referees.

General response to the second issue:

In particular referee #1 claims our methods performance should have been tested on more than one species and the other reviews support this criticism of ref. #1. As outlined below in our detailed response we are willing to provide additional data on

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oxygen isotopes on teak, as well as further FTIR analyses proving the purity of other tree species than teak. However, besides introducing a new device for particularly careful cellulose extraction on potentially very thin wood cross sections we do not recommend any changes to the "classical" procedure of chemical cellulose extraction. As a matter of fact, different chemical prescriptions of cellulose extraction from wood exist (e.g. Loader et al. 1997, Rinne et al. 2005, Brendel et al. 2000). The vast majority of extraction methods uses NaOH and NaClO2 as reagents and only Brendel et al. 2000 suggested a hydrolysis procedure with acetic acid. In the guideline presented in our MS we do not propose any new chemical treatment, i.e. we have used well tested option the "classical" NaOH and NaClO2 treatment. Its validity has been proven in international inter-laboratory comparisons (e.g. Boettger et al. 2007). We claim that there is negligible chance of failure applying the well tested and established chemical procedures with a newly designed Teflon device that allows more convenient, accurately and, particularly, more gentle sample handling than similar extraction approaches published earlier (e.g. Kagawa et al. 2015). The device introduced by us is versatile. On the one hand, it allows extraction of up to 150 cm of wood increment equaling 1500 tree rings of 1mm width in average. On the other hand, it can be adapted down to 1/6 in size to minimize the use of chemicals if number of samples is low. Furthermore, the Teflon device can even be used with the chemical protocol proposed by Brendel et al. 2000 or potentially any other future chemical procedure. Note, independent from the chemical protocol used, our guideline suggests testing the purity of tree-ring extracted cellulose by FTIR analysis. Nonetheless, we do accept and will address the referees' complaints. We are happily willing to provide additional FTIR spectra on the various tree species we have investigated in this study. We will be able to show that there is no significant difference between the different devices because the protocol of chemical treatment is the same. We suggest to display these FTIR spectra in the supplementary material section. Furthermore, we are willing to provide oxygen isotope data for teak trees derived from extraction with the classical devices (Wieloch et al. 2009) as well as with the new device. With our "guideline" study we do not intend to prove that

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testing the purity of cellulose is obsolete for any future study. On the contrary, we do suggest testing the purity of extracted cellulose by FTIR analysis whenever an operator suspects incomplete removal of resins or lignins. Some of the tree species studied here have a wide geographical and altitudinal distribution and relatively broad genetic variability. Hence, they may reflect a broad variety of chemical wood components that cannot be captured in a single study like ours. We propose that the purity of cellulose extraction from new sites/regions or new species may be tested, at least occasionally, prior to mass spectrometric analysis of stable isotopes.

Comments to Referee #3:

Comment 1: This paper aims to describe a new method for processing tree ring samples to cellulose for isotopic analysis. The paper is fairly well written and the method is interesting. I think that selling this method as novel may be overselling a bit because what is described here is essentially a description of further refinement of the lath processing techniques described by Li et al. (2011), Weiloch et al. (2011) and Kagawa et al. (2015). The two novel additions by this described technique are the use of a peristaltic pump during the processing and the use of a UV-laser disection microscope (although I am not sure this last is novel given that Schollaen et al. (2014) described the use of a UV-laser microdisection system to sample tree rings. I think a little more effort needs to be taken to detail how this new method improves on earlier versions of the lath technique. Overall I think this is an interesting paper and presents a method that if I had the funds to procure the speciallized equipment I might consider adopting. My two bigest issues with this paper are 1) That I don't feel the authors did an adequate job of representing how their method is different or better than the three other methods describing the lath technique and 2) That they only used a single species for all of their tests of their methods performance.

Response: We thank the reviewer for the general remarks on our manuscript and his

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critical comments. In the revised manuscript, we will clarify the original advances of our guideline. For further explanations we would like to refer to our response given above.

Comment 2: I am interested in whether the authors thought to consider comparing resinous vs nonresinous samples? Looking at the list of species Pinus sylvestris and Psudotsuga menziesii are the only two resinous speciments presented. In other methods studies comparing cellulose isolation techniques it was noted that often the biggest differences were either between heartwood and sapwood and between resinous and non-resinous species. From the looks of the FTIR comparision the authors only used Tectona grandis. Why are the FTIR spectra from all ten species not shown ?

Response: Previous studies have already confirmed that the chemical purity (through FTIR comparison) match between the classical and cross-section method for several different tree species (Kagawa et al. 2015). In particular, Rinne et al. 2005 have dealt with resinous vs non-resinous samples in an excellent study. Our chemical extraction protocol does not differ from that study. The reviewer is maybe forcing the issue a little bit too much. We do not think that repeating older studies using the same chemicals for cellulose extraction takes us a step further. This would be redundant to what was already checked by Kagawa et al (2015). Rather, we picked up the outcome of the previous publications and focused on our original advances, such as an improved semi-automatic cellulose extraction system and the application of the UV-laser microscope. Note, that our guideline does not require the use of a UV-laser microscope.

Comment 3: Why is Tectona grandis the only species used for their comparison of markings on the wood. Really what they shoud say is that there is no evidence for an effect from contamination in teak not all species.

Response: We evaluated the effects of contaminants (pencil marks, chalk and corn starch) on the oxygen and carbon isotope values of wood samples and focused our study on the species Tectona grandis. Furthermore, our guideline provides the necessary equations allowing anybody to calculate potential impacts of contaminants. In the

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revised MS we rephrase the discussion part of the potential implications of contaminating substances and think that this section will then be clearer.

Comment 4: Although the authors to mention the sapwood-heartwood difference it would appear they only looked at Tectona grandis to check this. I would like to see this test done in a more resinous species, for example, Pinus ponderosa, or some other conifer to see if there were any significant differences. They have the samples so I am confused again as to why this test was only done on one of their ten species.

Response: Please refer to comments above.

Comment 5: On page 11601, I am puzzled as to how the authors assess the relative proportion of earlywood to latewood ? I also imagine this is something that would vary not only based on species but based on year given that there can be large interannual variabililty in latewood density and amount. Also how much variability in earlywood-to-latewood proportions is significant ?

Response: In the revised MS we rephrase the sentence for clarification and add references.

Comment 6: In the last sentence on page 11601 the authors state that the calculated the potential effects of contaminants. This I would assume is based on their analysis of teak. I am concerned that this effect may not be the same for other species. Also I am confused as to why they needed to check the effect of contaminants again when apparantly they already did so in a previous experiment (Page 11604 first line) ?

Response: The previous experiment cited was a preliminary study. As outlined above we intend to provide a guideline. This includes literature review as well as new experimental results, e.g. on how a potential contaminants may impact the original isotope ratio of a substance. The equation given can be applied to any sample and may be applied to conifers (estimated average δ13C = -23per mil) as well as to C4 plants (estimated average δ13C = -12per mil). The equation is quite simple and we believe that

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it is not necessary to give any further examples.

Comment 7: Page 11605 paragraph 15. I am unclear what they mean by the sentence starting Â′nA disadvantage, like an evaporation of water resulting in enrichment: : : ÂËŹz Is this an effect that has been noted by other researchers ? If so could this not be a bigger problem for cellulose extraction techniques than is suggested. For example what if the isotope value of the deionized water used in the lab varies from day to day or season to season. This would be a huge problem for cellulose extraction if so. Or do they just mean higher concentrations of chemicals from evaporation. Please clarify.

Response: This sentence refers to increasing concentrations of chemicals from evaporation. Sentence will be rephrased in revised MS.

Comment 8: Page 11606 paragraph 15. Please further clarify the problem of shrinkage. I would think that shrinkage would occur no matter what size laths are used so please clarify what the issue is with thicker cross sections ? Also I would be curious if thicker cross sections increases the extraction times or leads to a gradient in purity of cellulose from the outside towards the center of the lath ?

Response: Reviewer is right, shrinkage can occur on wood laths of any thickness. Sentences will be rephrased. Kagawa et al. 2015 have proven the applicability of wood laths of at least up to 2mm to this cellulose extraction method. They do not report any gradient in purity, and, their FTIR analyses proved them right. Our device can handle much thinner wood cross sections without breaking them during the extraction and drying process.

Comment 9: On page 11608 you mention that you may not need to weigh samples. I am curious as to how small a ring you can sample ? I have worked on samples where some rings consited of one or two rows of trachieds only. Would this provide sufficent sample ?

Response: This would provide sufficient sample for modern IRMS. However, such tiny

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tree rings may only be dissected from cellulose cross section with the aid of a UV-Laser microdissection microscope.

Comment 10: I do find this to be an interesting technique, however I am curious about the cost to set up all the specialized equipment that this method would require ? In the conclusions you mention that this is a faster, cheaper method. I would imagine this method is only cheaper if your lab already has all of the eqipment. Not all of us have the resources to spend C0,000 to purchase the speciallized equipment necessary to adopt this new technique even if we wanted to. I've done some asking around and the UV-laser microdissection microscope the authors are fond of mentioning costs _ C0,000. I know it is not standard practice but perhaps some informaiton on the cost of equipment could be put in the supplementary material ?

Response: As mentioned above our guideline does not require the use of a UV-laser microscope per se (Zeiss Microbeam or Leica LMD7000). The new Teflon device presented here can be produced in almost any fine mechanics workshop. The design plans are provided in the supplement. So major costs will comprise purchase of the Teflon materials and workshop. The rest is standard laboratory equipment (balances, beakers etc.) and, last but not least, an IRMS or CRDS system.

Further minor comments, in which we generally agree, will be taken into account when redrafting the revised version of the manuscript.

On behalf of the authors; Yours sincerely

Karina Schollaen

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