

***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 #2

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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 $\delta^{13}\text{C}$ 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.

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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 $\delta^{18}\text{O}$ for the teak as well. Only $\delta^{13}\text{C}$ 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 $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, yet no $\delta^{18}\text{O}$ 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.

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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.

Specific comments:

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?

How were sub-samples selected for FTIR analyses? Was there any variation between the outer edges and internal portion of the wood sample? It seems like the batch processing approach may not extract cellulose uniformly

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

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

Pg 11591, Section 3.3: Only carbon isotope data/methods are presented yet oxygen

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isotopes are discussed throughout the manuscript. The manuscript would be improved by including $\delta^{18}\text{O}$ values.

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 $\delta^{13}\text{C}/\delta^{18}\text{O}$ 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.

Minor and Grammatical comments:

11593, Eq 1: What is n in the equation 1? It doesn't seem like it is a needed variable.

11591, Line 2: “-ecological” delete –

Pg 11591, Line 6: This sentence should be rewritten. “hence” and “well-elaborated” are not necessary.

Pg 11592, Line 8: apostrophe rather than single quote in “tree's”

* Typos – there are a number of typographical and other errors throughout the manuscript (e.g. Fig 1C “diamond” rather than “diamant”

Pg 11597, Line 9 -11: Step five does not describe the mounting of the wood-cross section to a microscope slide as in Figure 1e. Was the wood attached or glued to the slide? If so, how and with what substance (glue) and how does the glue react during the chemical extraction process?

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