Reply to the review of Anonymous Referee #2

The authors would like to thank anonymous referee #2 for the comments. In the following, referee's comments are given in bold, author's responses in plain text. Suggested new text is quoted in italics together with page and line numbers.

The paper by Ziehmer et al. highlights the possibility of using cellulose content in tree rings as a proxy for temperature. This paper is a rather technical paper that has two components: 1) a methodological aspect in which the authors discuss how to measure cellulose content in trees and 2) the application of using cellulose content as a proxy for temperature. While I believe that the approach of the authors is interesting and might even be promising, the authors have not convinced me of the accurate measurements of cellulose content. Lots of errors can be introduced in the method (which to a certain degree the authors discuss), but the paper lacks a clear estimation as to what the error on this method is. This could for example be accomplished by doing replicate sampling on the same tree. Another possibility is to split the paper in two papers, one which discusses the methodology and one which discusses the chronologies.

We appreciate the review of the anonymous referee #2, who evaluates our approach as interesting and potentially promising. Still, referee #2 states concern e.g. about the accuracy of the measurements. In the following, we would like to reply and clarify mentioned issues.

At first, referee #2 divides the paper into two parts, namely a methodological and an application part. In contrast to referee #2, we do not see these two sections as separate and independent parts. The methodology to determine the cellulose content (CC%) of tree rings is a conventional method used in the field of dendroclimatology containing of three major steps: (i) wood preparation, (ii) cellulose extraction (in our case α-cellulose extraction) and (iii) the calculation of the CC% based on the wood and cellulose dry weight (cf. sections 2.3 – 2.5, pp. 4-5). As mentioned in the introduction of the manuscript (p. 2, ll. 17-20), the method of CC% determination is mostly used as a tool for determining the degradation state in subfossil wood and for evaluating the quality of the cellulose extraction. Therefore, the methodology itself is not novel; however, the application in form of CC% series which are investigated over time and the potential for an additional supplementary proxy in tree rings is indeed novel.

The current study has been developed in the framework of the project *Alpine Holocene Tree Ring Isotope Records (AHTRIR)*. The aim of the project is to develop triple tree-ring isotope records (δ^2H, $\delta^{18}O$, $\delta^{13}C$) based on Holocene wood remains from glacier forefields, peat bogs and small lakes in the central European Alps to reconstruct climate by a multi-proxy approach for the past 9,000 years. Thereby, the framework of the project allowed the investigation of CC% series of both modern tree

rings and subfossil wood remains and their variability over large parts of the Holocene in order to gain a better understanding of CC% in tree rings and its temporal variation.

The presented study could benefit, but was also limited at the same time by the framework of the project: the vast advantage of the presented study are thousands of individual cellulose samples from both living and subfossil wood material distributed over large parts of the Holocene, which allowed the investigation of their CC% and served as a testbed for the temporal study of CC% in tree rings. However, we were at the same time limited by the high amount of samples, which so far did not allow the analysis of replicates within this project. Further, the high-Alpine tree species used in this project often reveal very narrow rings and the amount of extracted cellulose was just sufficient for further analysis. As the initial aims of the project did not include the closer analysis of CC% and its variation but was rather a concept that developed during the progress of the project, the sampling and analysis of replicates has not been conducted so far. Yet, in a study performed earlier from the Lötschental in Switzerland (unpublished measurements) we evaluated the natural variability of CC% on different larch tree-ring cores over time (see Fig. 1, 2 at the end of the replies). It documents a mean standard deviation of 3.7% in CC% for five individual cores from different trees of the same location. This standard deviation would even be significantly smaller when the values of the different cores would be adjusted according to their mean values. Therefore, we are confident that replications of larch samples of the present study would be the same within a few couple of percent (approx. 3 to 4 %).

Therefore, we do agree with referee #2 that for a robust error estimation a replicate sampling of the same tree would be preferential in the future. In the current study, we present first procedures to minimize and quantify the error, but we do agree that this is not yet complete and does not represent the accuracy of the method. Definitely, there is the need for another study on the influence and accuracy of the method.

Influences on CC% due to juvenile wood vs. mature wood, heart vs. sapwood, the influence of tree species and also the influence of preparation steps such as cutting vs. milling and the “storage” during extraction as well as the duration of the extraction need to be tested, as well as the chance of intercomparison between the individual laboratories. As suggested to reviewer #1, an intercomparison between those laboratories dealing with α-cellulose would be most suitable, as α-cellulose is well defined and its purity can be checked by FTIR determination (Galia, 2015).

As the current study presents preliminary results on the analysis of CC% time series and the described methods simply summarize the methods used at the university of Bern, we would rather not split the paper, but present it as an initial work and inspiration for further studies on CC%. Further, replicate testing is not possible any longer due to finalization of the PhD of Malin Ziehmer, and we estimated errors as best as we could here, but we agree further studies are needed for the determination of the uncertainties associated with CC% time series.

Major comments

While there are few grammatical and/or spelling mistakes, the paper should be improved for clarity. At times the paper is just very confusing. I suggest the authors try to shorten their paper and remove certain sections that make the paper unnecessary long and confusing (e.g. the discussion on whether to use dry weight before or after cutting, see more explanation below). In addition, the result section is also very confusing (see more details below)

We accept that the section on the dry weight determination may appear confusing to the reader. The aim was to make the reader aware of the fact that there is a loss of sample material during the process of cutting (which is potentially analogue to e.g. milling), so that it is essential to determine the weight after cutting. However, we will rephrase the section in order to simplify and clarify it (see details below).

Introduction

p2 L3-9: The authors argue that alpha-cellulose is the preferred substance for isotope analysis due to its long-term stability. I believe this is rather vague and the authors could give more details about the low mobility of cellulose, the fact that alpha cellulose is a singular chemical compound and the fact that it is also that the pathway from photosynthetic products to cellulose formation is more direct than the pathway to any of the other extractives (additional fractionations).

In fact, there is potential here to elaborate more on the role of (α-)cellulose as preferred substance for isotope analysis and discuss this fact in more detail. For example, McCarroll and Loader (2004) have addressed three major reasons for the shift from the analysis of whole wood to α-cellulose in stable isotope analysis: (i) the unambiguous link of tree ring CC% to a specific growth period, (ii) the isolation of cellulose as a single chemical component, which reduces potential problems caused by varying cellulose:lignin ratios and (iii) the greater level of homogeneity achieved during the purification of α-cellulose. In addition, Boettger et al. (2007) conducted an interlaboratory comparison on methods of cellulose preparation, as cellulose is traditionally used for isotopic analysis, which is underpinned by the interlaboratory comparison among nine stable isotope laboratories in Europe.
Due to the well-established role of cellulose for stable isotope studies in the field of dendroclimatology, we did not consider it necessary to elaborate in more detail its characteristics and advantages for the fact that it has been done earlier in well-known tree-ring stable isotope publications (Boettger et al., 2007; McCarroll and Loader, 2004) and we tried to keep our introduction compact.

However, following the referee's suggestions, we highlight the preferred role of cellulose by adding relevant references (Borella et al., 1999, 1998, Loader et al., 2013, 2003; Treydte et al., 2007).

p2 L21-37: In this section, the authors discuss the fact that subfossil or fossil wood can have degradation of different wood components. The authors stress how this influences the isotope ratios and can have an effect on the ratios of the individual components. This is a major limitation of the study, but although the authors mention this, they don't seem to be worried that this might affect their study and there is no further mention of this in the rest of the paper and not even in the discussion.

As mentioned earlier in this reply, the presented study is part of the project *Alpine Holocene Tree Ring Isotope Records (AHTRIR)*, where most of the tree-ring material is derived from Holocene wood remains from glacier forefields, peat bogs and small lakes, and only a small part of samples consists of modern living wood. Most of the Holocene wood samples are well preserved, but a degradation of samples cannot fully be excluded. This fact revealed the starting point for the investigation of CC% to see if modern and Holocene wood CC% are comparable.
In fact, the CC% in modern and Holocene wood samples is comparable; however, we found outliers in Holocene CC%, where CC% showed pronounced decreases (cp. section 2.6 Outlier detection and correction). We could attribute these low CC% values to the outermost rings of Holocene wood remains, where e.g. the exposition to weathering within glaciers, peat bogs or lakes could have led to a higher degree of degradation (see Fig. 2 in Reply_Referee1).
We do agree that the use of subfossil wood might be a limitation of this study; however, at the same time we could show that CC% levels in subfossil and modern wood are comparable and concluded that we could use long-term variations in Holocene CC% as an indicator of climate variations.
We further agree that we should discuss the potential influence of degradation on the CC% time series in the discussion, and reflect to what extent the degradation of individual CC% series could affect the potential of CC% as a potential supplementary proxy. In this regard, it is important to note that we have not detected a trend of CC% over time (i.e. towards the past) which would be expected when degradation would be a major driver of the variations.

Overall, the authors should bring in more discussion on the physiological aspects of the different components of wood formation in order to give the reader background into the possible limitations of the method. For example, cellulose/lignin/extractive ratios are known to differ between juvenile and mature wood and between heart wood and sapwood. It is also known to differ between normal wood and reaction wood (see for example Saka, 1991, Chemical composition and distribution, Ch 2 in Wood and Cellulosic Chemistry, Second Edition, Revised,

and Expanded, as well as Rowell et al 2012, Handbook of Wood Chemistry and Wood Composites (Second edition), CRC Press, London (2012), pp. 48-51)
The authors need to discuss this in the paper and need to address how this could affect their data.

This is a valid point and actually highlights why we have concentrated on the extraction of one single chemical component, i.e. the α-cellulose for our main purpose: the isotope investigations. It allows us to circumvent the biases that potentially could result from a changing composition (cellulose/lignin) when analyzing bulk wood since the different components exhibit significantly different isotope compositions (Borella et al., 1999, 1998). Indeed, our work currently displays a lack on the discussion of physiological aspects of wood formation and their potential influence on the CC% series. Again, our study is here limited by the availability of the sampling material, which in our case consists mainly of Holocene wood remains from glaciers etc. as mentioned above. Therefore, we are also limited here in the exploration of the influence of juvenile vs. mature wood, or heart vs. sap wood. Still, these limitations should be mentioned and further explored in a future study, e.g. in the framework of an interlaboratory comparison.
Regarding the use of reaction wood, we tried to avoid reaction wood; for modern trees, cores were taken in parallel to the slope, and for Holocene wood remains, stem discs were available, so reaction wood could mostly be identified and if possible avoided, as it is usually done in dendroclimatology.
Similar to the potential degradation of wood, these physiological influences need to be further investigated and will shortly be addressed in this study.

In addition, the authors also should research additional papers studied on similar subjects. The following paper discusses lignin content as a proxy for temperature. Since lignin and cellulose are the two main components of wood, it seems logic that a change in one will also affect a change in the other. Gindl, W., Grabner, M. & Wimmer, R. 2000. The influence of temperature on latewood lignin content in treeline Norway spruce compared with maximum density and ring width.Trees 14: 409-414.

In general, there is so far only little literature focusing on cellulose and lignin content in tree rings and their potential to reconstruct climate.
The results of the above-mentioned literature are indeed interesting; however, the analysis of lignin is only conducted over 10 consecutive years on modern wood samples. Further, the reconstruction of temperature based on lignin results in an autumn temperature reconstruction (Sept-Oct). Besides the fact that the reconstruction is very short, the authors describe the method as time-consuming (and potentially expensive?).

In contrast to the study of Gindl et al. (2000), the determination of CC% is somewhat a by-product when extracting α-cellulose for the analysis of stable isotopes in tree rings. The determination does neither add additional time consumption nor cost, but results in additional information on the individual tree rings. In our case, and due to the framework of the project, we worked with 5-year tree-ring blocks, in order to reduce cost and time to analyze Holocene climate variability within a feasible time (we are talking of thousands of measurements). Therefore, we create 5-year mean values and are able to investigate long-term trends in CC% which would not be possible by the method described by Gindl et al. (2000).

Another difference between the two components lignin and cellulose is the link to the growing season, where cellulose will be produced during most of the growing season, whereas lignin will be produced towards the end of the growing season. Therefore, cellulose will potentially incorporate a more homogenous temperature signal of large parts of the growing season (in particular for the evergreen pine trees growing at the tree-line for which photosynthesis is possible more or less throughout the year), whereas lignin will only record end of season temperature.

In general, further investigations are needed on how the ratios of the main components lignin, hemicelluloses and cellulose in a tree ring change and affect each other (cf. Borella et al., 1999, 1998 for isotope differences). A low lignin value could either result in an increased hemicellulose content or CC%. It would be worth to investigate these ratios in a tree ring also in relation to climatic factors.

Results

P6, L5: The authors discuss that a determination of sample weight after cutting is essential. Considering that the study relies on cellulose content measurements, I believe that this is rather obvious. It is more logical to use dry weight after cutting rather than before cutting. I think it is a good idea of the authors to point it out and to discuss it, but I suggest the authors remove it from the methods (section 2.5). This will make that section much less confusing.

We do agree that this is rather confusing. Therefore, we will rephrase section 2.5 and focus there only on the dry weight after cutting, and shortly discuss the loss during cutting in the results section (as currently done at the beginning of the results section).

P6, L 11: The authors discuss the fact that a systematic error is introduced while the samples are unpacked. This is indeed a good addition, but the authors don't mention what they consider this error to be. Since it is a systematic error, the authors argue that the variability between samples should not be affected. However, the error means that small differences in cellulose content between samples cannot be interpreted. Therefore, it is very important that the authors discuss/estimate the error. Especially considering that they are looking at rather small

differences in cellulose content. When looking at Table 4, it seems that the maximum weight loss during unpacking of the sample (after extraction) is 5.3 %. The authors could use this as a %error on their data. More accurately, the authors should determine the error by using replicate sampling of the same tree.

When unpacking the cellulose from filter bags, there is always the risk that smallest cellulose fibers remain in the filter bag or fly off during the removal; therefore, we assume the error to be systematic (especially since the samples were unpacked by the same person). Here we tried to estimate the error by investigating 42 individual filter bags with samples from one tree. However, we do see that the loss per sample varies in the range from 0.2% up to 7.7% at maximum, which results in a mean loss of 3.2 ± 1.4% (percent of extracted cellulose weight and not dry weight) for these 42 cellulose samples. This relative uncertainty in cellulose weight transfers directly to the relative uncertainty of the CC% determination (relative uncertainties are additive with the uncertainty of the dry weight after cutting is negligible). Relevant for the CC% variation is the variation of the relative uncertainty (± 1.4%) and not the relative uncertainty itself (3.2%), which only yields a mean offset of the whole curve.
Although the relative uncertainty slightly limits the interpretation of small differences between the individual 5-year CC% samples, they do not limit the investigation of trends in CC% time series. For example, modern CC% agree in their trends, even though they might not perfectly agree in every single data point.

P 6 section 3.1 and further: the use of % for cellulose content as well as % to express differences between sites is rather confusing and makes the paper difficult to read. Is there any way the authors can make this clearer? For example: p 6 L 22: UAZR1 and UAZR2 values are 10 percent lower than the other two trees. This could mean that their cellulose content drops from 40% to 30% (which is not the case), or that the cellulose content drops from 38 to 34 % (roughly 10% of 38% ~4, so a 4 percent drop, which seems to be the correct interpretation here (?)). Another example: P6 L30: an increase in CC % over time by ~5%. What does this mean? from 35 to 40 % or from 35 to 36.8% (reasoning that 5% of 35 is ~1.3)

Indeed, this could be made clearer by using CC% instead of %, e.g. p.6, ll. 23-25:

> *"These differences are a result of low minimum values for UAZR-1 and UAZR-2, which are up to 10 CC% lower than for the other two trees (Table 5)."*

Apart from the very first part of the results section (p.5, l. 34 – p.6, l.15), we always meant CC% when there is written % throughout the entire results and discussion section, so we are consistent here, but we agree that this is not obvious. Therefore, we would change all % into CC% in order to be correct and avoid any misunderstanding.

Discussion and Conclusion

The authors should revisit the methods used and include a discussion on the limitations of their method. Also, a discussion on the practical aspects of this method should be included: It is definitely not easier than measuring ring widths, so what is the advantage? Is there other information that has been revealed?

We already agreed earlier in this reply, that limitations such as the use of subfossil wood, i.e. physiological aspects, as well as methodological aspects concerning the extraction and their potential influence on our dataset should shortly be discussed.
Obviously, the extraction of α-cellulose from tree rings is not easier than measuring tree-ring width, and usually α-cellulose is only extracted in the course of the stable isotope determination in tree rings. However, we see a significant potential that CC% series, which often already exist in many tree-ring laboratories in large quantities, could be used as an additional supplementary proxy. This is especially the case for multi-proxy studies with the aim to reconstruct climate, as the presented project AHTRIR, where there is potential to compare the variability of the CC% with other climate-dependent tree-ring proxies such as tree-ring width, density and isotopes.

Minor comments

P4 section 2.5: this section is extremely confusing. If possible it should be rewritten.

We agree that this section might be confusing due to the elaboration on the role of dry weight of wood before and after cutting. We will simplify this section by reducing the content simply to the dry weight after cutting, which will clarify the calculation of CC%.

P4 L32: I think the authors mean "1. dry weight" in the equation?

No, here we presented the general formula without focusing on 1. or 2. dry weight. However, being rewritten, we will eliminate this issue and define dry weight as the dry weight after cutting of the wood sample.

P4, L33 "weighing" instead of weighting

Indeed, this is a spelling mistake and will be corrected.

P5, L3: replace cellulose with sample

We would rather replace cellulose by cellulose material than by sample.

P5, L19: add the . . .obtained in the form. . .

Indeed, the article is missing and will be filled in.

Figures

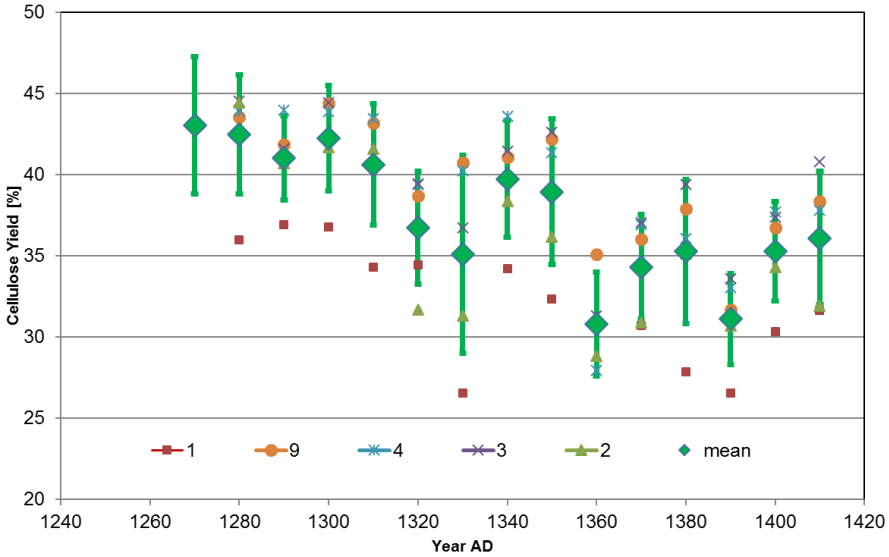

Figure 1. Variability of CC% in larch tree rings from Lötschental (CH). The numbers correspond to tree cores from different trees.

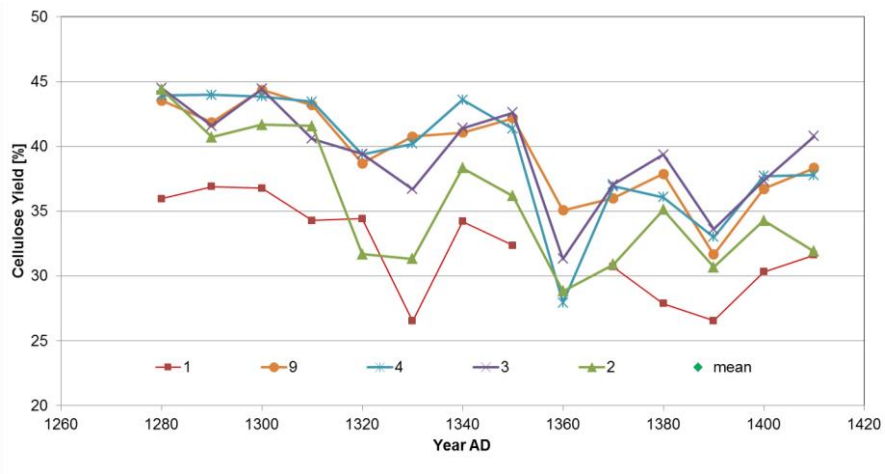

Figure 2. Temporal variability of CC% in larch tree ring series from Lötschental (CH). The numbers correspond to tree cores from different trees.

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