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. Changes in the manuscript and suggested new text is quoted in red and italics together with page and line numbers of the revised and track-mode manuscript.

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 heartwood vs. sapwood. 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.

The importance of a future interlaboratory comparison off CC [%] has been added to the conclusion and outlook of the manuscript (**p. 14, ll. 7-13**) and corresponds to our response to referee #1 made earlier in this reply:

"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 heartwood vs. sapwood. 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."

- 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.

In order to clarify that data points in our correlation analysis are independent, the following sentence has been added to the manuscript (**p.6, ll. 22-23**):

“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.

The consideration of age trends in CC [%] series has been added to the discussion of the manuscript (**p.11, ll. 1-6**):

“In order to exclude any age-related biases, possible age trends in CC [%] series have been investigated, even though they were not expected as the trends in modern CC [%] series of the two tree species diverge and do not reveal a common increase/ decrease over time. The age-alignment of both modern and Holocene 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 (Fig. S5).

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.

To clarify the link between correlations with winter months and CC [%], the following lines have been added to the discussion (**p. 12, ll. 4-9**):

“This might explain the found correlations with winter temperatures especially for PICE trees, i.e. potential photosynthetic activity at low temperatures in winter, where tissue formation is no longer possible (Hoch et al., 2002 and references therein). Thereby, the concentration of NSC is increasing and is 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.

In order to clarify the influence of biological factors and climate, the following section has been added to the discussion of the manuscript (**p. 11, ll. 7-16**), adapted from the reply to referee #1:

"Further, the two different species Larix decidua Mill. and Pinus cembra L. at the sampling site Val Roseg (VRR) exhibit different trends in their mean chronologies. As they experience the same climate, the role of the biological factors here is undoubted. This is rather obvious as two coniferous species are compared, where Larix decidua Mill. is a deciduous species, whereas Pinus cembra L. is an evergreen species; therefore, differences in their metabolism are to be expected. Although the two species are both found at the upper tree-line and known to be adapted to the harsh environmental conditions, LADE is characterized as the light-demanding pioneer species which is often found in open settings, e.g. on glacier forefields, whereas PICE is, under undisturbed conditions, the highest rising species in the inner sections of the Alps and with that adapted to short vegetation periods (Ellenberg, 1996). The fact that individual CC [%] series from the same species at different sites are similar calls for a common 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 [%].

As the potential link between CC [%] and NSC is described in detail in the discussion section, no further changes have been made to this section.

Indeed, it would be highly interesting to analyse 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.

The potential of investigating the link between CC [%] and wood density in future studies is shortly discussed in the conclusion and outlook section (**p. 14, ll. 14-21**):

"Further, the analysis of a relationship between CC [%] and wood density would be highly interesting. These two proxies are usually not investigated within the same research project. However, 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. Although the evaluation of α-CC [%] is obviously not easier than measuring tree-ring width, there is a significant potential of using it as an additional supplementary proxy, especially in those cases where CC [%] series are already existent in tree-ring laboratories and where climate is to be reconstructed in a multi-proxy approach."

- 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.

A short description of the project, in which the CC [%] study has been conducted, was added to the introduction of the manuscript (**p. 3, ll. 24-31**):

"The current study is embedded in the project "Alpine Holocene Tree Ring Isotope Records (AHTRIR)" which aims at reconstructing Holocene climate variability using a multi-proxy approach for the past 9000 years. Therefore, tree-ring material consists of living wood material, but the largest part is based on findings of Holocene wood remains from glacier forefields, peat bogs and small lakes in the central European Alps. Initially, the determination of CC% was used to determine the quality of the cellulose extraction before performing analysis of triple isotopes (δ^2H, $\delta^{18}O$, $\delta^{13}C$) on the tree-ring cellulose. However, it offers the unique opportunity to investigate the α-CC [%] and its variations in long-living trees from two high-Alpine coniferous tree species […]"

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).

The potential influence of degradation on CC [%] series from Holocene wood remains is discussed on **p. 12, ll. 26-37**:

"As the framework of the project AHTRIR included both the analysis of living and subfossil wood, Holocene wood remains were also investigated for signs of degradation. Most samples were well preserved, and for the period from 9,000 to 3,500 years b2k, the CC [%] also varied between 30-40 CC%. Therefore, the CC [%] in living and subfossil wood samples is comparable. Only a small number of outliers was found (see also 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 (Fig. S6). Although the potential degradation subfossil wood might have been a limitation of this study, CC [%] of modern and subfossil wood is comparable despite a few outliers, which leads to the conclusion that long-term variations in Holocene CC [%] could serve as an indicator of climate variations. Moreover, there is no trend detected in CC [%] over time (i.e. towards the past) which would be expected in case degradation would have been a major driver of CC [%] variations."

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*".

The title has been changed to the proposed new title.

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.

The importance of an interlaboratory comparison is highlighted in the conclusion and outlook section (**p. 14, ll. 7-13**, shown earlier in this reply to referee #1). Further, a sentence has been added on the intention and motivation of this paper to stimulate further research on tree-ring CC [%].

"This study represents a first step into this direction and 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

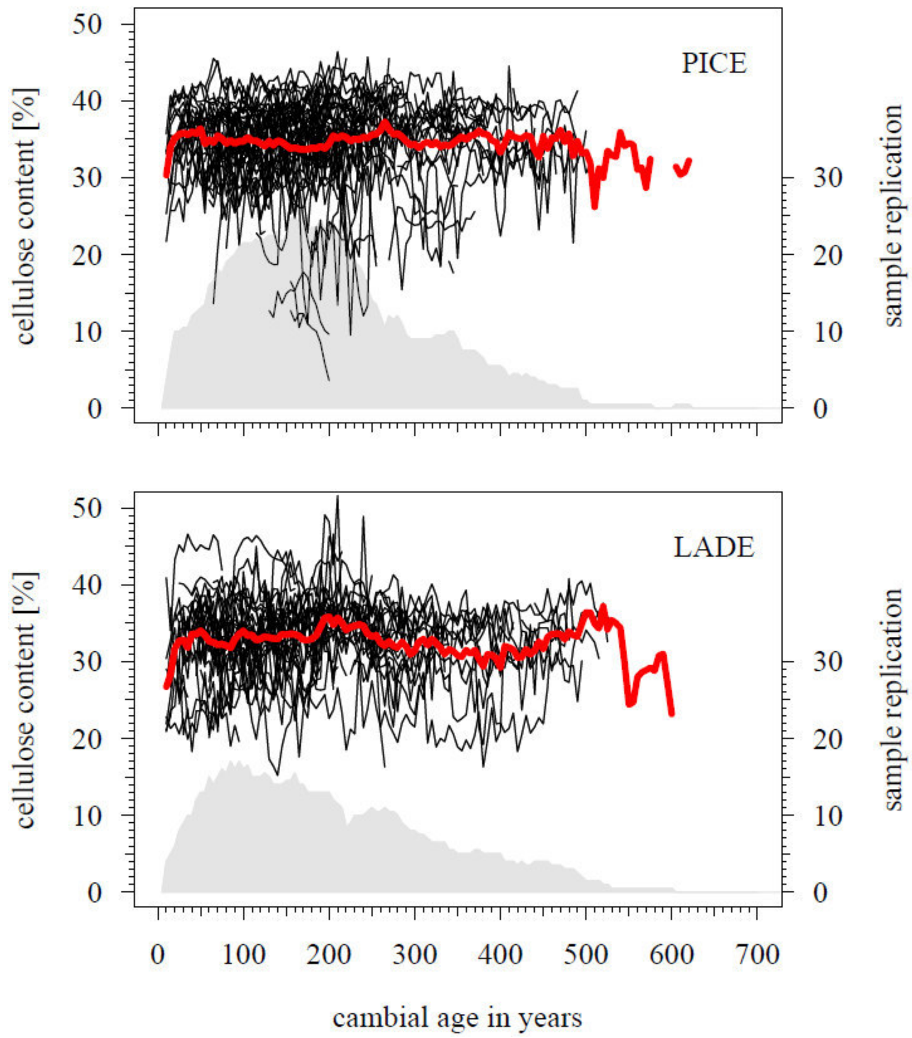

Figure 1. Cellulose content (CC [%]) in *Pinus cembra* L. (PICE) and *Larix decidua* Mill. (LADE) aligned according to their cambial age in years (pith offset estimation is considered here). Shown are the individual series in black and the mean in red, as well as the sample replication indicated by the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

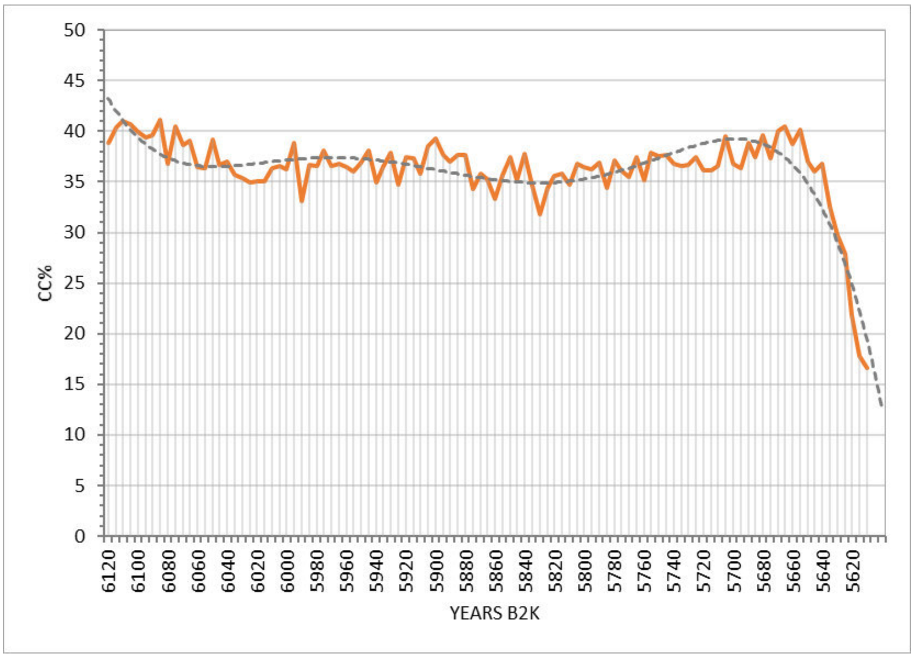

Figure 2. Example of CC [%] variations and degradation in a *Larix decidua* Mill. tree (ULFI-47). The tree exhibits a long-term trend in its CC [%] series, followed by a rapid decrease of CC [%] in its outermost rings, which is attributed to degradation of CC [%] due to exposition to weathering. Still,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

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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. Changes in the manuscript and suggested new text is quoted in red and italics together with page and line numbers of the revised and track-mode manuscript.

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 ($\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$) 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.

A short description of the project, in which the CC [%] study has been conducted, was added to the introduction of the manuscript (**p. 3, ll. 24-31**):

"The current study is embedded in the project "Alpine Holocene Tree Ring Isotope Records (AHTRIR)" which aims at reconstructing Holocene climate variability using a multi-proxy approach for the past 9000 years. Therefore, tree-ring material consists of living wood material, but the largest

part is based on findings of Holocene wood remains from glacier forefields, peat bogs and small lakes in the central European Alps. Initially, the determination of CC [%] was used to determine the quality of the cellulose extraction before performing analysis of triple isotopes (δ^2H, $\delta^{18}O$, $\delta^{13}C$) on the tree-ring cellulose. However, it offers the unique opportunity to investigate the α-CC [%] and its variations in long-living trees from two high-Alpine coniferous tree species [...]"

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.

A comment on the limitation in error estimation and replicate sampling has been added to the discussion of the manuscript (**p. 13, ll. 5-21**):

"The presented study could benefit, but was at the same time limited by the framework of the project in which it was performed: 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 (Fig. S7, S8). 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 %).
In the current study, first measures to minimize and quantify the error of CC [%] have been presented; however, in future studies, it will be essential to accomplish a robust error estimation by a replicate sampling of the same tree."

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).

The importance of a future interlaboratory comparison off CC [%] has been added to the conclusion and outlook of the manuscript (**p. 14, ll. 7-13**) and corresponds to our response to referee #1 made earlier in this reply:

"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 heartwood vs. sapwood. 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."

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).

In order to clarify the paper and shorten it at the same time, section 2.5 has been rewritten (see details further 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).

The relevant references elaborating on the preferred role of cellulose in dendroclimatology have been added to the manuscript (**p.2, ll. 3**):

"[...] the most preferred component is α-cellulose as a single chemical component extracted from tree-rings (Borella et al., 1999, 1998, Loader et al., 2013, 2003; McCarroll and Loader, 2004; 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.

The limitation of using subfossil wood samples in the framework of the project AHTRIR and the influence of degradation on CC [%] are discussed on **p.12, ll. 26-37**:

"As the framework of the project AHTRIR included both the analysis of living and subfossil wood, Holocene wood remains were also investigated for signs of degradation. Most samples were well preserved, and for the period from 9,000 to 3,500 years b2k, the CC [%] also varied between 30-40 CC [%]. Therefore, the CC [%] in living and subfossil wood samples is comparable. Only a small number of outliers was found (see 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 (Fig. S6). Although the potential degradation subfossil wood might have been a limitation of this study, CC [%] of modern and subfossil wood was comparable despite a few outliers, which led to the conclusion that long-term variations in Holocene CC [%] could serve as an indicator of climate variations. Moreover, there were no trend detected in CC [%] over time (i.e. towards the past) which would be expected in case degradation would be a major driver of 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 heartwood 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).

As we focus on α-cellulose in this study, we highlighted its preferred role in dendroclimatology by commenting on its advantage of being a single chemical component (**p. 2, ll. 3-7**):

"A major advantage of using α-cellulose in isotope research is the extraction of one single chemical component of a tree-ring. Thereby it allows researchers to circumvent the biases that potentially could result from a changing composition (i.e. in the cellulose/lignin ratio) when analysing bulk wood since 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.

As our study is limited by the availability of sampling material, physiological aspects of wood formation and their potential influence on CC [%] cannot be properly explored in the framework of this study and would be part of future interlaboratory comparison studies, which we shortly discuss in the conclusion and outlook section (**p. 14, ll. 7-13**):

"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 heartwood vs. sapwood. 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."

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.

This has been clarified in section 2.1 (**p. 4, ll. 12-20**):

Cores are taken at breast height from three radial sections; two in parallel to the slope, and one up-slope, thereby avoiding any kind of compression wood on the down-slope side of the tree.
The Holocene wood remains stem from glacier forefields, peat bogs and small lakes, which have been continuously collected over the last two decades (Joerin et al., 2006, 2008; Nicolussi, et al., 2009; Nicolussi et al., 2005; Nicolussi and Patzelt, 2000b). Wood material of the EACC (Eastern Alpine Conifer Chronology) has been merged and updated with subfossil samples of the same species and

altitude collected by continued sampling of wood remains and stem discs in glacier forefields (Nicolussi and Schlüchter, 2012). Similar to modern wood samples, the use of compression wood in these Holocene samples was avoided during the further analysis.

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 analyse 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.

The study by Gindl et al. (2000) establishes a relationship between a major tree ring component and climate; however, the time period they cover is rather short and the method rather expensive. Therefore, we added a short comment on this study in the introduction of our study in order to emphasize the advantages of using α-cellulose as an additional proxy (**p. 3, ll. 4-10**).

"For instance, Gindl et al. (2000) discussed lignin content as a temperature proxy for the late growing season (Sept-Oct). As lignin and cellulose are among the major components of a tree ring, and changes in one component will affect the cellulose/lignin ratio, a link between CC [%] and temperature can be expected as well. However, the study of Gindl et al. (2000) covers only a short

time period of 10 years and the method used is rather time-consuming, whereas the production of α-CC [%] is often a by-product when extraction α-cellulose for stable isotope analysis in tree rings."

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).

Section 2.5 (**p.5, ll. 15-29**) has been rephrased as follows:

"The CC [%] is calculated from the dry weight of wood and the cellulose weight of the sample:

$$CC\ [\%] = \left(\frac{\alpha\text{−cellulose weight}}{\text{wood dry weight}}\right) \times\ 100\% \qquad (1)$$

The wood dry weight is thereby defined as the sample weight of the individual wood sample after cutting, and the cellulose weight refers to the weight of the extracted α-cellulose after being removed from the ANKOM filter bag.

A remaining source of error is the collection of dry α-cellulose from the ANKOM filter bags. The described slicing of wood samples facilitates the removal of α-cellulose from filter bags compared to ground wood samples and thereby reduces the loss of α-cellulose material when being collected from the filter bags. In this study, the resulting loss is examined for 42 samples, where the filter bag including the α-cellulose and the emptied filter bag are scaled in addition to the α-cellulose weight to give an accurate estimate of the observed loss."

The loss during cutting and the importance of determining the dry weight of the wood sample after cutting is shortly discussed at the beginning of the results section (**p. 6, ll. 28-33**):

"The mean sample loss during the cutting process amounts to 2.6 ± 1.7 % of the dry weight of an individual sample, where mean loss values range from 0.3 % up to 11.1 % per tree (data not shown here). Besides, very few individual samples experienced losses between 10 % and 30 % caused by wood pieces bouncing off during cutting or loss of material due to powdery wood substance as a result of degradation. Hence, for the precise calculation of CC [%], a determination of sample weight after cutting or milling is essential."

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.

According to our reply to referee #2, an elaboration on the relative uncertainty of CC [%] series has been added to the beginning of the results section (**p. 6-7, ll. 33-14**):

"Second, when unpacking the cellulose material from the filter bags, there is always a risk that smallest fibers remain in the filter bag or fly off during the removal; therefore, we assume this error to be systematic. In this study, we estimated this error by the investigation of 42 filter bags, which revealed a mean loss of α-cellulose of 0.378 ± 0.163 mg (3.2 ± 1.4 %) (Table 4). Therefore, the calculation of the CC [%] results in the minimum CC [%] of the sample, as losses of cellulose in the order of 3.2 ± 1.4 % of the cellulose weight are to be expected, which would result in slightly increased CC [%] value. However, as the unpacking is accomplished equally for all samples, a systematic error is assumed which affects the CC [%] calculation in the same manner. It can be assumed that the systematic error does not influence the variability of the individual CC [%] series.
Further, the relative uncertainty in cellulose weight transfers directly to the relative uncertainty CC [%] determination, as relative uncertainties are additive, with the uncertainty of the dry weight after cutting being 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 slight differences between the individual 5-year CC [%] samples, it does not limit the investigation of trends in CC [%] series.
The improvement in the CC [%] calculation and the estimation of the relative error justify the use of the term "CC [%]" rather than "cellulose yield", as sources of error are reduced and well estimated, and resulting calculations are close to the true values."

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.

In order to clarify the use of units, CC [%] is given in the unit CC% (instead of % only). Absolute variations are given in CC% as well. This should avoid any confusion on absolute and relative changes which have occurred before by only using %. In the manuscript, the following sentence has been added to clarify the use of units (**p. 7, ll. 16-18**):

"For a better understanding of CC [%] variations, modern CC [%] series are analysed in their temporal variability per species and site, where the absolute values and absolute variations in CC [%] are given in the unit CC%."

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.

The limitations of the study such as potential limitation by degradation of subfossil wood samples or the missing information from replicate measurements are elaborated towards the end of the discussion (**p.12-13. ll. 26-21**):

"As the framework of the project AHTRIR included both the analysis of living and subfossil wood, Holocene wood remains were also investigated for signs of degradation. Most samples were well preserved, and for the period from 9,000 to 3,500 years b2k, the CC [%] also varied between 30-40 CC%. Therefore, the CC [%] in living and subfossil wood samples is comparable. Only a small number of outliers was found (see also 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 (Fig. S6). Although the potential degradation subfossil wood might have been a limitation of this study, CC [%] of modern and subfossil wood is comparable despite a few outliers, which leads to the conclusion that long-term variations in Holocene CC [%] could serve as an indicator of climate variations. Moreover, there is no trend detected in CC [%] over time (i.e. towards the past) which would be expected in case degradation would have been a major driver of CC [%] variations.

The low-frequency trends exhibited in the mean series of Holocene α-CC [%] in the period from 9000-3500 years b2k illustrate the potential of CC [%] as an additional proxy. The arithmetic mean CC [%] series shows pronounced decreases after known cold events in the Early and Mid-Holocene, whereas a continuous increase is observed between 7350 and 6250 years b2k, which could be the result of increased temperatures and more favorable growing conditions for trees at the upper tree-line. However, the investigation of the individual species also illustrates differences in variations between LADE and PICE approving the observed differences in species within modern samples. A complete understanding of CC [%] on tree species and environmental conditions will help to further improve the robustness of this novel proxy.

The presented study could benefit, but was at the same time limited by the framework of the project in which it was performed: 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 (Fig. S7, S8). 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 %).

In the current study, first measures to minimize and quantify the error of CC [%] have been presented; however, in future studies, it will be essential to accomplish a robust error estimation by a replicate sampling of the same tree."

Further, the conclusion and outlook section has been complemented by comments on the potential of future research on CC [%] and the following sentence has been added (**p. 14, ll. 18-21**):

"Although the evaluation of α-CC [%] is obviously not easier than measuring tree-ring width, there is a significant potential of using it as an additional supplementary proxy, especially in those cases where CC [%] series are already existent in tree-ring laboratories and where climate is to be reconstructed in a multi-proxy approach."

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 [%].

Section 2.5 has been rewritten (see comment earlier in this reply).

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.

This has been eliminated as the entire section has been rewritten.

P4, L33 "weighing" instead of weighting

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

Due to rewriting section 2.5, this spelling mistake is no longer existing.

P5, L3: replace cellulose with sample

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

This has been corrected and reads now as follows:

"[…] thereby reduces the loss of cellulose material when being collected from the filter bags."

P5, L19: add the . . .obtained in the form. . .
Indeed, the article is missing and will be filled in.

This has been corrected and reads now as follows:

"Data is obtained in the form of Coarse Resolution Subregional Means (CRSM) […]"

Figures

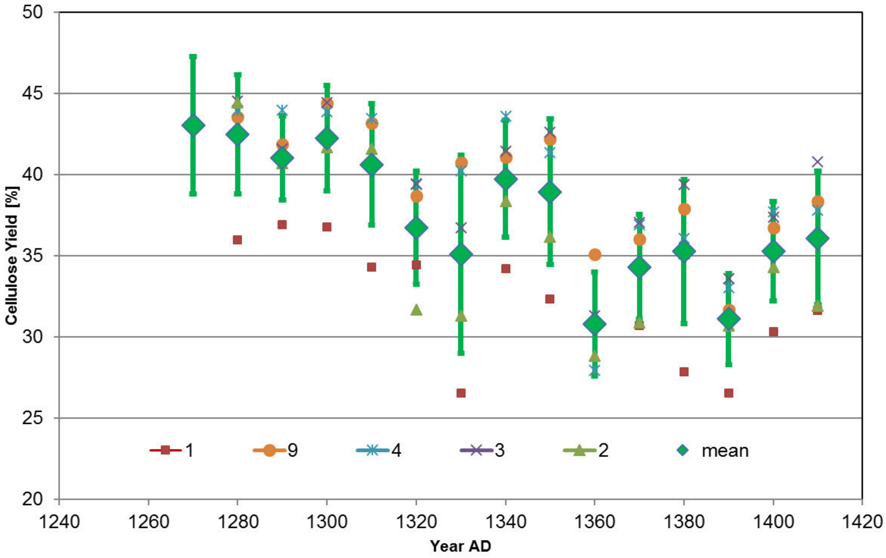

Figure 1. Variability of CC [%] in larch tree rings from Lötschental (CH). The numbers correspond to tree cores from different trees.

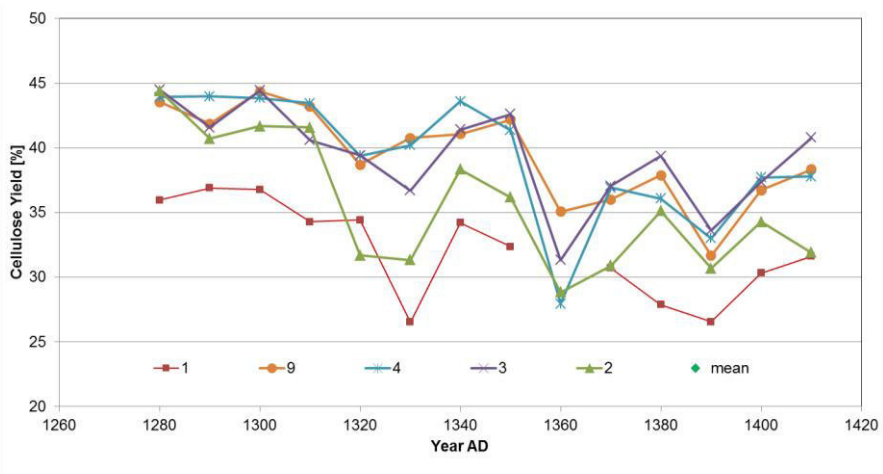

Figure 2. Temporal variability of CC [%] in larch tree ring series from Lötschental (CH). The numbers correspond to tree cores from different trees.

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Preliminary evaluation of ~~T~~the potential of tree-ring cellulose content as a novel supplementary proxy in dendroclimatology

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Abstract. Cellulose content (CC [%]) in tree rings is usually utilized as a tool to control the quality of the α-cellulose extraction from tree ~~-~~rings in the preparation of stable isotope analysis in wooden tissues. Reported amounts of CC [%] are often limited to mean values per tree. For the first time, CC [%] series from two high Alpine species, *Larix decidua* Mill. (European Larch, LADE) and *Pinus cembra* L. (Swiss stone pine, PICE) are investigated in modern wood samples and Holocene wood remains from the Early and Mid-Holocene. Modern CC [%] series reveal a species-specific low-frequency trend independent from their sampling site over the past 150 years. Climate-cellulose relationships illustrate the ability of CC [%] to record temperature in both species, but for slightly different periods within the growing season.

The Holocene CC [%] series illustrate diverging low-frequency trends in both species, independent of sampling site characteristics (latitude, longitude and elevation). Moreover, potential age trends are not apparent in the two coniferous species. The arithmetic mean of CC [%] series in the Early and Mid-Holocene indicate low CC [%] succeeding cold events. In conclusion, CC [%] in tree rings show high potential to be established as novel supplementary proxy in dendroclimatology.

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1 Introduction

Highly-resolved proxy records from the tree-ring archive contribute significantly in reconstructing and understanding past climate variability on versatile temporal and spatial scales (Büntgen et al., 2011; Esper et al., 2002; Mann et al., 1998). The annually-resolved and calendar-dated tree-ring records are particularly useful in disentangling natural climate variability from the anthropogenic imprint on the global climate and its impact on the climate system, which enables the improvement of models in view of projecting future climate change (Keel et al., 2016; Keller et al., 2016).

Most dendroclimatological reconstructions are based on tree-ring width (TRW) and maximum latewood density (MXD) records (Büntgen et al., 2006; Ljungqvist et al., 2016; Trouet et al., 2009). However, the determination of stable isotope ratios of carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$) and hydrogen (δD) isotopes in tree components and their ability to reconstruct past climate has gradually opened a new field of dendroclimatology during the past four decades (Frank et al., 2015; Leuenberger, 1998; McCarroll and Loader, 2004; Treydte et al., 2006). In addition, the potential of stable isotope records in tree rings has been increased by improvements in both sample preparation and measurement techniques (Boettger et al., 2007; Filot et al., 2006; Laumer et al., 2009; Loader et al., 2015). Even though the isotopic composition has been determined in various components of a tree, e.g. bulk wood samples, leaves, methoxyl groups of lignin and leaf waxes (Anhäuser et al., 2017; Borella et al., 1999; Kahmen et al., 2011; Kimak et al., 2015), the most preferred component is α-cellulose extracted from tree -rings (Borella et al., 1998, 1999, Loader et al., 2003, 2013; McCarroll and Loader, 2004; Treydte et al., 2007). A major advantage of using α-cellulose in isotope research is the extraction of one single chemical component of a tree ring. Thereby it allows researchers to circumvent the biases that potentially could result from a changing composition (i.e. in the cellulose/lignin ratio) when analysing bulk wood since different components exhibit significantly different isotope compositions (Borella et al., 1998, 1999).

The α-cellulose is the dominant component in tree -rings and its extraction follows a standardized procedure (Boettger et al., 2007). The woody annual growth layers of trees, i.e. the tree rings, are composed of lignin (phenolic polymer), hemicellulose (heterogenous polysaccharides) and cellulose (β-1,4 glucan) in a ratio of 1:1:2 (Freudenberg, 1965; Hu et al., 1999). Thereof, the part of cellulosic material which is insoluble in a 17% sodium hydroxide (NaOH) solution is defined as α-cellulose (Burton and Rasch, 1931; Cross and Bevan, 1912). The various tree-ring components are known to differ in their isotopic composition, whereas α-cellulose reveals to be the most stable component as the polymer is of long-term stability (Boettger et al., 2007; Leavitt and Danzer, 1993; McCarroll and Loader, 2004).

The quantity of the α-cellulose content (hereinafter referred to as CC [%]), generally calculated from the dry weight and the α-cellulose weight of a wood sample, is usually determined and used as a tool to test the quality of the cellulose extraction and the purity of the cellulose (Burton and Rasch, 1931).

However, variations in the amount of α-cellulose and the determined CC [%] (also: cellulose yield; used rather simultaneously in literature) are scarcely mentioned in dendroclimatological literature. Merely few publications provide numbers on the mean extracted CC [%] (Cullen and Macfarlane, 2005; Gaudinski et al., 2005; Leavitt and Danzer, 1993; Loader et al., 1997). While reported results on the α-CC [%] are scarce in literature, the importance of the CC [%] calculation as a tool for the determination of degradation state in subfossil wood is addressed within several studies (Brenninkmeijer, 1983; Loader et al., 2003 and references therein; Schleser et al., 1999).

Schleser et al. (1999) point out that the determination of isotopic ratios in cellulose from woody plant tissues often assumes constancy in the CC [%], which may hold true for modern samples from living trees. However, samples from subfossil or fossil wood have presumably experienced degradation processes with potential influence on the α-CC [%]. The artificial degradation of samples revealed a discrimination of $\delta^{13}C$ values up to 0.3‰ with decreasing CC [%], where the change in degradation behaviorbehaviour over time splits into initially increased degradation of cellulose with a strong depletion in $\delta^{13}C$, followed by an inverse effect comprising an enrichment in $\delta^{13}C$ with advancing degradation (Schleser et al., 1999).

Loader et al. (2003) discusses the use of whole wood versus α-cellulose in subfossil tree samples as the various wood components (lignin, hemicelluloses, cellulose) experience variable degrees of decomposition changing the ratio of the individual components within a tree ring. The polysaccharide components, i.e. cellulose and hemicelluloses, decompose more rapidly than lignin, which leads to an altered ratio of cellulose and lignin in whole wood samples which in turn modifies the isotopic composition of whole wood samples (Borella et al., 1998; Loader et al., 2003; Schleser et al., 1999). Therefore, cellulose is the preferred wood component in subfossil wood studies; however, the effect of partial decay of cellulose on the isotopic signature of tree rings and its implications for paleoclimate reconstructions is insufficiently investigated, although results from naturally degraded subfossil wood show no evidence for a change in the isotopic composition of cellulose due to degradation (Loader et al., 2003).

While studies on CC [%] and its implication for dendroclimatological studies are scarce, even less is known on the influence of environmental conditions on the annual variability of the ~~α-~~CC [%] (Genet et al., 2011). For instance, ~~(~~Gindl et al.~~,~~ (2000) discussed lignin content as a temperature proxy for the late growing season (Sept-Oct). As lignin and cellulose are among the major components of a tree ring, and changes in one component will affect the cellulose/lignin ratio, a link between CC [%] and temperature can be expected as well. However, the study of Gindl et al. (2000) covers only a short time period of 10 years and the method used is rather time-consuming, whereas the production of CC [%] is often a by-product during the extraction of α-cellulose for stable isotope analysis in tree rings. A study on CC [%] in roots revealed influences of topography and soil moisture status on root CC [%] (Hales et al., 2009). However, variations in CC [%] over time may as well be related to tree metabolism and the availability in non-structural carbon (NSC), which occurs in the form of starch and sugars, where the sugars provide the nutrients for cell wall components such as cellulose (Haigler et al., 2001; Hoch et al., 2002). Studies on NSC concentrations at the Alpine tree-line provide evidence that trees growing in the tree-line ecotone are not depleted in carbon; on the contrary, NSC concentrations are found to increase with elevation, implying that the limitation of tree growth at the upper tree-line does not result from insufficient nutrition, but rather thermal conditions, i.e. temperature, which limits the sink activity and thereby growth (Hoch et al., 2002; Hoch and Körner, 2003, 2009, 2012; Körner, 2003). Applied to the CC [%] of a tree ring, it can be hypothesized that temperature acts as the main control, as it is steering the sink activity, i.e. the tissue formation.

This manuscript presents results of a pilot study, where time series of modern and subfossil tree-~~-~~ring cellulose are established, allowing a novel insight into a tree-ring component which is commonly used in dendroclimatology, but hardly examined itself in its variability over time and in the factors driving its variability. The current study is embedded in the~~a~~ project *Alpine Holocene Tree Ring Isotope Records* (AHTRIR) which aims at reconstructing Holocene climate variability using a multi-proxy approach for the past 9000 years. Therefore, tree-ring material consists only partly of living wood material; the by far largest part is based on findings of Holocene wood remains from glacier forefields, peat bogs and small lakes in the central European Alps. Initially, the determination of CC [%] was used to determine the quality of the cellulose extraction before performing analysis of triple isotopes (δ^2H, $\delta^{18}O$, $\delta^{13}C$) on the tree-ring cellulose. However, ~~I~~it offers the unique opportunity to investigate the ~~α-~~CC [%] and its variations in long-living trees from two high-Alpine coniferous tree species (*Larix decidua* Mill. and *Pinus cembra* L.) in Holocene wood remains found in glacier forefields and peat bogs in the European Alps as well as in modern trees sampled at the current Alpine tree line. The case study aims at (i) identifying common trends in time series of ~~α-~~CC [%] in tree rings from Early- and Mid-Holocene subfossil and modern wood material; (ii) investigating dependencies of CC [%] in relation to their sampling site (latitude, longitude, elevation), their age and the species; (iii) ~~analyzing~~analysing climate-CC [%] relationships and finally (iv) determining the ability to utilize CC [%] as a supplementary proxy in dendroclimatological reconstructions.

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2 Material and methods

2.1 Samples and sampling sites

Holocene wood remains and modern tree-ring material are sampled with the intent to reconstruct Holocene climate variability in a multi-proxy approach. Thereby, samples from two Alpine tree-line species, namely the Swiss stone pine (*Pinus cembra* L., PICE) and the European larch (*Larix decidua* Mill., LADE) are collected as they represent the typical tree-line species present at modern sampling sites in form of living trees, and in glacier forefields and peat bogs as subfossil wood remains (Joerin et al., 2008; Nicolussi, et al., 2009; Nicolussi and Patzelt, 2000a). The sampling sites are located along a SW-NE transect in the Central European Alps (46°02'N - 47°03'N, 7°33'E - 12°15' E; Fig. 1, Table 1 and 2). The modern tree-ring material has been cored at three upper tree-line sites close ~~in the proximity of~~to glacier forefield sites during fieldwork in 2015 (Figure 2, Table 1). At each site, four trees per species have been cored; however, at the sites UAZR and FPCR, only one of the two species is present. Cores are taken at breast height from three~~four~~ radial sections; two in parallel to the slope, ~~one up~~ and one up~~down~~-slope, thereby avoiding any kind of compression wood on the down-slope side of the tree.~~.~~ The Holocene wood remains stem from glacier forefields, peat bogs and small lakes, which have been continuously collected over the last two decades (Joerin et al., 2006, 2008; Nicolussi, et al., 2009; Nicolussi et al., 2005; Nicolussi and Patzelt, 2000b). Wood material of the EACC (Eastern Alpine Conifer Chronology) has been merged and updated with subfossil samples of the same species and altitude collected by continued sampling of wood remains and stem discs in glacier forefields (Nicolussi and Schlüchter, 2012). Similar to modern wood samples, the use of compression wood in these Holocene samples was avoided during the further analysis.

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2.2 Tree ring width and dating

Total tree-ring width~~s~~ (TRW) is measured for all samples. At least two radii are measured per tree with a precision of 0.001 mm by using a LINTAB device in connection to TSAPWin software (Rinn, 1996). For each sample, a mean TRW series is established by crossdating and averaging the radii per tree. In case the inner part of the stem is missing in a tree-ring series, the tree-ring number between the pith and the first tree ~~-~~ring measured is estimated (pith-offset (PO), Table 3). For the modern series, dating is simple as the exact sampling date is known. For the Holocene wood remains, the mean series are compared to local tree-ring series and reference chronologies (Nicolussi, et al., 2009; Nicolussi and Schlüchter, 2012). Crossdating is achieved by calculating statistical parameters, and applying visual controls.

2.3 Sample preparation

Samples for CC [%] analysis are prepared as 5-year tree-ring blocks per tree, thus no pooling between trees is applied. For the Holocene samples, radial wedges are cut out of stem disks by using a bandsaw. Subsequently, blocks are prepared from the wedges and the cores under the microscope by using a commercial scalpel. The dry weight of each block is scaled and is defined to be between 20 mg and 50 mg, as a 60 % weight loss is expected during α-cellulose extraction. Further, the incipient degradation in samples might have additionally reduced the CC [%] in the samples. The blocks are further ~~sliced~~ cut into smallest slices of wood material by first slicing in parallel to the ~~fiber~~fibre direction, and subsequently against the ~~fiber~~fibre direction. The resulting size of the cut sample is < 1 mm. Again, the dry weight of the cut sample is scaled to estimate the loss during the cutting procedure. For the following chemical treatment, the samples are packed into ANKOM Filter Bags (Type F57), heat-sealed and labelled individually.

2.4 Cellulose extraction

The cellulose extraction is based on a modified Jayme-Wise procedure (Boettger et al., 2007; Borella et al., 1999; Leuenberger, 1998). The bleaching (delignification) of samples in a 1 % $NaClO_2$ + CH_3COOH solution with a pH value of 4 is conducted at 70 °C for at least 30 hours to remove lignin. In a second step, samples are treated in a 17 % NaOH solution at 25 °C for 45 minutes to extract the pure α-cellulose (Burton and Rasch, 1931; Cross and Bevan, 1912). Subsequently, samples are neutralized in a 1 % HCl solution using 25 % HCl and washed intensely with heated and cold distilled water until the pH of the washing water is ~ 7. Samples are dried in an oven at 50 °C; the duration of the drying is thereby dependent on the sample size in the individual filter bags.

2.5 Calculation of CC [%]

The CC [%] is ~~usually~~ calculated from the dry weight of wood and the α-cellulose weight of a~~the~~ sample:

$$CC\ [\%] = \left(\frac{\alpha\text{−cellulose weight}}{\text{wood dry weight}}\right) \times 100\% \quad (1)$$

~~In order to optimize the approximation of the CC [%], the current study includes the weighting of 1. dry weight, 2. dry weight and α-cellulose weight, whereas the 1. dry weight defines the weight of the 5-year tree ring block, the 2. dry weight the weight of the wood sample after cutting. Thereby, the potential loss during the cutting process is determined and considered in the CC [%] calculation resulting in a precise estimation:~~

$$CC\ [\%]' = \left(\frac{\text{cellulose weight}}{\text{2. dry weight}}\right) \times 100\% \quad (2)$$

The wood dry weight is thereby defined as the sample weight of the individual wood sample after cutting, and the cellulose weight refers to the weight of the extracted α-cellulose after being removed from the ANKOM filter bag.

A remaining source of error is the collection of dry α-cellulose from the ANKOM filter bags. The described slicing of wood samples facilitates the removal of α-cellulose from filter bags compared to ground wood samples and thereby reduces the loss of α-cellulose material when being collected from the filter bags. In this study, ~~T~~the resulting loss is examined for 42 samples, where the filter bag including the α-cellulose and the emptied filter bag are scaled in addition to the α-cellulose weight to give an accurate estimate of the observed loss.

2.6 Outlier detection and correction

Outliers in CC [%] are observed for Holocene wood remains, where the outermost rings have experienced a higher degree of degradation, e.g. due to abrasion at the glacier sites or the exposition of wood to environmental influences and associated weathering of the sample. The differential grades of degradation lead to pronounced decreases in CC [%], appearing within the outermost rings, but also along cracks between individual rings and seldom on the innermost parts of trees, which complicate the comparison of individual time series. Therefore, detection and elimination of outliers is performed by visual and statistical application of boxplots (Tukey, 1977).

Tukey (1977) defines outliers as samples, which lie outside the whiskers, whereby the extension of the whisker is limited by the 1,5 inner-quartile range (IQR). In addition to the boxplots, visual comparisons of suspicious samples with other cellulose series is applied; thereby outliers close to the whiskers revealed to be true values and the loss of samples by outlier detection and elimination could be minimized to the most extreme values.

2.7 Meteorological data

CC [%] series of modern sampling sites are correlated with meteorological data from the HISTALP database to investigate the relationship between climate and CC [%] (Auer et al., 2007). Data is obtained in the form of Coarse Resolution Subregional Means (CRSM), which are calculated as arithmetic means from homogenized individual station series for five defined subregions within the Greater Alpine Region (GAR), which have been identified by EOF-based regionalization. Auer et al. (2007) recommend CRSM-series especially for lower frequency analysis for all climate parameters. Series from the sampling sites FPCR and UAZR are correlated to meteorological data from the NW subregion, and from VRR to data from the SW region, respectively, due to their geographical location.

For these subregions, monthly anomalies (calculated for the reference period 1961-1990) of temperature (T [°C], 1760-2008), precipitation (prec [mm], 1800-2008), cloud cover (cloud [%], 1840-2008) and sunshine (sun [h], 1880-2008), including the arithmetic seasonal (MAM, JJA, SON, DJF), semi-annual (AMJJAS, ONDJFM) and annual means are extracted. For further analysis, 5-year mean values are calculated for the annually resolved climate data as CC [%] series were established in 5-year resolution and which thereby integrate the mean climatic signal over five consecutive years.

Pearson's correlation coefficient is calculated between the mean CC [%] series per site and species, and the monthly, seasonal and annual values for the four climate variables for the time period 1865-2008 (1880-2008 in case of sunshine). 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.

3 Results

As the precise calculation of the CC [%] depends on error estimates for the dry weight of samples and the cellulose weight, two steps in the calculation of CC [%] are here improved: the precision of dry weight and the loss estimation in cellulose weight.

The mean sample loss during the cutting process amounts to 2.6 ± 1.7 % of the dry weight of an individual sample, where mean loss values range from 0.3 % up to 11.1 % per tree (data not shown here). Besides, very few individual samples experienced losses between 10 % and 30 % caused by wood pieces bouncing off during cutting or loss of material due to powdery wood substance as a result of degradation. Hence, for the precise calculation of CC [%], a determination of sample weight after cutting or milling is essential.

Second, when unpacking the cellulose material from the filter bags, there is always a risk that smallest fibres remain in the filter bag or fly off during the removal; therefore, we assume this error to be systematic. In this study, we estimated this error by analysing 42 filter bags, which revealed

~~Second, a further reduction of error is achieved by the estimation of α-cellulose loss when the extracted cellulose is removed from the ANKOM filter bags, as there is the potential of small fibers getting caught in the filter bag. The investigation of 42 filter bags and the contained CC [%] revealed~~ a mean loss of α-cellulose of 0.378 ±

0.163 mg (3.2 ± 1.4 %) (Table 4). Therefore, the calculation of the CC [%] results in the minimum CC [%] of the sample, as losses of cellulose in the order of 3.2 ± 1.4 % of the cellulose weight are to be expected, which would result in slightly increased CC [%] values. However, as the unpacking is accomplished equally for all samples, a systematic error is assumed which affects the CC [%] calculation in the same manner. It can be assumed that the systematic error does not influence the variability of the individual CC [%] series.

Further, the relative uncertainty in cellulose weight transfers directly to the relative uncertainty in CC [%] determination as relative uncertainties are additive, with the uncertainty of the dry weight after cutting being 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 minor differences between the individual 5-year CC [%] samples, it does not limit the investigation of trends in CC [%] series.

The improvement~~s~~ in the CC [%] calculation and the estimation of the relative error justify the use of the term "CC [%]" rather than "cellulose yield", as sources of error are reduced and well estimated, and resulting calculations are close to the true values.

3.1 Modern CC [%] series

For a better understanding of CC [%] variations, modern CC [%] series are ~~analyzed~~analysed for~~in~~ their temporal variability per species and site, where the absolute values and variations in CC [%] are given in the unit CC%. Trees sampled at UAZR consist solely of PICE samples, as the tree-line at that site is only composed of one tree species. Measured tree-ring width series vary in length between 150 and 205 years; the pith offset estimation varies between 32 and 82 for the individual trees (Table 3). The mean segment length (MSL) amounts to 173 years and the period covered by all four samples spans from 1860 to 2014 A.D. (Table 1). The mean cellulose content per tree varies between 31.6 ~~%~~CC% and 39.7 ~~%~~CC%, where UAZR-1 (33.2 ~~%~~CC%) and UAZR-2 (33.2 ~~%~~CC%) show distinctly lower mean CC [%] values as UAZR-3 (38.6 ~~%~~CC%) and UAZR-4 (39.7 ~~%~~CC%). 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). Further, the time series of CC [%] for UAZR -1 and -2 show more variability in their series compared to UAZR-3 and -4, which show a high common variability and merely small fluctuation in their CC [%]. This is further confirmed by the calculated Pearson's correlation coefficient (r) with highest values for UAZR-3 and -4 (r=0.64) (Table 6). A significant correlation is also found between UAZR-2 and -3 (r=0.36), whereas the remaining correlation coefficients are non-significant ($p > 0.05$). The arithmetic mean calculated from four samples in their common period (1860-2015 AD) indicates an increase in CC [%] over time by ~ 5 ~~%~~CC% (red line, Fig. 3, top left).

In contrast to UAZR, the sampling site FPCR consists solely of LADE trees, where tree~~-~~-ring series contain between 155 and 335 years; the MSL amounts to 208 years, raised by the sample FPCR-1 (335 years), whereas the residual tree-ring series consist of 155 and 170 years (Table 1, Table 3). The four tree samples cover a common period from 1860 to 2015 A.D. (Table 1). The mean CC [%] per tree varies between 28.3 ~~%~~CC% and 32.9 ~~%~~CC%, showing overall lower mean CC [%] values than observed for the PICE trees at UAZR (Table 5). Further, the range of CC [%] per individual tree is large, as LADE trees show low minimum CC [%] values between 15.2 ~~%~~CC% and 21.8 ~~%~~CC% as well as high maximum values of up to 51.6 ~~%~~CC% (Table 5). In their common period, trees at FPCR show a decrease in CC [%] from 1860 to 1945 A.D. and a strong increase of nearly 20 ~~%~~CC% from 1980 to 2014 A.D, which is common among the individual trees (Fig. 3, top right).

Pearson's correlation coefficient illustrates the common variability among the samples FPCR-1, -5 and -6, showing high correlation coefficient between 0.67 and 0.79, whereas non-significant and lower correlation coefficients are found for FPCR-3 (Table 6).

The sampling site VRR located in the Val Roseg in Eastern Switzerland, and in the proximity of the Tschierva glacier, is the only sampling site where both tree species, LADE and PICE are found, thereby allowing a direct comparison of species under same climatic growing conditions.

PICE trees at VRR exhibit tree-ring series of 170 to 255 years, with a common period between 1845 and 2015 A.D. and a MSL of 209 years (Table 1, Table 3). Mean CC [%] varies between 32.9 ~~%~~CC% and 37.2 ~~%~~CC%, where the samples VRR-1 and VRR-1-2 (same tree) reveal a range of > 20~~%~~CC%, whereas the other PICE trees at VRR exhibit sizes between 13 ~~%~~CC% and 16.3 ~~%~~CC% (Table 5). Minimum values are around 20 ~~%~~CC% for VRR-1 (and 1-2) and around 30 ~~%~~CC% for all the other trees, and maximum values exceed 40 ~~%~~CC% with maximum values > 45 ~~%~~CC% (Table 5). Calculated correlation coefficients are almost all significant ($p < 0.05$) and are between 0.41 and 0.75 (Table 6). The arithmetic mean series for VRR-PICE displays an increase in mean ~~cellulose content~~CC [%] of > 10 ~~%~~CC% in the common period (Fig. 3, bottom left).

The LADE tree-ring series at the same location, with a common growth period between 1865 and 2015 A.D. and MSL of 219 years, consist of two times 150 years, 190 and 385 years (Table 1, Table 3). Mean CC [%] is between 26.3 ~~%~~CC% and 36.0 ~~%~~CC% with minimum values ranging from 16.3~~%~~CC% to 30.4 ~~%~~CC%, whereas the maximum values per tree show a lower spread between 39.4 ~~%~~CC% and 46.3 ~~%~~CC% (Table 5). As for PICE, LADE CC [%] series reveal significant correlation coefficients among the individual trees (Table 6). The arithmetic mean series shows a decrease in CC [%] of approximately 5~~%~~CC% in the period 1865-1980 A.D., followed by a strong increase of more than 10 ~~%~~CC% during the recent three decades (Fig. 3, bottom right).

The investigation of individual tree-ring CC [%] series from three sampling sites (UAZR, FPCR, VRR) and two coniferous tree species (PICE, LADE) display common variability of trees from the same species, independent from the sampling site. Individual CC [%] per site and species show in general good agreement, represented in their correlation coefficients, which allows the establishment of arithmetic mean chronologies for their common growth periods (comparable time periods for all sites).

Common trends in the tree species, independent of the sampling site, indicate a common influence from environmental factors on CC [%]. All PICE series reveal a continuous increase of CC [%] over time, whereas the LADE samples show a decrease over the common period, replaced by a rapid increase over the past three decades (Fig. 4, left). The close relationship between the site series of the same tree species is also revealed by the calculated Pearson's correlation coefficient, where $r=0.86$ for LADE and $r=0.76$ for PICE mean series.

3.2 Climate-cellulose relationships

To test the influence of environmental conditions on the CC [%], Pearson's correlation coefficient is calculated between the mean CC [%] series per site and species and the climate variables temperature (cf. Fig 4, right), precipitation, sunshine as well as cloud cover (Fig. 5-6, Fig. S1-2).

A temperature signal is clearly recorded in both tree species, independent from the sampling site (Fig. 5). Both PICE sites show significant correlations with temperature throughout the year, with high correlation during the growing season and interestingly in October ($r=0.67$ for UAZR-PICE, $r=0.65$ for VRR-PICE). Seasonal temperature averages result in highest correlations for PICE; correlations for the summer season (JJA) result in $r=0.68$-0.71 and for autumn season (SON) in $r=0.72$-0.75 for UAZR-PICE and VRR-PICE, respectively. In

LADE, correlations with temperature are lower than for PICE and significant correlations are found mostly during the growing season (May-August). Seasonal averages in temperature anomalies result in highest correlations for the summer season (JJA) for both FPCR-LADE (r=0.55) and VRR-LADE (r=0.69).

Precipitation is best recorded in PICE at UAZR during the early growing season, revealing highest correlation coefficients in May (r=0.68). In contrast, LADE mean series at both sites and PICE at VRR mostly show non-significant correlations with precipitation records (Fig. 6).

The influence of cloud cover is highest in PICE trees during the early as well as towards the end of the growing season (Fig. S1). Significant positive correlations are found in MAM for UAZR-PICE (r=0.49), and for JJA in both UAZR-PICE (r=0.41) and VRR-PICE (r=0.42). Interestingly, the correlation coefficient for cloud cover and LADE mean series reveals significant negative correlations during the winter season (DJF, r=-0.56 (FPCR-LADE), r=-0.63 (VRR-LADE)), especially in January for FPCR-LADE (r=-0.57) and in February for VRR-LADE (r=-0.46).

High, significant correlations with sunshine are observed for the late autumn and winter season, whereas negative correlations are found during summer season, being significant at both PICE sites in August, where VRR-PICE reveals significant negative correlations with sunshine from May to August (Fig. S2). For UAZR-PICE, highest correlation with sunshine are found in January (r=0.86) as well as in February (r=0.76) and the winter season (DJF, r=0.87), whereas for VRR-PICE, highest correlations are found for November and December (r=0.84 and r=0.82, respectively) as well as for the winter season (r=0.79). The pattern is similar for FPCR; significant positive correlations occur in late autumn and winter (N, D, J, FD) and are highest in January (r=0.62). In contrast, VRR-LADE shows highest correlations in late autumn (Nov) and in February (r=0.66 and r=0.55, respectively).

In summary, the results of the Pearson's correlation coefficient analysis between HISTALP data and mean CC [%] per species and site indicate partly significant influence of environmental factors (temperature, precipitation, cloud cover, sunshine duration) on the CC [%] in tree rings and the potential to reconstruct past climate from this novel supplementary proxy. However, the comparison of the two coniferous species further indicates a species-specific response of CC [%] on the environmental conditions.

3.3 Holocene CC [%] series

In a further step, CC [%] series are established for LADE and PICE tree-–ring samples from glacier forefields, peat bogs and small lakes for the period from 8550 to 3500 years b2k (Fig. 7). To investigate trends in the CC [%], the individual series are averaged per species (PICE in blue, LADE in green) and an arithmetic mean is also calculated over all series (black), which is smoothed by a spline (orange) to illustrate long-term changes (Fig. 7). The arithmetic mean series shows the high variability in the CC [%] over time, fluctuating between 22 %CC% and 40 %CC%. Further, the series exhibits interesting low--frequency trends with rapidly decreasing CC [%] in the periods 8250-7950 years b2k (Δ CC [%] ~ 5 %CC%), 6250-5950 years b2k (Δ CC [%] ~ 2 %CC%), 5650-5450 years b2k (Δ CC [%] ~ 4 %CC%), 5300-5000 years b2k (Δ CC [%] ~ 5.5 %CC%) and 4500-4000 years b2k (Δ CC [%] ~ 6 %CC%). Besides these phases of rapid decreases, CC [%] is increasing on a multi-centennial scale between 7350 and 6250 years b2k (Δ CC [%] ~ 4.5 %CC%) and more rapidly after low CC [%] phases, e.g. in the periods 7950-7750 years b2k (Δ CC [%] ~ 3.5 %CC%), 5450-5300 years b2k (Δ CC [%] ~ 7 %CC%) and 4000-3650 years b2k (Δ CC [%] ~ 8.5 %CC%). The calculated arithmetic mean series per species exhibit mostly lower mean values in the LADE series (green) and more positive values for PICE (blue). LADE shows extremely low CC [%] between 4700 and 4200 years b2k, and extreme high values between 4000 and 3900 years b2k. The PICE average series shows extremely low values around 8000 years b2k as well as around 4000 years b2k; otherwise both the LADE and PICE series mostly fluctuate between 30 ~~%~~CC% and 40 ~~%~~CC%.

For most of the investigated time period, the sample replication amounts to 3 trees; two gaps at 7300 and 6300 years b2k are solely covered by a single tree; partly a replication of ≥ 4 trees is achieved (Fig. 7, bottom).

Fig. 7 further displays identified cold phases (light-blue bars) ~~analyzed~~analysed by Wanner et al. (2011), where the darker bars illustrate the cold phases which were identified by three different phenomena (cold phases, glacier advances, Bond cycles) and the lighter areas mark the entire length of the cold phases; additional cold periods are marked as blue vertical lines (Wanner et al., 2011, 2015). Interestingly, the strong decreases in cellulose content emerge mostly after the indicated cold phases.

As modern wood samples already displayed differential trends in the CC [%], mean series of LADE and PICE and their low-frequency trends are investigated closer in Fig. S3, where the contribution of individual trees per species is revealed in the sample replication (Fig. S3 (d)). The mean series reveal distinct offsets over time (Fig. S3 (a)). In the periods 8100-7800 years b2k and 5600-4600 years b2k, the smoothed values exhibit a common long-term trend, whereas in the remnant phases, series even show opposed trends. The difference between the two arithmetic mean series emphasizes that the differences between the species are~~is~~ not constant over time (Fig. S3 (b)).

Although the Holocene samples stem from various sites, the phases 8900-8200 years b2k and 7500-6700 years b2k are dominated by glacier forefield sites at Mont Miné and Tschierva glacier, respectively (Fig. S3 (c)). For both intervals, the two coniferous species are present, and as the sampling site is equal, similar as well as divergent trends in the species result presumably from the impact of environmental factors on CC [%].

In order to confirm the non-existing influence of the sampling site location on CC [%] series also in Holocene samples, the arithmetic mean of CC [%] per site and species is calculated and compared to latitude, longitude and elevation of the individual sites (Fig. S4). Calculated linear regressions (dotted lines) per species do not indicate a dependence of CC [%] and sampling site locations, as linear regressions are non-significant ($p > 0.05$).

To further exclude non-climatic influences on CC [%], individual CC [%] series per tree are aligned according to their biological age by taking the estimated pith~~-~~offset into account (Fig. S5). Both PICE (upper panel) and LADE (lower panel) do not reveal any distinct low-frequency trend in the age-aligned series.

4 Discussion

The determination of ~~α-~~CC [%] is a common procedure in tree-ring laboratories that measure stable isotope ratios in tree-ring cellulose. However, to our current knowledge, existing records of CC [%] were simply used as a tool to ascertain the quality of the extracted cellulose; the CC [%] is, if mentioned in literature, given as a mean value and its temporal variability is not regarded any further.

The investigation of CC [%] in modern tree samples of two high-Alpine coniferous species (LADE, PICE) at three sites in the Swiss Alps reveals common variations ~~in the CC [%]~~ within the tree species, independent of the sampling site, pointing out a common environmental driver. While PICE trees show a continuous increase over the investigated common period, ~~larch~~ LADE trees are characterized by a decrease throughout the 20th century followed by a strong increase in CC [%] from the 1980s until today.

In order to exclude any age-related biases, possible age trends in CC [%] series have been investigated, even though they were not expected as the trends in modern CC [%] series of the two tree species diverge and do not reveal a common increase/ decrease over time. The age-alignment of both modern and Holocene 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 (Fig. S5).

Further, the two different species *Larix decidua* Mill. and *Pinus cembra* L. at the sampling site Val Roseg (VRR) exhibit different trends in their mean chronologies. As they experience the same climate, the role of the biological factors here is undoubted. This is rather obvious as two coniferous species are compared, where *Larix decidua* Mill. is a deciduous species, whereas *Pinus cembra* L. is an evergreen species; therefore, differences in their metabolism are to be expected. Although the two species are both found at the upper tree-line and known to be adapted to the harsh environmental conditions, LADE is characterized as the light-demanding pioneer species which is often found in open settings, e.g. on glacier forefields, whereas PICE is, under undisturbed conditions, the highest rising species in the inner sections of the Alps and with that adapted to short vegetation periods (Ellenberg, 1996). The fact that individual CC [%] series from the same species at different sites are similar calls for a common driving factor of regional extent, such as temperature.

~~Although the two species are both found at the upper tree-line and known to be adapted to the harsh environmental conditions, LADE is characterized as the light-demanding pioneer species which is often found in open settings, e.g. on glacier forefields, whereas PICE is, under undisturbed conditions, the highest rising species in the inner sections of the Alps and with that adapted to short vegetation periods (Ellenberg, 1996). Further, both species are coniferous; however, LADE trees are also deciduous, as they lose their needles at the end of the growing season. Therefore, differences in the tree metabolism are to be expected.~~

The investigation of the climate-cellulose relationships by correlating mean CC [%] series with climate variables (temperature, precipitation, cloud cover, sunshine duration) extracted from the HISTALP database (Auer et al., 2007) over a common period of 140 years (1865-2008 A.D.; 1880-2008 for sunshine) reveals interesting correlation coefficients between CC [%] and climate. Temperature is recorded in both species, revealing highest correlations during summer season, and additionally for the autumn season in PICE trees. An increase in CC [%] in trees with increasing temperatures may be explained by the enhanced sink activity in trees at the upper tree-line. In the attempt to establish a functional explanation of the Alpine tree-line, Körner (1998) compiled five hypotheses, where the climate-driven tree-line is either driven by stress, disturbance, reproduction, carbon balance or growth limitation (Körner, 1998). Since then, several publications have shown that concentrations of non-structural carbon (NSC) in trees at the upper tree-line increase with elevation, thereby indicating no evidence that trees at high elevations experience a carbon shortage, but rather implying a lowered sink activity at the upper tree-line resulting in a growth-limitation (Hoch et al., 2002; Hoch and Körner, 2009, 2012; Körner, 2003). As photosynthetic activity is less sensitive to cold temperatures than growth, it may still be active at 0 °C, where temperatures are too cold for a tree to grow (minimum temperatures for tree growth are ~ 5-7 °C) (Hoch and Körner, 2012; Körner, 1998; Tranquilini, 1979). Therefore, the carbon~~C~~ acquisition in a tree can still be active, although low temperatures prevent the tissue formation. In consequence, the concentration of NSC in trees at the upper tree-line is increasing, as the source is still active, but the sink is not. In conclusion, colder temperatures during the growing season reduce the tissue formation in tree rings, resulting in ~~a~~ less CC [%] in the tree ring as the source activity is low. Regarding the investigated species in this study, the LADE as a pioneer species, exploring the upper tree-line, highly benefits from the increase in temperature associated with global warming and which is especially pronounced since the 1980s (Rebetez and Reinhard, 2008). As temperatures during the growing season increase and the growing season is potentially prolonged, the sink activity and the CC [%] in tree rings are~~is~~ increased. This might explain the found correlations with winter temperatures especially for PICE trees, i.e. potential photosynthetic activity at low temperatures in winter, where tissue formation is no longer possible (Hoch et al., 2002 and references therein). Thereby, the concentration of NSC is increasing and is 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.

In PICE trees, the ~~cellulose constant~~CC [%] is slowly and steadily increasing over time, potentially reacting more slowly to increased temperatures, prolonged growing seasons and rising mean annual temperatures. In contrast, LADE series from modern trees exhibit a rapid increase over the past 30 years, obviously benefitting from increased temperatures during the growing season. The CC [%] in Holocene wood remains and modern wood samples could therefore serve as a novel additional proxy in multi-proxy approaches, offering the opportunity to test the temperature sensitivity of diverse tree species, before combining them into chronologies. A combination of differently reacting tree species allows a complementation of temperature-CC [%] relationships, which may result in more robust climate reconstructions.

Moreover, the PICE trees at two modern sampling sites showed highly significant correlation coefficients with sunshine duration especially during the winter season. As evergreen conifers are known to be photosynthetically active throughout the year as long as temperatures permit photosynthetic activity (Hoch et al., 2002 and references therein), PICE trees may potentially benefit from increased sunshine duration and be able to increase the carbon acquisition during the winter season. Therefore, the CC [%] in PICE trees may even represent an annual signal. On the other hand, significant correlations found for sunshine duration and cloud cover with LADE CC [%] series may represent artefacts, as LADE trees lose their needles in autumn and carbon acquisition is only possible during the growing season.

As the framework of the project AHTRIR included both the analysis of living and subfossil wood, Holocene wood remains were also investigated for signs of degradation. Most samples were well-preserved, and for the period from 9,000 to 3,500 years b2k, the CC [%] also varied between 30-40 CC%. Therefore, the CC [%] in living and subfossil wood samples is comparable. Only a small number of outliers was found (see also 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 (Fig. S6). Although the potential degradation of subfossil wood might have an impact, CC [%] of modern and subfossil wood is comparable despite a few outliers, which leads to the conclusion that long-term variations in Holocene CC [%] could serve as an indicator of climate variations. Moreover, there is no trend detected in CC [%] over time (i.e. towards the past) which would be expected in case degradation would have been a major driver of CC [%] variations.

The low-frequency trends exhibited in the mean series of Holocene ~~α-~~CC [%] in the period from 9000-3500 years b2k illustrate the potential of CC [%] as an additional proxy. The arithmetic mean CC [%] series shows pronounced decreases after known cold events in the Early and Mid-Holocene, whereas a continuous increase is observed between 7350 and 6250 years b2k, which could be the result of increased temperatures and more favorable growing conditions for trees at the upper tree-line. However, the investigation of the individual species also illustrates differences in variations between LADE and PICE approving the observed differences in species within modern samples. A complete understanding of CC [%] variations in ~~on~~different tree species and the influence of environmental conditions on CC [%] will help to further improve the robustness of this novel proxy. The presented study could benefit, but was at the same time limited by the framework of the project in which it was performed: 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 number 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 α-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, we evaluated the natural variability of CC [%] on different LADE tree-ring cores over time (Fig. S7, S8). 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 if the values of the different cores would be adjusted according to their mean values. Therefore, we are confident that replicates of LADE samples of the present study would be the same within a few couple of percent (approx. 3 to 4 %). In the current study, first measures to minimize and quantify the error of CC [%] have been presented; however, in future studies, it will be essential to accomplish a robust error estimation by a replicate sampling of the same tree.

5 Conclusion and outlook

For the first time, ~~α-~~CC [%] and its temporal variability of the two coniferous Alpine tree-line species *Larix decidua* Mill. (European larch, deciduous) and *Pinus cembra* L. (Swiss stone pine, evergreen) have been investigated on a centennial scale for modern samples from living trees and on a multi-millennial scale for the Early and Mid-Holocene including the transition to the Late Holocene. The established CC [%] series revealed species-specific long-term trends, independent of the location of the sampling site along the Swiss Alps in both past and present tree samples. First investigations of climate-cellulose relationships display the ability of CC [%] to record temperature at the Alpine tree-line.

As higher ~~α-~~CC [%] values have been observed for low-elevation tree-ring material (unpublished data), the influence of elevation ought to be examined along an altitudinal gradient to quantify the decrease in α-cellulose and thereby verify the hypothesis that not carbon, but temperature is the limiting factor resulting in limited sink activity which results in turn in lower CC [%] values. This goes along the line of non-structural carbon investigations.

In a succeeding study, the site- and species-sensitivity of multiple proxies (TRW, ~~CC%~~CC [%], δD, δ^{13}C, δ^{18}O) and the correlations between the individual proxies will be examined in relation to the environmental conditions per site to quantify their ability for multi-millennial reconstructions based on a multi-proxy approach from tree rings (Ziehmer et al., in prep.). Thereby, the effects of variations in ~~α-~~CC [%] on triple isotope records will be tested.

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Moreover, ~~α-~~CC [%] series ought to be established in annual resolution to establish a robust calibration and verification of the CC [%]-climate relationship as the reduced resolution of our samples (5-year tree-ring blocks) prohibits the proper analysis of the climatic response of cellulose and a robust calibration against instrumental data. The existence of numberless CC [%] series in dendro-laboratories all over the world will allow a broad investigation of CC [%] in various species from wide-spread sampling sites in temporal resolution reaching from intra-annual to decadal tree-ring samples.

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 heartwood vs. sapwood. 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.

Further, the analysis of a relationship between CC [%] and wood density would be highly interesting. These two proxies are usually not investigated within the same research project. However, 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. Although the evaluation of CC [%] is obviously not easier than measuring tree-ring width, there is a significant potential of using it as an additional supplementary proxy, especially in those cases where CC [%] series are already existent in tree-ring laboratories and where climate is to be reconstructed in a multi-proxy approach.

A complete comprehension of environmental factors controlling the ~~α-~~CC [%] in diverse tree species will result in an improved understanding of physiological and biochemical processes in forming annual growth layers, and thus serve as an additional proxy in dendroclimatology. This study represents a first step into this direction and 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.

Data availability

At present, data can be obtained upon request. As agreed among the project participants, datasets will be made available to the public after the official completion of the Alpine Holocene Tree Ring Isotope Records (AHTRIR) project.

Author contribution

M. M. Z. and M. L. designed the study. The subfossil wood sampling was mainly done by K. N and C. S whereas the living wood sampling was performed by all authors. The preparation of the experiments as well as the statistical analysis and interpretation were carried out by M. M. Z. The drafting of the manuscript was accomplished by M. M. Z. with contributions from all co-authors.

Competing interest

The authors declare that they have no conflict of interest.

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site code		FPCR	UAZR	VRR
sampling site		Val d'Hérens, Mont Miné glacier	Haslital, Unteraar glacier, north shore Grimsel reservoir	Val Roseg
site character	site type	near timberline	near timberline	near timberline
	latitude °N	46°04' N	46°34' N	46°26' N
	longitude °E	7°33' E	8°17' E	9°51' E
	aspect	WSW	SSO	E
	elevation (m a.s.l.)	1930-2000	1968-1986	2127-2190
	number of trees	4	4	8
	period A.D.	1680-2015	1810-2015	1630-2015
	period A.D. with replication ≥ 4	1860-2015	1865-2015	1845-2015 (PICE) / 1865-2015 (LADE)
	mean segment length [years]	208	173	209 (PICE) / 219 (LADE)

Table 1. Site characteristics of modern sites. Coordinates, aspect and elevation describe their geographical distribution from west to east. Four trees are sampled per site and species, respectively, resulting in eight trees cored at VRR, where both species are present at the tree-line. Site characteristics are complemented by the maximum growth period and the period covered by a sample replication of four trees in years A.D. as well as the mean segment length (MSL) in years.

site code	sampling site	site character				
		site type	latitude °N	longitude °E	aspect	elevation (m a.s.l.)
ahmo	Ahrntal, Moaralm	peat bog	47°02' N	12°05' E	SE	1995
ahst	Ahrntal, Starklalm	peat bog	47°03' N	12°07' E	S	2080
bih	Paznaun, Bielerhöhe	peat bog	46°55' N	10°06' E	N	1930-2020
eba	Ötztal, Ebenalm	lake/peat bog	47°01' N	10°57' E	NO	2060-2170
g	Ötztal, Gurgler Zirbenwald	peat bog	46°51' N	11°01' E	NW	2060
gdm	Kaunertal, Daunmoränensee	lake/peat bog	46°53' N	10°43' E	O	2295
ggua	Ötztal, Gurgler Alm	peat bog	46°51' N	11°00' E	W	2150-2200
gli	Kaunertal, Ombrometer	peat bog	46°53' N	10°43' E	NO	2135-2160
gp	Kaunertal, Gepatschferner	glacier foreland	46°52' N	10°44' E	W	2060-2275
hib	Defereggental, Hirschbichl	lake/peat bog	46°54' N	12°15' E	E	2140
kofl	Ahrntal, Kofler Alm	peat bog	46°57' N	12°06' E	S	2165-2190
mazb	Vinschgau, Marzoneralm/B	peat bog	46°35' N	10°57' E	N	2125
mazc	Vinschgau, Marzoneralm/C	peat bog	46°35' N	10°57' E	N	2120
maze	Vinschgau, Marzoneralm/E	peat bog	46°35' N	10°57' E	N	2125
mm	Val d'Hérens, Glacier du Mont Mine	glacier foreland	46°02' N	7°33' E	NNE	1980-2010
mort	Morteratschgletscher	glacier foreland	46°25' N	9°56' E	W	2040-2050
rt	Rojental	lake/peat bog	46°48' N	10°28' E	SO	2400
tah	Passeier, Timmeltal	peat bog	46°54' N	11°08' E	S	1975-2260
tsc	Val Roseg, Tschiervagletscher	glacier foreland	46°24' N	9°53' E	NW	2115-2210
ua	Haslital, Unteraargletscher	glacier foreland	46°34' N	8°13' E	E	1950
ulfi	Ultental, Fiechtsee	lake/peat bog	46°28' N	10°50' E	N	2110
uwba	Ultental, Weißbrunnalm	peat bog	46°28' N	10°49' E	NE	2330
zer	Mattertal, Zermatt, Findelengletscher	glacier foreland	46°03' N	7°47' E	N	2300-2330

Table 2. Site characteristics of Holocene sites. Given are the site codes as well as the geographic location expressed by latitude, longitude and aspect. Elevation of sampling sites is comparable with modern sampling sites, occasionally exceeding the current tree-line.

sample	species	start [years b2k (A.D.)]	end [years b2k (A.D.)]	years	PO	PO (5-year)	number of samples (5-year)
UAZR-1	PICE	135 (1865 A.D.)	- 14 (2014 AD)	150	50	10	30
UAZR-2	PICE	140 (1860 A.D.)	- 14 (2014 AD)	155	82	17	31
UAZR-3	PICE	165 (1835 A.D.)	- 14 (2014 AD)	180	61	13	36
UAZR-4	PICE	190 (1810 A.D.)	- 14 (2014 AD)	205	32	7	41
FPCR-1	LADE	320 (1680 A.D.)	- 14 (2014 AD)	335	150	30	67
FPCR-3	LADE	155 (1845 A.D.)	- 14 (2014 AD)	170	30	6	34
FPCR-5	LADE	155 (1845 A.D.)	- 14 (2014 AD)	170	45	9	34
FPCR-6	LADE	140 (1860 A.D.)	- 14 (2014 AD)	155	55	11	31
VRR-1	PICE	205 (1795 A.D.)	- 14 (2014 AD)	220	62	13	44
VRR-1-2	PICE	195 (1805 A.D.)	- 14 (2014 AD)	205	77	15	41
VRR-3	PICE	175 (1825 A.D.)	- 14 (2014 AD)	190	12	3	38
VRR-4	PICE	155 (1845 A.D.)	- 14 (2014 AD)	170	49	10	34
VRR-5	PICE	240 (1760 A.D.)	- 14 (2014 AD)	255	13	3	51
VRR-6	LADE	135 (1865 A.D.)	- 14 (2014 AD)	150	6	2	30
VRR-7	LADE	135 (1865 A.D.)	- 14 (2014 AD)	150	13	3	30
VRR-8	LADE	370 (1630 A.D.)	- 14 (2014 AD)	385	27	6	77
VRR-9	LADE	175 (1825 A.D.)	- 14 (2014 AD)	190	25	5	38

Table 3. Individual tree characteristics per modern sampling site. Given are the tree species, start and end date of the α-cellulose content series in years b2k and years A.D. in brackets, respectively. The length of the individual series is enlisted in years and the estimation of the pith offset (PO) in annual resolution and calculated as number of 5-year blocks. The number of 5-year cellulose blocks covering the length of the cellulose series is shown.

sample	bag incl. sample [mg]	emptied bag [mg]	expected sample weight [mg]	sample weight [mg]	loss [mg]	loss [%]
1	138.250	126.552	11.698	11.473	0.225	1.9
2	138.112	128.480	9.632	9.307	0.325	3.4
3	129.500	116.654	12.846	12.293	0.553	4.3
4	147.350	134.918	12.432	11.980	0.452	3.6
5	129.060	118.279	10.781	10.405	0.376	3.5
6	133.301	123.375	9.926	9.731	0.195	2.0
7	147.471	132.364	15.107	14.758	0.349	2.3
8	136.276	123.247	13.029	12.486	0.543	4.2
9	147.790	134.199	13.591	13.011	0.580	4.3
10	136.433	125.743	10.690	10.341	0.349	3.3
11	132.212	121.153	11.059	10.708	0.351	3.2
12	133.778	119.853	13.925	13.553	0.372	2.7
13	145.170	133.002	12.168	11.667	0.501	4.1
14	140.314	128.114	12.200	11.804	0.396	3.2
15	118.253	108.009	10.244	10.211	0.033	0.3
16	127.516	115.089	12.427	12.004	0.423	3.4
17	124.031	111.314	12.717	11.733	0.984	7.7
18	123.409	113.444	9.965	9.704	0.261	2.6
19	127.960	119.475	8.485	8.228	0.257	3.0
20	154.157	136.382	17.775	17.348	0.427	2.4
21	138.214	126.419	11.795	11.321	0.474	4.0
22	114.291	101.147	13.144	12.823	0.321	2.4
23	136.587	125.258	11.329	11.305	0.024	0.2
24	121.803	107.166	14.637	14.383	0.254	1.7
25	130.931	116.707	14.224	13.969	0.255	1.8
26	138.189	126.357	11.832	11.503	0.329	2.8
27	128.379	114.667	13.712	13.430	0.282	2.1
28	132.578	120.533	12.045	11.402	0.643	5.3
29	131.704	119.411	12.293	11.726	0.567	4.6
30	113.811	102.525	11.286	10.972	0.314	2.8
31	120.800	109.288	11.512	11.310	0.202	1.8
32	116.480	102.682	13.798	13.321	0.477	3.5
33	125.114	112.714	12.400	12.036	0.364	2.9
34	119.056	107.090	11.966	11.530	0.436	3.6
35	121.489	109.201	12.288	11.951	0.337	2.7
36	106.796	92.591	14.205	13.770	0.435	3.1
37	126.301	111.446	14.855	14.389	0.466	3.1
38	127.248	113.117	14.131	13.642	0.489	3.5
39	156.524	143.516	13.008	12.529	0.479	3.7
40	130.215	121.632	8.583	8.347	0.236	2.7
41	144.788	140.079	4.709	4.362	0.347	7.4
42	123.144	109.257	13.887	13.681	0.206	1.5

Table 4. Calculated sample loss during unpacking of extracted α-cellulose from ANKOM Filter Bags (Type F57) for 42 samples (sample tree MM-602, PICE). The expected sample weight is calculated by subtracting the scaled weight of the emptied filter bag from the weight of the filled bag. The difference between the expected weight of α-cellulose and the scaled weight of unpacked cellulose defines the loss, which is given here in [mg] and [%].

site	sample number	species	mean CC [%]	σ CC [%]	minimum CC [%]	maximum CC [%]	range CC [%]
UAZR	UAZR-1	PICE	33,2	3,9	26,7	42,6	15,9
	UAZR-2	PICE	31,6	3,4	24,8	36,8	12,0
	UAZR-3	PICE	38,6	1,6	34,8	42,8	8,0
	UAZR-4	PICE	39,7	2,5	34,3	46,4	12,1
FPCR	FPCR-1	LADE	29,0	6,5	18,3	48,9	30,6
	FPCR-3	LADE	29,1	6,9	15,2	38,5	23,3
	FPCR-5	LADE	32,9	6,7	20,9	51,6	30,7
	FPCR-6	LADE	28,3	5,6	21,8	46,6	24,8
VRR	VRR-1	PICE	32,9	5,2	21,2	41,9	20,7
	VRR1-2	PICE	33,0	4,5	20,8	42,0	21,2
	VRR-3	PICE	34,3	3,2	29,0	42,0	13,0
	VRR-4	PICE	37,2	3,9	29,2	45,5	16,3
	VRR-5	PICE	35,4	3,3	30,2	45,7	15,5
	VRR-6	LADE	30,6	5,4	19,9	39,5	19,6
	VRR-7	LADE	36,0	4,4	30,4	44,5	14,1
	VRR-8	LADE	26,3	6,0	16,3	39,4	23,1
	VRR-9	LADE	32,5	5,5	26,4	46,3	19,9

Table 5. General statistics of the individual α-cellulose content series per tree from the modern sites. Given are the arithmetic mean values, the standard deviation as well as maximum and minimum α-cellulose content values as well as the range per tree. To simplify the comparison between species, the species per site and tree individuum are enlisted once more.

	UAZR-1	UAZR-2	UAZR-3	UAZR-4
UAZR-1	1			
UAZR-2	-0.10	1		
UAZR-3	0.35	0.36	1	
UAZR-4	0.08	0.32	0.64	1

	FPCR-1	FPCR-3	FPCR-5	FPCR-6
FPCR-1	1			
FPCR-3	0.31	1		
FPCR-5	0.74	0.18	1	
FPCR-6	0.79	0.39	0.67	1

	VRR-1	VRR-1-2	VRR-3	VRR-4	VRR-5
VRR-1	1				
VRR-1-2	0.75	1			
VRR-3	0.66	0.44	1		
VRR-4	0.75	0.62	0.63	1	
VRR-5	0.75	0.41	0.70	0.68	1

	VRR-6	VRR-7	VRR-8	VRR-9
VRR-6	1			
VRR-7	0.78	1		
VRR-8	0.35	0.54	1	
VRR-9	0.55	0.61	0.60	1

Table 6. Pearson's correlation coefficient calculated for the individual trees per site and species calculated in their common growth period (sample replication ≥ 4 trees). Bold numbers indicate that correlations coefficients are significant ($p < 0.05$).

Figures

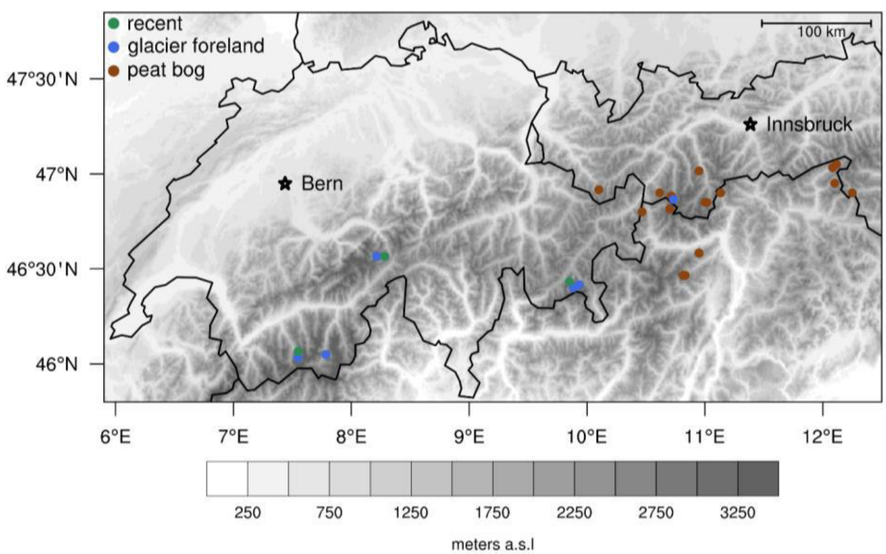

Fig. 1. Sampling sites of Holocene wood remains and living tree individuals. The sites are located along a SW-NE transect along the central European Alps and contain glacier forefields (blue), peat bogs and small lakes (maroon), where Holocene wood remains are collected, and modern sites, where living trees were cored (green). The modern sites are situated in the proximity of glacier forefield sites, thereby resembling similar growth conditions and serving as a connection between Holocene and present-day tree growth and environmental conditions.

Fig. 2. Location of modern sampling sites at the Alpine tree-line at Mont Miné glacier (FPCR, left), in front of Unteraar glacier (UAZR, center) and in the Val Roseg close to the Tschierva glacier (VRR, right). (Source: K. Nicolussi)

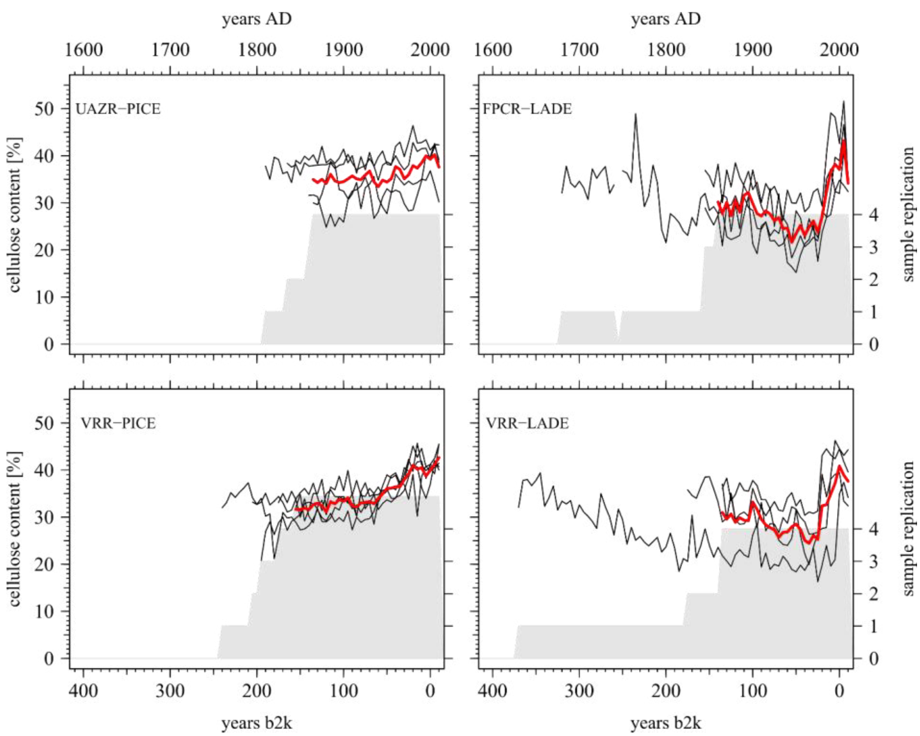

Fig. 3. Individual cellulose content series for two coniferous species (LADE, PICE) at the modern sites. The individual cellulose content time series (black lines) are supplemented by the mean cellulose content per species and site (red line), calculated as the arithmetic mean of the individual trees for the common growth period (sample replication ≥ 4). The sample replication (grey area) displays the temporal coverage per site.

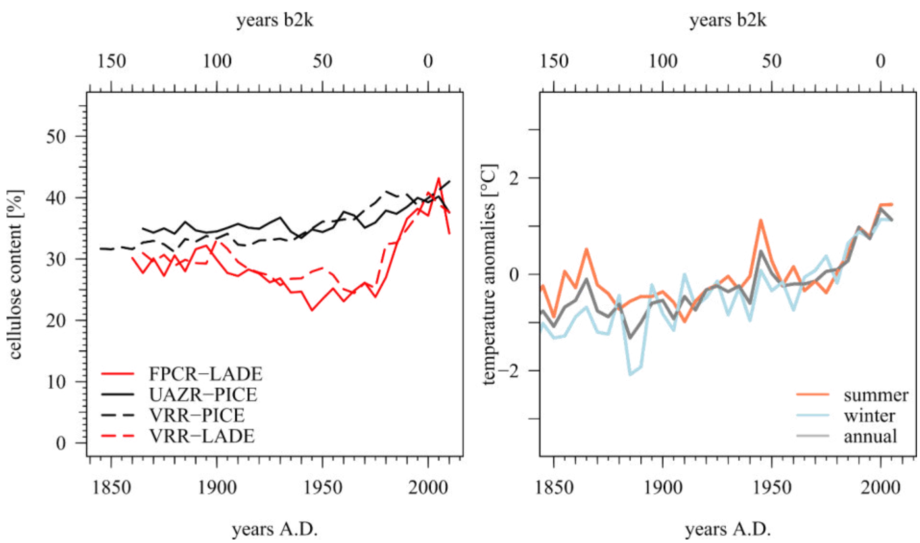

Fig. 4. Modern cellulose content chronologies per site and species (left) and temperature anomalies shown for the NW and SW subregions within the Greater Alpine Region from HISTALP for summer (April-September) and winter (October-March) half-year and per year (right). The CC [%] chronologies are calculated as arithmetic means from individual series for their common period (sample replication ≥ 4). HISTALP data is given for summer season (April-September), winter season (October-March) and annual anomalies calculated as 5-year averages with respect to the sample resolution of CC [%] series. Temperature anomalies for SW and NW subregion in the displayed season means are equal.

Temperature

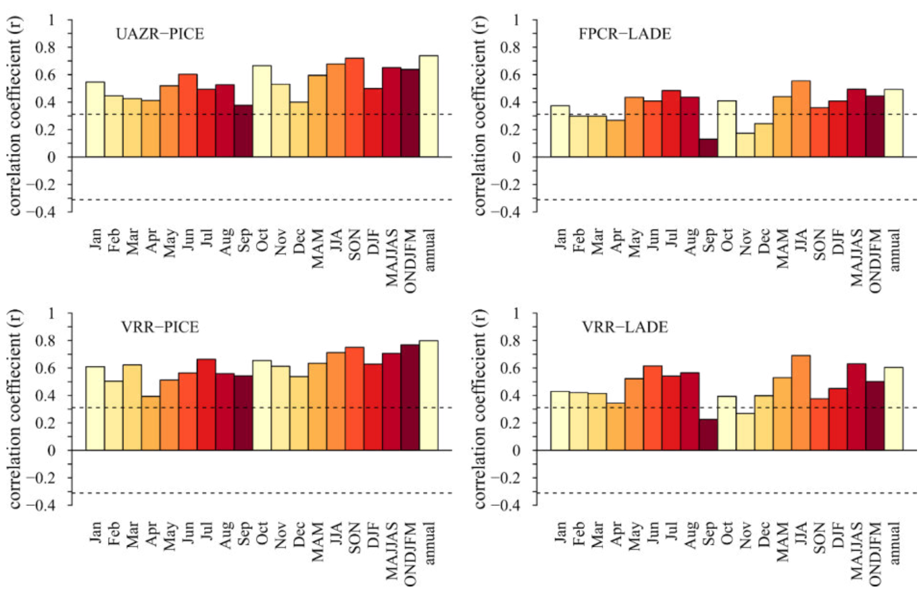

Fig. 5. Pearson's correlation coefficient for mean CC [%] and temperature anomalies (w.r.t. 1961-1990) for the period 1865 – 2005 A.D. UAZR and FPCR are correlated with HISTALP NW region data, whereas VRR species are correlated with the SW region dataset. The dashed horizontal lines indicate the level of significance ($p < 0.05$).

Precipitation

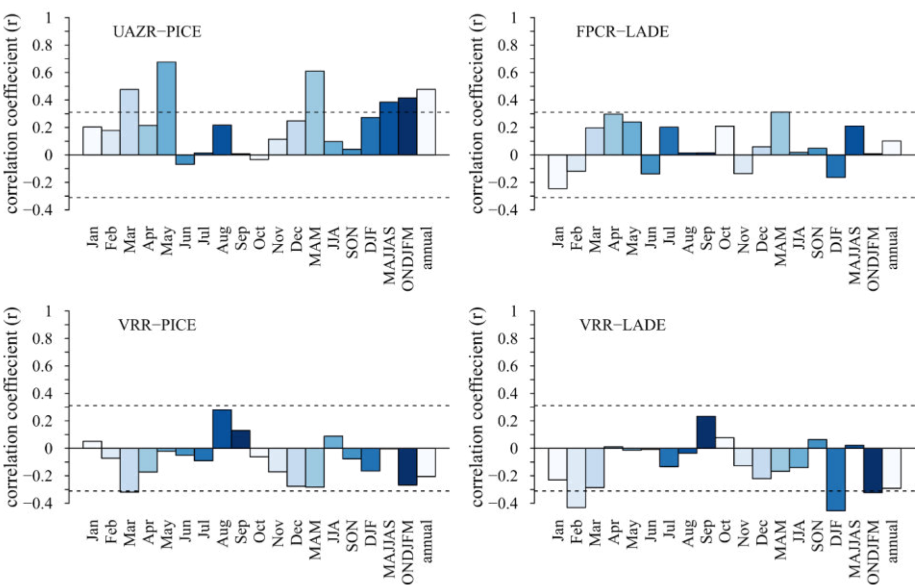

Fig. 6. Pearson's correlation coefficient for mean CC [%] and temperature anomalies (w.r.t. 1961-1990) for the period 1865 – 2005 A.D. UAZR and FPCR are correlated with HISTALP NW region data, whereas VRR species are correlated with the SW region dataset. The dashed horizontal lines indicate the level of significance (p < 0.05).

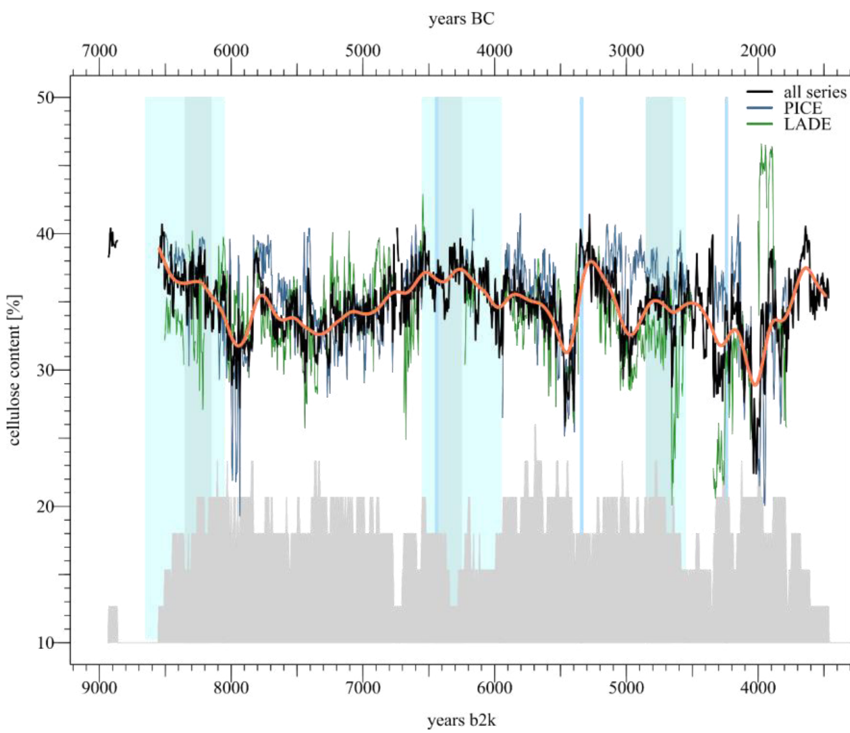

Fig. 7. Progression of mean cellulose content during the time period 9000 to 3500 years b2k. Mean cellulose content is calculated for the two species *Larix decidua* Mill. (LADE, green line) and *Pinus cembra* L. (PICE, grey line); the overall mean series is shown in black and its smoothed values in orange. The sample depth is on the right side (grey polygon). Major cold phases are indicated as cyan rectangles, where darker colors implicit the cold events and the lighter colors their entire duration (Wanner et al., 2011). Additional cold events are marked as blue vertical lines (Wanner et al., 2015)

Preliminary evaluation of tThe potential of tree-ring cellulose content as a novel supplementary proxy in dendroclimatology

SUPPLEMENT

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Cloudiness

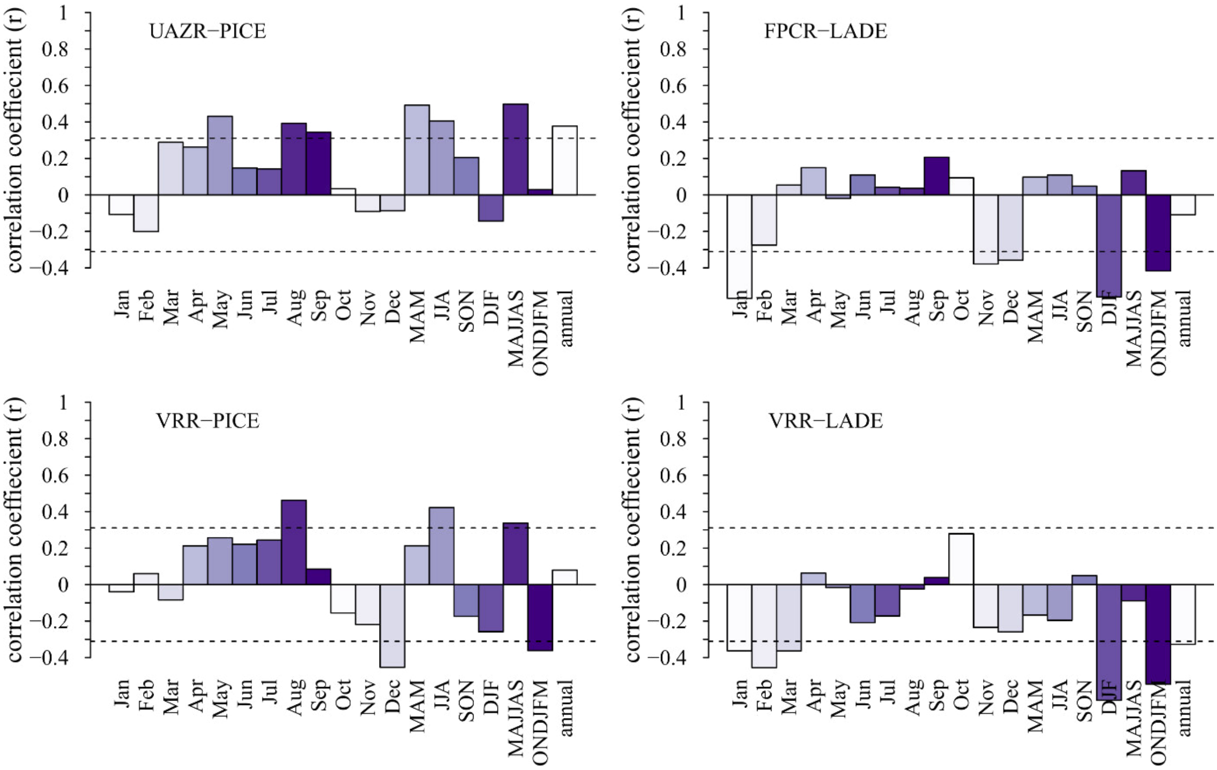

Fig. S1. Pearson's correlation coefficient for mean CC [%] and cloudiness anomalies (w.r.t. 1961-1990) for the period 1865 – 2005 A.D. UAZR and FPCR are correlated with HISTALP NW region data, whereas VRR species are correlated with the SW region dataset. The dashed horizontal lines indicate the level of significance ($p < 0.05$).

Sunshine

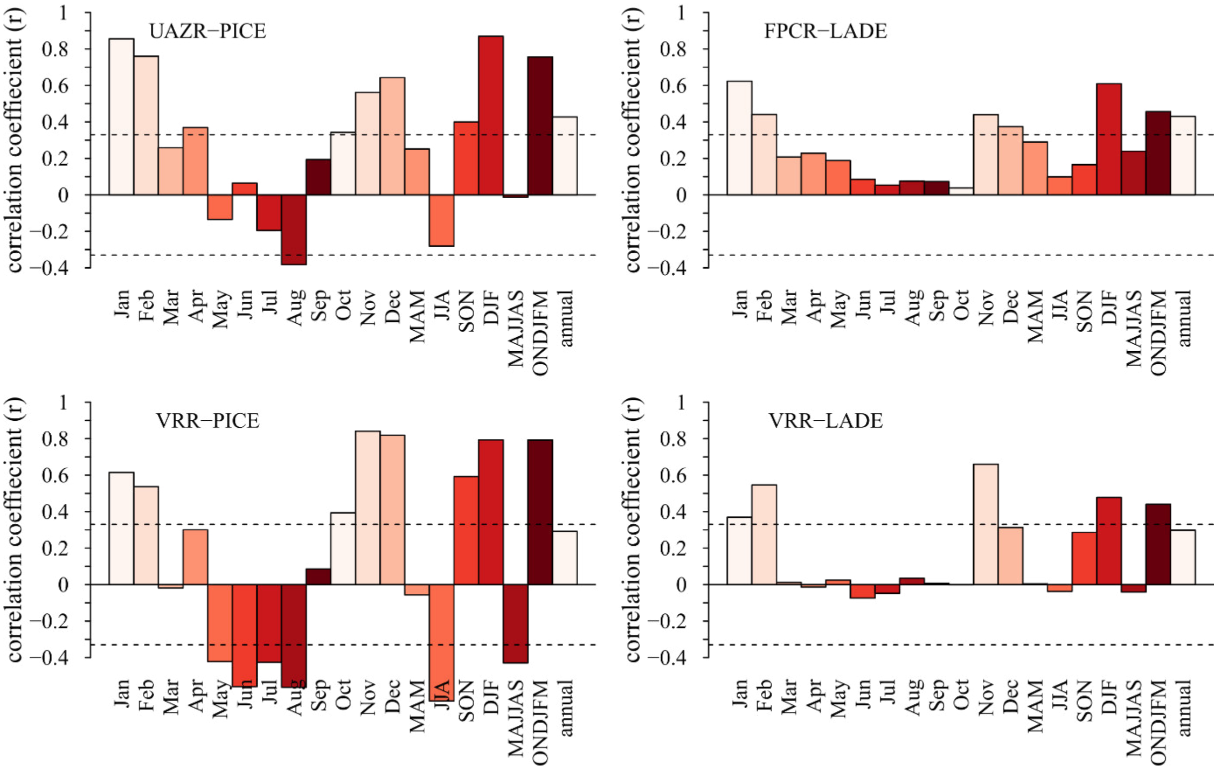

Fig. S2. Pearson's correlation coefficient for mean CC [%] and sunshine anomalies (w.r.t. 1961-1990) for the period 1865 – 2005 A.D. UAZR and FPCR are correlated with HISTALP NW region data, whereas VRR species are correlated with the SW region dataset. The dashed horizontal lines indicate the level of significance ($p < 0.05$)

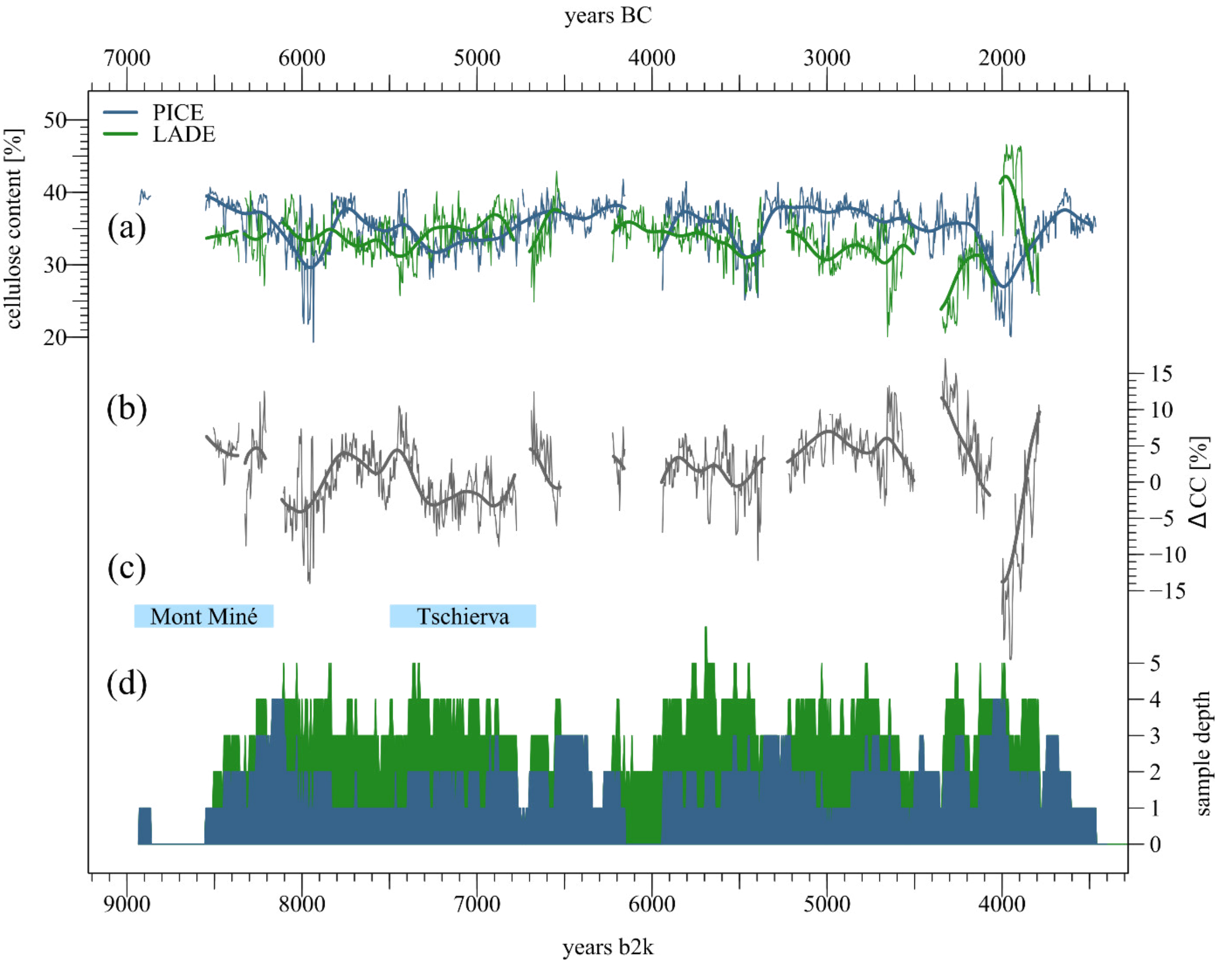

Fig. S3. Tree-species related cellulose content variability during the Holocene. Shown are (a) the mean cellulose content series for LADE (green) and PICE (blue) with corresponding smoothing lines indicating their low-frequency trends, (b) the mean difference between the species given as delta CC [%] (dark grey, on top) and (d) the sample replication (bottom) indicating the number of samples contributing to the mean cellulose content series per species. Blue rectangles (c) mark phases where wood sample stem almost exclusively from a single glacier site.

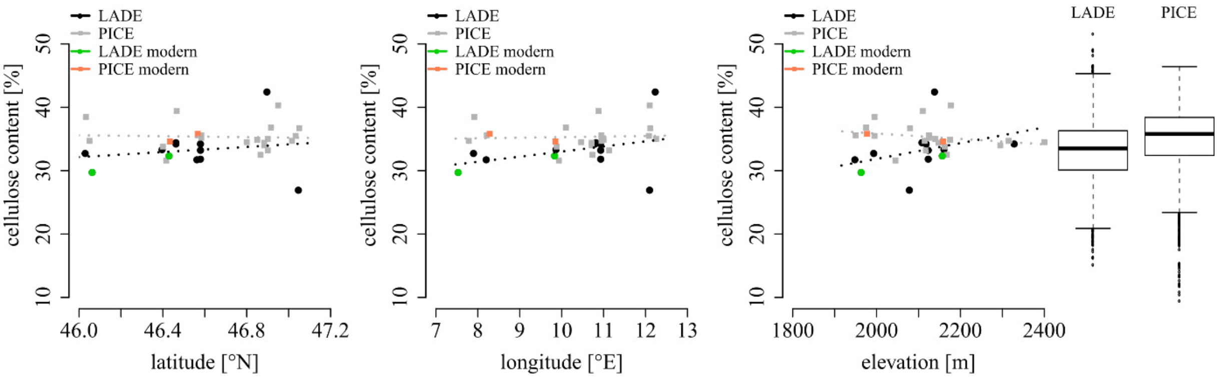

Fig. S4. Effects of site gradients in latitude (left), longitude (center) and elevation (right) on the mean α-CC [%] of both individual modern (colored) and Holocene wood samples. Mean α-CC [%] is calculated as the arithmetic mean of tree average values per site. Shown linear regressions per species are non-significant ($p > 0.05$). Boxplots (right) indicate the distribution of CC [%] values for the two tree species over all sites and samples.

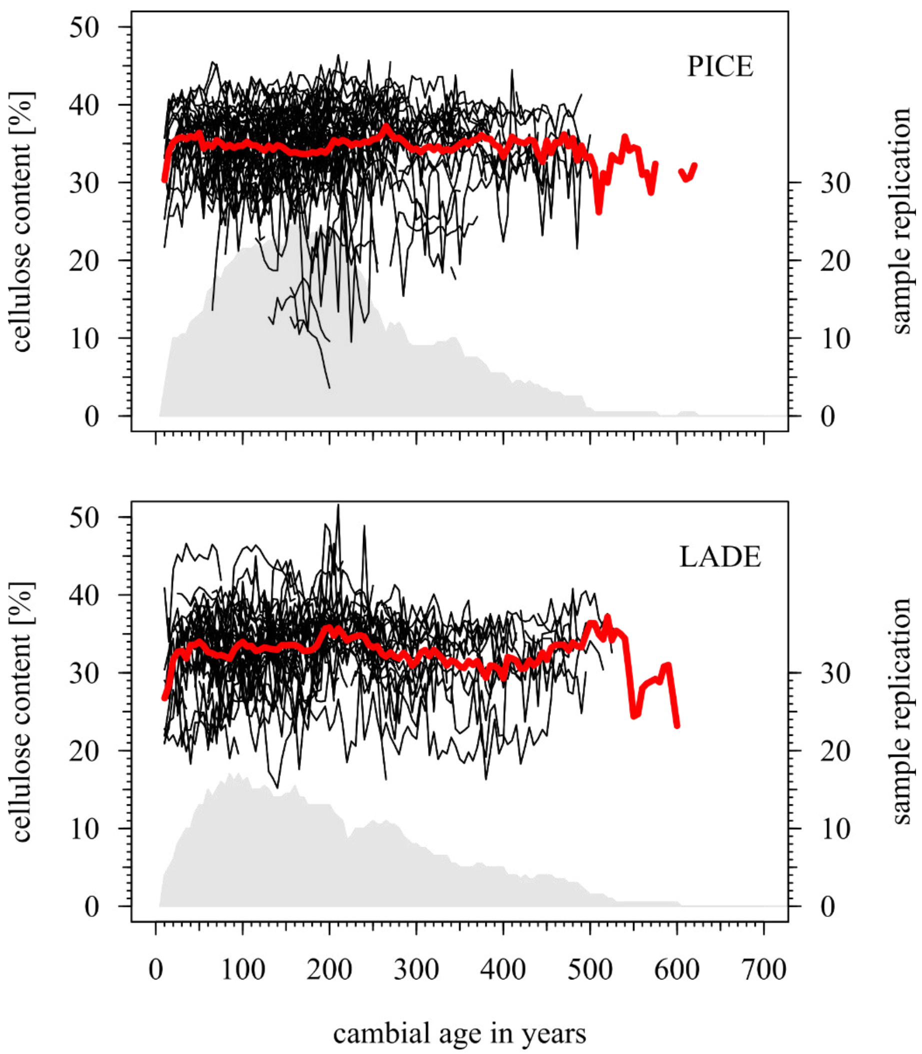

Fig. S5. Cellulose content (CC [%]) in *Pinus cembra* L. (PICE) and *Larix decidua* Mill. (LADE) aligned according to their cambial age in years (pith offset estimation is considered here). Shown are the individual series in black and the mean in red, as well as the sample replication indicated by the grey area at the bottom of each graph.

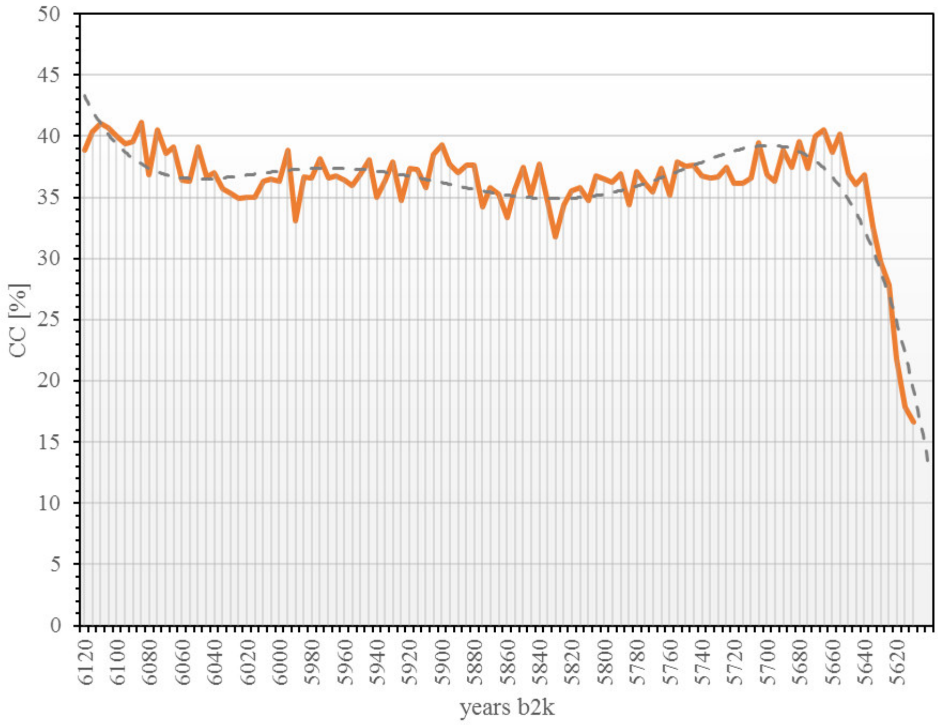

Fig. S6. Example of CC [%] variations and degradation in a Larix decidua Mill. tree (ULFI-47). The tree exhibits a long-term trend in its CC [%] series, followed by a rapid decrease of CC [%] in its outermost rings, which is attributed to degradation of CC [%] due to exposition to weathering. Still, most of the tree is well preserved and suitable for CC [%] analysis.

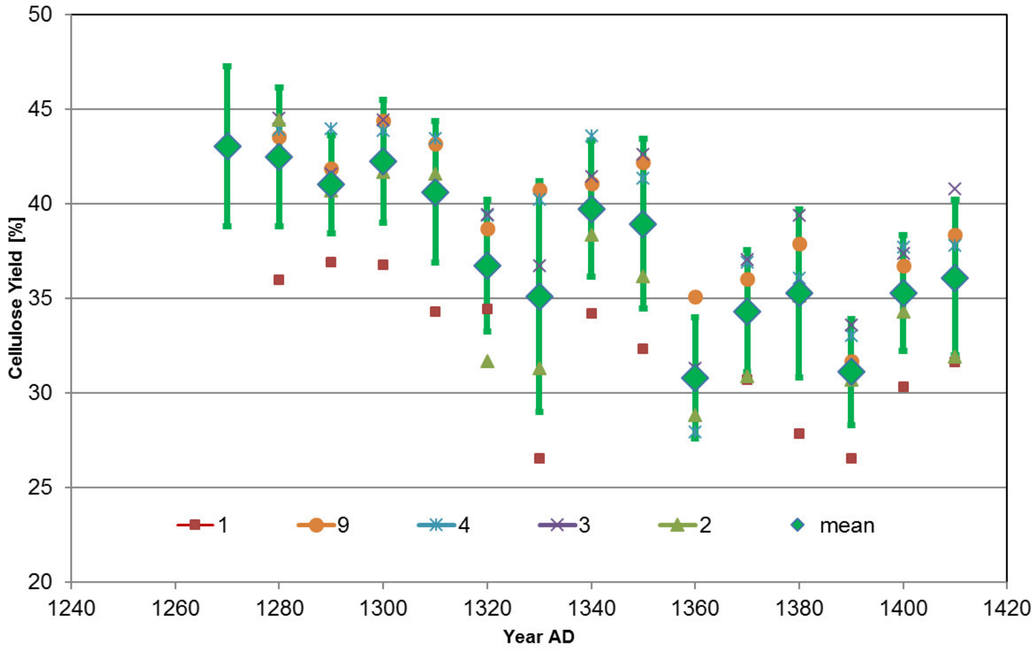

Fig. S7. Variability of CC [%] in larch tree rings from Lötschental (CH). The numbers correspond to tree cores from different trees.

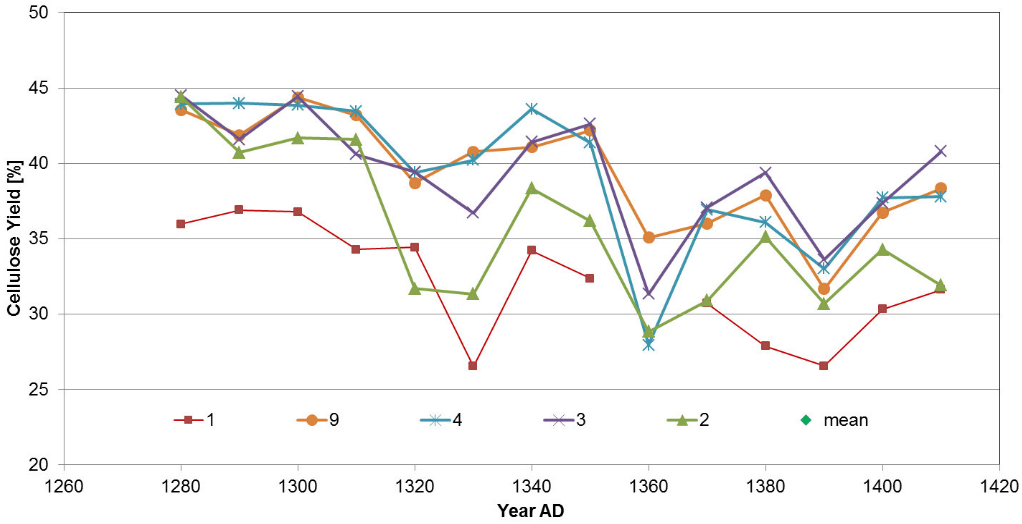

Fig. S8. Temporal variability of CC [%] in larch tree ring series from Lötschental (CH). The numbers correspond to tree cores from different trees.