Reply to the BG the Anonymous Referee #2:

In blue the reviewer's comments and suggestions, in black our answers, in red the sentence added to the revised manuscript text

Anonymous Referee #2 Received and published: 7 January 2021

Review of "Cambial-age related correlations of stable isotopes and tree-ring widths in wood samples of tree-line conifers" by Tito Arosio et al.

This study presents the correlations between different tree ring parameters as tree-ring width (TRW) and stable isotopes (δD, δ18O and δ13C) from samples collected from the Eastern Alpine Conifer Chronology for different cambial-age groups. The scientific output of this paper is very low. Since the authors already presented the cambial-age analysis in another paper, published last year in the same journal (doi.org/10.5194/bg-17-4871-2020), I did not understand why the authors did not include analyses presented here, in that paper and decided to publish them separately.

The analysis of the cambial age of the trees of the dataset have been analyzed before, showing that age trends are present at cambial age <100 yr, and not later. Here we used a different approach that is based on the analysis of statistical correlations between the different tree rings parameters aiming at obtaining data for the interpretation of the biological causes of the juvenile age trends. This approach was started after the publication of the previous paper and stimulated by more recent papers that study the influence of cambial metabolism on the cellulose isotopes. Therefore, this work is a new analysis how the TRW and cellulose isotopes are connected to each other in relation to age.

My feeling is just they want to artificially increase their number of publications, because the data and the scientific output presented here are not enough for an independent research paper, especially in such high impact journal as Biogeoscineces.

This is not the case, see statement to your previous remark.

More than that, the presented paper cannot be read independently, as an individual research paper, in order to understand which kind of data were used. To understand the actual age-related trends of the presented data, it is necessary to read another paper, of the same authors, which was published in the same journal.

It is stated in the abstract and introduction that this is a continuation of the previous paper published in Biogeoscience (Arosio et al, 2020a) and of that published in Frontier (Arosio et al, 2020b) by the same authors. Furthermore, we described the dataset used in the Materials and Methods section of this manuscript, therefore it is a stand-alone paper. In the present study, we analyze the correlations in relation to the cambial age and not the age-related trends.

The authors present the correlation between TRW and i)δ18O, ii)δD and iii)δ13C.Such kind of correlations are rather useless, first of all, due to the fact that the authors already presented in another paper the cambial age trend of these four different tree ring parameters

That the "study of the correlations between TRW and the isotopes is useless" goes against recent literature, in which these correlations are more and more studied for a better understanding of the signals that the isotopes communicate. In the manuscript we quote several papers where the correlations are studied with contradictory results; moreover a very recent paper analyses the correlation difference between TRW and δD, linking them to different climatic conditions (Lehman et al. 2021 https://doi.org/10.1016/j.dendro.2020.125788). The correlations between TRW and carbon isotope were also studied (Shestakova et al, 2017, Shestakova et al, 2019). Even Biogeoscience recently published a paper where the authors used the correlations between TRW

and carbon isotopes (Deshpande et al. 2020 - https://doi.org/10.5194/bg-17-5639-2020). Our work increases the knowledge of this phenomenon, looking at how the correlations change according to cambial age and to various types of detrending methods. The relationship between the different isotopes in cellulose is also under investigation, and the combinations of them are used in paleoclimatic study (Nakatsuka et al. 2020, Xu et al 2020). Our data show that they can change as function of the cambial age. The correlations do not have transitive properties if R is < 1.0, so it is important to look not only at the correlations between TRW and isotopes but also among the isotopes.

We have added some sentences in the revised manuscript to underline the usefulness of the study of the relationships between TRW and isotopes:

"Only a few studies have analysed the correlations among TRW values and stable isotope fractionation with rather contrasting results, and some of them have used this relationship to extract climatic and physiological information (Deshpande et al. 2020, Lehmann et al 2021, Shestakova er al. 2017 Shestakova et al. 2019). Also, some combinations of the different isotopes have been used for paleoclimatic studies (Xu et al. 2020, Nakatsuka et al 2020). Most of these previous works used detrended TRW values and the calculated values of the correlation R factors were generally in the range 0.2 - 0.3 (Hafner et al. 2011; Kirdyanov et al. 2008; Schollaen et al. 2013; Shestakova et al. 2019; Weigl et al. 2007), but one work found correlations to be non-significant (Sidorova et al. 2010). However, none of the studies has taken into consideration how the cambial age of isotope samples can affect such correlation analyses. To fill this gap, we reanalyzed our database and divided the samples in classes of cambial age, with attention to the juvenile phase, i.e. the first 100 cambial years, and we studied the correlations between TRW and stable isotopes, using raw data as well as detrended data applying different detrending methods."

and secondly, because between these parameters does not exist any links.

In fact the presence of links among these parameters has been shown before. For example Szejner et al. (2020) indicated a "link between cambial activity and triose phosphate cycling", and TRW is known to be an index of cambial activity (Srivastava, 1976) and triose phosphate cycling is known to affect δ18O and δD values (Sternberg & DeNiro, 1983; Hill et al., 1995; Barbour & Farquhar, 2000, Augusti et al. 2006), in good agreement with the data we present.
Moreover, TRW and $\delta^{13}C$ are linked via carbon allocation strategies (Shestakova et al. 2019) and are affected by climatic and regional variations, but not by cambial age (our results).
In addition, we also studied the correlations among the isotopes sharing the same source, i.e. δD and $\delta^{18}O$. We found that the correlation between δD and $\delta^{18}O$ change with cambial age and they correlate differently for the two species (negatively for pine and positively for larch).

The TRW does not influenceδ18O andδ18O does not influence TRW, the same with other parameters. The variation of the TRW is independent of the δ18O,δD, or δ13C variations.

We agree that there is no direct causal influence of TRW on $\delta^{18}O$ and δD and viceversa, however these values may be influenced similarly by external or internal drives, and our work is focused to better understand these connections. A linkage between TRW and cellulose isotopes has been shown before, as stated above (Szejner et al. 2020, Shestakova et al 2019). This link can be caused by climatic driver or biochemical events, looking at our large database we are able to identify the biochemical linkage out to the common climatic driver. The aim work is to understand how these relationships change with respect to the cambial age, regardless of climatic factors.

The correlations are made between parameters that do not have a cause-and-effect relationship. When correlation analyses are performed, it is supposed to be a connection or a relationship between those two parameters, but in this case, the only connection can be the presence or absence of the trend in juvenile cambial age of the trees, and this aspect was showed in the previous paper.

The reviewer has to consider not only a time dependence (juvenile effect) but also a dependence on biochemical processes related to the metabolism. In order to disentangle these different influences we applied statistical methods. Correlation is a statistical measure of the strength of a relationship between two quantitative variables, and it does not imply causation! It is a tool aimed at identifying a common driver of two variables, and this may lead to novel interpretations. Our aim was to understand the change in relationship between the variables during cambial age for a better understanding of the causes of the age trend, that so far are just hypothesized. In the previous paper we did not analyse correlations and we did not propose interpretations for the juvenile age trends.

The obtained correlations are due mainly to the trend of the data, and the trend is already a demonstrated fact of these series. When you correlate two data sets with similar or opposite trends, automatically you will get a correlation coefficient (positive or negative). And the explanation of such correlations, correlations between parameters that do not have a link between them, based on the physiological processes of trees are only speculations.

We agree with the reviewer that correlations can be caused by common trends of the data. In fact, exactly to correct this problem, we made a linear detrend of each cambial age class (fig 1 d,e,f and 2d,e,f). The finding that after detrending the correlations remained significant shows that they are not driven by the age-related trends of the juvenile phase. Moreover, we did not observe a correlation TRW- $\delta^{13}C$ in the juvenile phase using both raw and detrended data, even though we previously showed that carbon presents an age-related trend in cembran pine. Also, δD has species-specific age trends but its correlation with TRW in the juvenile phase is not species-specific.

We add the figure S3 in supplement to better show the three different scenarios (raw, linear detrending, and spline for TRW):

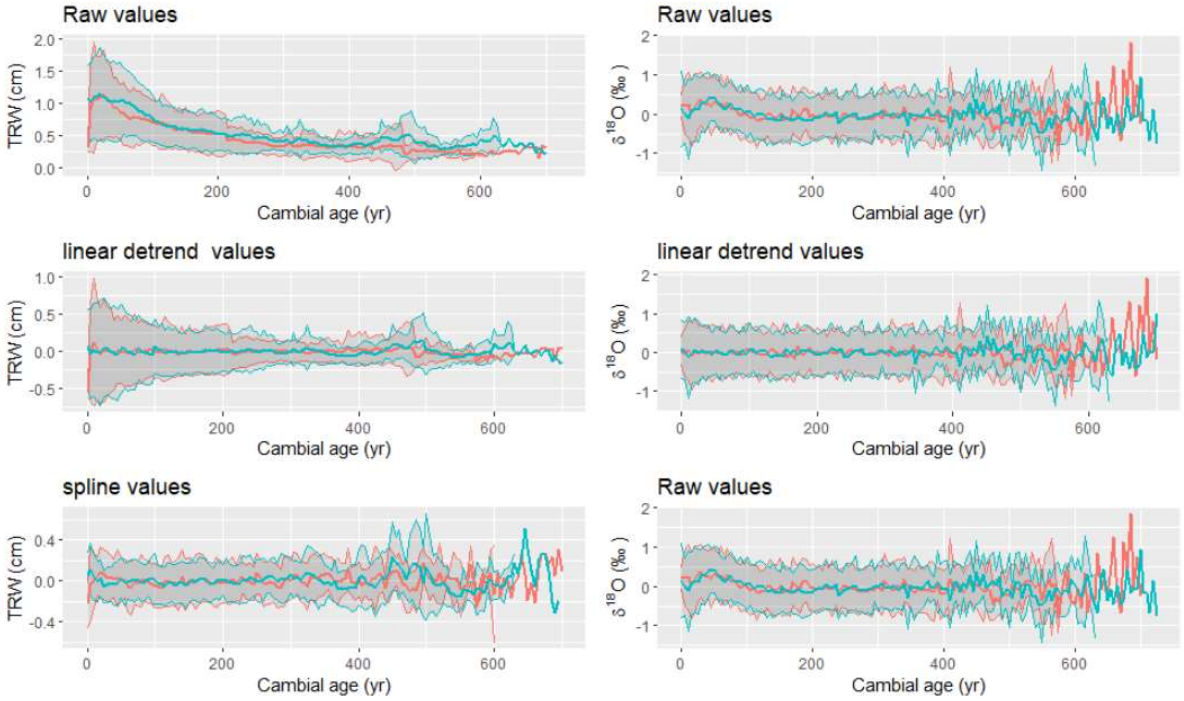

Figure S3. TRW and d18O values during tree cambial age expressed in the three forms used for the correlation analysis. The upper panels show raw data of TRW (a) and d18O (b); the middle panels show linear detrended data of TRW (c) and d18O (d) ; the lower panels show linear spline detrended data of TRW (e) and raw data of d18O (f). The values of larch trees are in red, those of cembran pine are in turquoise

The paper even does not have a conclusion section. The last two sentences (three rows) of the discussion summarize the conclusions of the paper, but which does not bring anything new from the last published paper by the authors. (Line 158: In conclusion, our results confirm the existence of a juvenile phase in the δD andδ18O isotopes...).

We agree with reviewer and we significantly have rewritten the conclusion section:

"In conclusion, we studied the effect of cambial age on the modification of the relationships between tree rings components (TRW and cellulose isotopes). Our results indicate that during the juvenile phase the change of the δD and $\delta^{18}O$ isotopes of cellulose can be attributed to a high cambial activity that decreases with higher age. This indicates a different climatic sensitivity of the age groups, in accordance with Xu et al. (2020), implying that a simple detrending of the values of water isotopes may not be sufficient for paleoclimatic studies. The absence of a substantial change of trends between $\delta^{13}C$ and TRW indicates that plants do not modify the mechanisms of use of reserves as a function of age. We provide novel information for the use of the relationships between trees ring components (TRW and isotope compositions) to extract climatic information, in particular alerting readers that the cambial age and the TRW detrending methods can influence the results. The relationship between the isotopes of water (δD, $\delta^{18}O$) in cellulose shows a difference between juvenile phase and adult phase, but a different behavior is present in the two species. In adult phase there is a stronger correlation in larch than in cembran pine, in agreement with Arosio et al. (2020b)"

The figures are of very poor quality. Moreover, the paper contains only 2 simple figures(bar figures), while the figures from supplementary contain too many very small figures, which are hard to follow.

We have redrawn fig 1 and 2 to improve their quality. The supplementary have been simplified following reviewer's suggestion.