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Biogeosciences Discussions

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

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.

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

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

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 #2:

Comment 1: This manuscript summarizes cellulose extraction procedures for stable isotope analyses and provides a proposed guideline for "modern tree-ring isotope research." The authors present a semi-automated extraction system for batch processing cellulose and new data to test the assumptions of these recent studies and examine the effects of different methods and potential contaminants (e.g. pencil marks, chalk, and corn starch) on the _13C values of the extracted cellulose. A number of recent studies (e.g. Li et al., 2001; Kagawa et al., 2015) have focused on cellulose extraction from wood slats, with the emphasis on standardizing the chemical procedures and increase sample throughput. Although the manuscript is thorough and well written, the manuscript is somewhat limited with respect to the contribution of new ideas, data, or methods. Details of potential improvements of to the manuscript are discussed below. Minor to major revisions are recommended prior to publication. The new extraction system and procedure described by the authors appears to streamline the cellulose extraction process and increase throughput; however, part of the authors' stated goal is to assess the chemical purity and reproducibility of batch cellulose extraction across a broad range of sample types. The authors discuss 10 different tree species and the application of the new method to these different tree types; however, only the teak data are presented here. This seems like a glaring omission. Either the other 9 species

should be left out of the discussion entirely, or isotope data should be reported for all of them. The manuscript would be improved by the addition of _18O for the teak as well. Only _13C values are reported here. Additionally, it is not clear why the authors only report purity (FTIR) results for the teak sample (Pg 11602; Section 5.3). The application of the cellulose extraction system to other tree species seems like a central component of the study, but the section (Pg 11606; Section 6.3 and again Pg 11608; Line 28 on) is vague and needs to be supported by data. Other than designing a new apparatus, the procedure outline in this manuscript does not represent a significant improvement or development from the other cited procedures (e.g. Li et al., 2001; Kagawa et al., 2015). I recommend minor – major revisions that include an explicit discussion of how the cellulose extraction procedure presented here performs on the 9 samples included in the discussion. The authors imply that their method is better for both _18O and _13C, yet no _18O values are discussed. Additionally, no purity data are included for samples other than the teak sample. As written, the manuscript does not appear complete. The inclusion of the additional data and a comparison of the isotope data between different the species would dramatically improve the manuscript. The discussion of the UV laser is only in passing. The manuscript could be greatly improved if the use of the UV laser is discussed in further detail. Perhaps some of these data are discussed in Schollaen et al., (2014), but since this method is discussed throughout the manuscript the authors should explicitly discuss the UV laser sampling method.

Response: We thank the reviewer for the general remarks and critical comments regarding our manuscript, which are very similar to the recommendations of Referee # 1. Please refer to our responses to comments 1 and 2 of Referee #1 as well as our general response given in the beginning.

Comment 2: Pg 11590, Line 6: Much emphasis has been placed on batch processing and "providing the same chemical conditions for all samples." Standardizing chemical processing and insuring reproducibility is critical; however, batch processing does not necessarily improve the reproducibility of chemical processing between batches. The

authors imply that their method is better than other extraction procedures because the samples are processed in larger batches. The data presented do not support this. The batch processing may be more efficient and therefore require less time, but that is different than saying that batch processing is superior. It seems like batch processing has the potential to produce large datasets of bad data if wood samples are not properly extracted. One way conventional isotope data is assessed is to look for outliers within a time series that could represent a mistake during processing (i.e. incomplete extraction). The authors should discuss a practical assessment of purity. Does every sample need to be examined via FTIR? Reproducibility between batches?

Response: We are using our new extraction device in combination with a reaction vessel that contains approx. 1.5 l of NaOH or NaClO2 solution. Our new device can hold up to 15 g of wood laths, similar to the chemicals/wood ratio of the “classical” devices used.

Comment 3: Pg 11592; Line 3 – 9: There are a lot of assumptions in this statement that need to be cited or quantified.

Response: The sentence has been rephrased. Relevant citations will be added.

Comment 4: Pg. 11592, Line 9: What is a “herbivore attack”?

Response: We do mean any damage or decay of wood brought about by bacteria, fungi or larvae (of wood worms).

Comment 5: Pg 11591, Section 3.3: Only carbon isotope data/methods are presented yet oxygen isotopes are discussed throughout the manuscript. The manuscript would be improved by including _18O values.

Response: We will add oxygen isotope data. C.f. comments above.

Comment 6: Pg 11600; Line 15 – 19. Cite Brookeman and Whittaker, 2012 – Their data seem to contradict some of these statements. Why mention the extra alpha-cellulose step if is not necessary? Either is should be done or it shouldn’t. It seems

like the relationship between holocellulose, alpha-cellulose, and _13C/_18O values would need to be verified for every tree species; therefore, omitting alpha-cellulose step doesn’t save time and it reduces precision. No data are presented in this manuscript showing how the new method that uses holocellulose applies to classic methods that almost always utilize alpha-cellulose.

Response: We will cite Brookeman and Whittaker 2012 in the revised MS. However, we do not want to prescribe whether or not alpha cellulose extraction is required for stable isotope analysis of tree-rings. Our paper does not intend to provide an new protocol to cellulose extraction. Moreover, our guideline includes relevant steps of sample preparation and tests that need to be applied before and after chemical extraction of cellulose. However, the device proposed in our MS is suitable for performing the extra step of applying 17% NaOH at room temperature for obtaining alpha-cellulose from holocellulose. From our point of view, the attractiveness of our guideline or approach is that it is applicable to cellulose obtained from a variety of existing chemical protocols.

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.