Reply to the review of Anonymous Referee #1

The authors would like to thank anonymous referee #1 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 authors have investigated if the cellulose content (CC) of tree-rings could be used as an additional palaeo-climatic proxy. The idea is indeed novel and the presented results are interesting. On the other hand, I have concerns regarding the reliability of the method.

We appreciate that anonymous referee #1 recognizes the novelty of tree-ring cellulose content (CC%) as an additional supplementary proxy for paleo-climate reconstructions and that he evaluates our presented results as interesting. In the following, we would like to resolve his concerns regarding the reliability of our method by replying to referee #1's comments point by point.

- In my opinion and from my experience, I would expect the CC-values to be "operatordependent", meaning that different laboratories would get to different results from the same tree-ring material. Various cellulose extraction protocols are used in different labs, see Boettger et al. 2007. An inter-lab comparison would therefore be crucial, in particular as there are no standards for verification. Further interfering methodological factors are also the preparation of the wood, e.g. milling (loss of powder during cellulose extraction) versus no milling (using slivers) and the recovery from filter/containers (also different methods are in use in different labs).

Indeed, different tree-ring laboratories utilize different routine methods in order to produce tree-ring cellulose from wood material as shown in the interlaboratory comparison by Boettger et al. (2007) in the framework of the ISONET project. Boettger et al. (2007) described the extraction procedure in three main steps, namely (i) pretreatment, (ii) delignification and (iii) purification. Differences in the extraction procedure between the individual laboratories existed in the concentration of reagents, the treatment time as well as the temperatures (Boettger et al., 2007). The study showed that four out of nine laboratories produced holocellulose rather than α-cellulose, where holocellulose represents a combination of hemicelluloses and α-cellulose. Therefore, we agree that CC% values can be operator-dependent as the extraction method used in the individual laboratories determines if holocellulose or α-cellulose is extracted.

In our study, we focus on the investigation of α-cellulose which is defined as the part of cellulosic material being insoluble in 17% NaOH solution (Burton and Rasch, 1931; Cross and Bevan, 1912). In Boettger et al. (2007), five laboratories already used 17% NaOH solution in order to purify their samples. We would expect that α-CC% series among laboratories are comparable, whereas the holocellulose would reveal a positive offset compared to α-cellulose series, as the holocellulose series include hemicelluloses.

A future interlaboratory comparison comparable to the study by Boettger et al. (2007) could confirm the comparability of α-cellulose series between individual laboratories. Such a comparison should also include investigations on the influence of preparation methods (milling vs. cutting wood), the individual steps and duration of the extraction, the role of the sample size, as well as the influence of tree species, juvenile vs. mature tree rings, and heart wood vs. sap wood. Further, purity of the extracted α-cellulose can be checked by FTIR spectra (Galia, 2015). Such an interlaboratory

comparison is essential and prerequisite for the assessment of the accuracy of CC% and comparison of CC% series among different tree-ring laboratories.
However, the presented study is part of a project investigating Holocene climate variability in a multi-proxy approach, where we focused on the extraction of α-cellulose following a standardized extraction procedure as described in the methods section. Here, we tried to assess e.g. the loss during the recovery of cellulose from the filter bags of sliced samples (see results section) in order to estimate the error of our CC% time series.
The presented study benefits from the continuity of the applied methodology; however, we do agree that further investigation of the method by an interlaboratory comparison would be the essential next step in order to gain a better understanding of CC% series variations, its dependencies on parameters mentioned above and to allow comparisons of existing CC% series obtained from individual tree-ring laboratories.

- Climate correlations with temperature may not be reliable as it seems to me that unrealistic significance levels have been used. The mean chronologies (Fig. 3) display a very high autocorrelation mainly because of the use of 5-yr blocked data. Therefore, it is absolutely essential to correct the degree of freedom for autocorrelation, which seems not to have been done. This would increase the correlation coefficients needed to be significant. Obviously, increasing trends in the data will correlate with increasing temperature trends. Accordingly, even winter months appear to correlate significantly with CC, but I cannot believe that all months of the year influence CC as suggested by results in Fig. 5. Trends could be related to ageing, for instance, rather than climate.

As described in section 2.7, we obtained meteorological data from the HISTALP database. For the individual climate parameters, we calculated monthly anomalies with the reference period 1961-1990. In order to obtain Pearson's correlation coefficients, five-year mean values were calculated for the individual climatic parameters, which matched the time step in our CC% series. We assume here that each data point (5-year blocked data) in the climate variable datasets and the CC% series is independent, since there is no data point overlap.
We also investigated the possibility of age trends in our CC% series, although we did not expect to find any age trends as the modern CC% series of the two tree species did not reveal a common increase/ decrease over time (cf. p. 27, Fig. 4 in the manuscript). The age-alignment of all CC% series by their biological age, taking their estimated pith offset (PO) into account, illustrates that CC% series are not biased by age trends, which leads us to the conclusion that the trends which we find in CC% series are most probably driven by climatic variables (see Fig.1 at the end of the replies). The independence of the individual CC% samples and the lack of autocorrelation in the CC% series allow the presented calculation of Pearson's correlation coefficients between the 5-year CC% content series and the 5-year-average climate variable data sets.
Regarding correlations with winter months: Indeed, especially the PICE trees show significant correlations also with winter temperatures. A potential explanation could be the fact that photosynthesis is still possible in winter (p. 10, ll.37-40). Thereby, the concentration of NSC is increasing and already available for tissue formation as soon as temperatures allow for it. Future studies on CC% on an annual and even intra-annual resolution could help improve our understanding of the influence of winter temperatures on the CC% in tree rings.

- There are strong differences in the mean chronologies between Larix and Pinus for the same site (VRR), while chronologies from the same species but rather distant sites are similar. This points to the importance of biological factors rather than climate.

Indeed, the two different species *Larix decidua* Mill. and *Pinus cembra* L. at the sampling site in Val Roseg (VRR) exhibit different trends in their mean chronologies. As they both experience the same climate, the role of biological factors here is undoubted. This is kind of obvious, as we compare two coniferous species, where *Larix decidua* Mill. is a deciduous species, whereas *Pinus cembra* L. is an evergreen species; therefore, we would expect differences in their metabolism.
The fact that the individual CC% series from the same species at different sites are similar calls for a driving factor of regional extent, such as temperature.

- The mechanisms resulting in varying CC are rather unclear. Some link to NSC and sink activity was proposed, but was not very understandable for me. The relationship between CC and wood density might be rather interesting to explore. Late-wood density is known as strong temperature indicator and it would be plausible to expect a relationship between CC-content and density.

In this preliminary evaluation of CC%, we investigated its variability, its link to climate and looked for potential mechanisms in a tree which could lead to variations in CC% and which are in turn dependent on climate. As we investigate two tree species growing at the Alpine tree-line, we know that their growth and therefore the cell formation is mostly restricted by temperature.
As discussed in the manuscript, research conducted by Körner and Hoch on the drivers of the climate-driven tree-line revealed that trees at the upper tree-line do not experience carbon shortage, but rather experience a lowered sink activity which results in a growth limitation (Hoch et al., 2002; Hoch and Körner, 2012, 2009; Körner, 1998). This simply means that a tree at the upper tree-line is still able to conduct photosynthesis, even though temperatures might be too low to allow tissue formation.
This represents the link to the variability in our CC%: short and cool growing seasons will lead to less tissue formation and a lower CC%, whereas warm and prolonged growing seasons will lead to an increased tissue formation and therefore to a higher CC%.

Indeed, it would be highly interesting to analyze the relationship between CC% and wood density. Within the scope of this project, we tried to determine the density of our samples by Blue Intensity (BI) measurements, as the conventional determination of density by X-ray images was not feasible due to the high amounts of individual samples (> 8,000 individual samples for the entire project).
However, the determination of density by BI measurements in Holocene wood samples remains a challenge due to the different coloring of wood samples.

Still, future studies should further focus on the link between CC% and wood density and explore their relationship. Usually the determination of wood density and the determination of CC% for stable isotope analysis do not occur within the same research project. Establishing a link between these two variables might allow to draw conclusions on wood density by determining the CC% and vice versa. Hence, further research on the relationship between CC% and other tree-ring proxies (tree-ring width, maximum latewood density, stable isotopes) is essential.

- Due to the degradation of cellulose in old wood, the reliability of the subfossil CC series seems not so clear to me.

The investigation of CC% series has been conducted in the framework of the project *Alpine Holocene tree ring isotope records (AHTRIR)*, where the determination of CC% was initially used to determine the quality of the cellulose extraction before performing analysis of triple stable isotopes on the tree-ring cellulose. As the project aims at establishing triple isotope records for the past 9,000 years for the central European Alps, tree-ring material consists both of living wood material, but the largest part is based on findings of Holocene wood remains from glacier forefields, peat bogs and small lakes.
Although most samples were well preserved, we expected a certain grade of degradation in the Holocene wood remains. But for the period from 9,000 to 3,500 years b2k, we see that the CC% varies between 30-40 %, so the CC% of the subfossil samples is comparable to what we find in modern CC% series from living trees. Only a small number of outliers was found (as described in section 2.6), where CC% values showed pronounced decreases mostly appearing in the outermost rings as well as along cracks in the wooden material. We assume that these tree-ring sections have been affected by weathering and therefore reveal a high degree of degradation, whereas the other rings have been well preserved.
In order to clarify this issue and illustrate the occurrence of degradation in the outermost rings vs. well-preserved inner rings, a supplementary graph will be added (see a first example graph in Fig. 2 at the end of the replies).

Overall, I find it worthwhile to investigate these data, but it seems premature to me to propose them as a palaeo-climate proxy.

We agree with referee #1 that further tests are necessary to evaluate the potential of CC% in tree rings as a paleo-climate proxy. Therefore, we already suggested in the title of the paper that the current manuscript discusses the potential of tree-ring CC% as an additional supplementary proxy rather than stating that we found a new proxy. Yet, we further emphasize this point by changing the title to: "*Preliminary evaluation of the potential of tree-ring cellulose content as a novel supplementary proxy in dendroclimatology"*. This highlights the importance that this potential has to be further explored and verified by interlaboratory comparisons and our study is a first step into this direction that intends to motivate other tree-ring researchers in the field of dendroclimatology and stable isotope analysis to investigate their existing CC% series and to perform further research on these data.

Figures

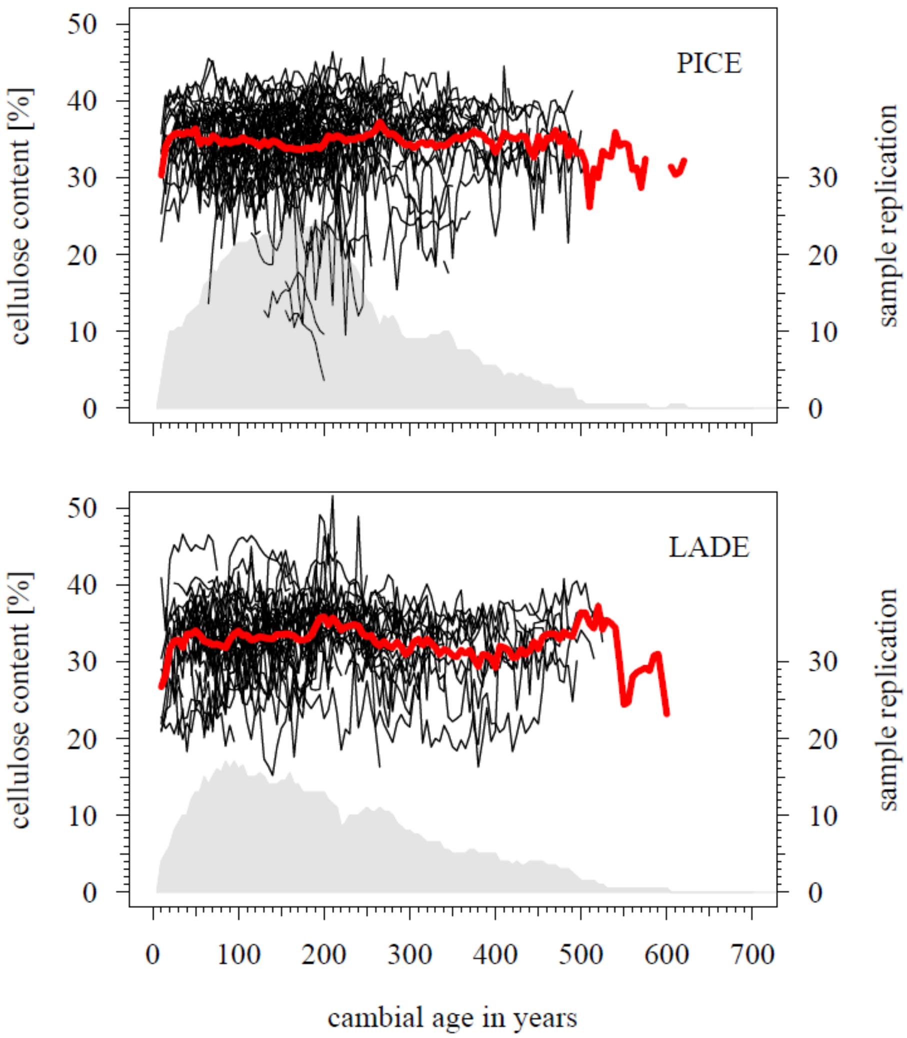

Figure 1. Cellulose content (CC%) in *Pinus cembra* L. (PICE) and *Larix decidua* Mill. (LADE) aligned according to their cambial age in years (pith offset estimation is considered here). Shown are the individual series in black and the mean in red, as well as the sample replication indicated by the grey area at the bottom of each graph.

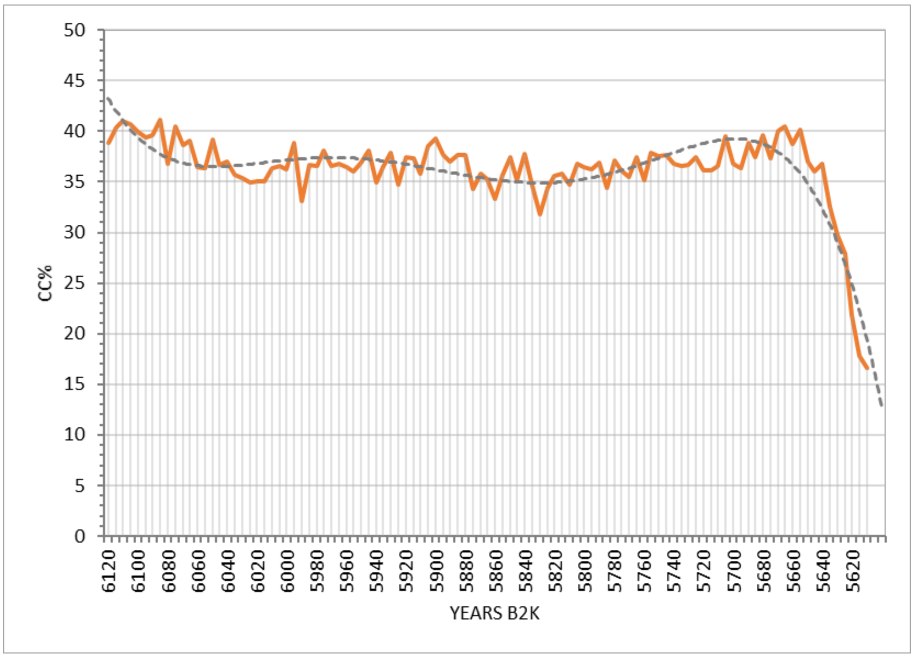

Figure 2. Example of CC% variations and degradation in a *Larix decidua* Mill. tree (ULFI-47). The tree exhibits a long-term trend in its CC% series, followed by a rapid decrease of CC% in its outermost rings, which is attributed to degradation of CC% due to exposition to weathering. Still, most of the tree is well preserved and suitable for CC% analysis.

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