### Reply to the BG the Anonymous Referee #1 :

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

This study takes advantage of the large Alpine Holocene Tree-Ring Dataset to examine effects of age on the correlation between tree-ring width (TRW) and stable isotope ratios of hydrogen. oxygen and carbon. The manuscript extends an excellent study by a similar group of authors (Biogeosciences 17, 4871-4882, 2020) which found an influence of age on stable isotope ratios of oxygen and hydrogen but not carbon. Correlations between stable isotope ratios and TRW are relevant and within the scope of BG, but this paper seeks to explore the effect of age on these correlations, a narrow and difficult subject given the known strong and non-linear effects of age on TRW. The dataset is apparently well-collected and impressive in its temporal coverage (9000 years) and replication (7604 samples). The approaches are standard, but the methods and assumptions appear to be valid and clearly outlined. The main conclusion, well supported by the data, is that the correlation between TRW and isotope ratios of hydrogen and oxygen is affected by age in the first 100 years. This is not surprising given that TRW is known to be strongly affected by age and that the same authors have already shown that isotope ratios of oxygen and hydrogen are affected by age. More detail in some of the methods would be helpful as described below. The authors give proper credit to related work, the title clearly reflects the contents of the paper, and the abstract is reasonably clear. The overall presentation is concise and well-structured, but problems with the English grammar make much of the writing unclear. Mathematical formulae, symbols, abbreviations and units are correctly defined and used. I have no suggestions for combining or eliminating major sections. The references and the supplementary material are appropriate.

## We thank the reviewer for the kind and constructive comments.

Specific Comments. The authors find that the correlation between TRW and isotope ratios of hydrogen and oxygen, but not carbon, are affected by age in the first 100 years. These correlations are strongly affected by the method used to detrend the TRW data for age. More precisely there is a strong difference between the results of linear and spline detrending. The spline but not linear detrending is addressed in lines 143-144. The authors need to explain the difference between these two detrending approaches. Is it possible the cause of this difference is that in the linear case detrending was applied to both the isotope and TRW data, while in the spline case detrending was applied only to the TRW data?

We investigated the relationship between TRW and isotope in three different scenarios, (i) raw data,(ii) linear detrending of each age class of TRW and isotope series, and (iii) spline TRW detrending and raw isotopes. Scenario (iii) is the most used approach for climatic investigations.

In the first two scenarios, the variance of the values did not change after detrending, while in the spline detrend of TRW the variance changed, remaining constant through all the cambial age. The spline detrend of isotopes is not necessary because they do not change the variance in respect to the cambial age.

We included the following part in the revised text in the Material and method section, subsection 2.1:

"The p-values (statistical significance) were calculated for each correlation and are displayed as bars in Figure 1 and 2. If P < 0.05, they are labeled as "ns" (not significant) in the graphs. To identify possible correlations derived by common trends, we calculated the correlations in three different scenarios: (i) by using the raw values of isotopes and of TRW (Fig. 1 a,b,c and 2 a,b,c); (ii) by applying in R a linear function to both the isotope and TRW values, separately for each cambial age class in function of the cambial age (Fig. 1d,e,f and 2d,e,f); and (iii) by using the raw, un-detrended, isotope values and applying a spline detrending to all TRW values not subdivided into classes, as done normally in climatic studies (Fritts 1976) (Fig. 1g,h,i). Spline detrending is a common methodology used in dendroclimatology to remove the ageing signal. In this study we calculated it by applying a linear spline detrending function, using the default sets (using 67% of total series length and frequency response of 50%), to the raw TRW utilizing the R library dplR (Bunn 2008). A visual representation of the data in the three different scenarios is in the supplementary (fig. S3)."



We also added the following figure S3 in the supplementary:

**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  $\delta^{18}$ O (b); the middle panels show linear detrended data of TRW (c) and  $\delta^{18}$ O (d); the lower panels show linear spline detrended data of TRW (e) and raw data of  $\delta^{18}$ O (f). The values of larch trees are in red, those of cembran pine are in turquoise.

For both species in the first 75 cambial years, the hydrogen isotope ratio is negatively correlated with TRW and the oxygen isotope ratio is positively correlated with TRW for both raw and linearly detrended data (Fig. 1, panels a, b, d, and e). The authors present an interesting and well-referenced explanation of this pattern in the Discussion.

On the other hand, this pattern leads to the expectation that there should be a negative correlation between the hydrogen and oxygen isotope ratios for both species over the same time period. Fig. 2 panels a and d show this for pine but not larch. Please explain.

We have shown before that deuterium fractionation in larch is different in respect to other conifers leading to a uniquely high depletion in Deuterium (Arosio et al, 2020,

<u>https://doi.org/10.3389/feart.2020.523073)</u>. Since the origin of this difference of larch is still unclear, we are presently unable to explain the correlation between  $\delta D$  and  $\delta^{18}O$  in larch. Yet, the interpretation presented in our manuscript fits well with cembran pine correlation.

We added the following sentence in the discussion:

"This interpretation also explains the negative correlation between the  $\delta D$ - $\delta^{18}O$  in the juvenile phase in cembran pine and the absence of correlation in the adult phase. In larch the correlations  $\delta D$ - $\delta^{18}O$  are different from those in cembran pine. Arosio et al. (2020b) showed that deuterium is uniquely depleted in larch, probably because of a different biological fractionation. Therefore, our interpretation does only hold Cembra pine. The correlation between the two isotopes in larch is presently difficult to interpret."

Line 13 states "no trends" were found in adult trees. Please be more specific. Trends against what variable? Cambial age?

We changed changed the sentence into: " $\delta D$  and  $\delta^{18}O$  exhibited no age-related trends in adult trees older than 100 yr."

Line 55 states "To avoid a geographical effect that can lead to artificial trends, we have normalized the isotope values of each tree by subtracting the tree mean from each value". This is a reasonable approach as long as the variance does not change as a function of the mean. Is that the case?

In previous analysis we have shown that the geographical effect influences the mean but not the variance. In any case, we tried to compute the analysis also with scaled values (correcting the data also with the variance), but this did not modify the results compared to the normalized results.

Line 63 describes the linear detrending of the isotope and TRW values, but omits the variable against which this detrending was applied. Is this variable cambial age?

Yes, we used the cambial age. We add this information to the sentence: "by applying a linear detrend, in function of the cambial age, to the isotope and TRW values removing linear trends in each age class."

Line 67 says default settings were used for the spline detrending. Given that this de-trending is central to the results, it would be useful to provide details of these settings. For example, what was the spline stiffness? Did you use the ratio or difference method to calculate residuals, etc.?

We used the spline detrend function using 67% of total series length and frequency response of 50%. We tested different detrend parameters with minor differences, we also tried a negative exponential detrend that caused an increase of non-significant correlation. We added this information to the manuscript:

"Spline detrending is a common methodology used in dendroclimatology to remove the ageing signal. In this study we calculated it by applying a linear spline detrending function, using the default sets (using 67% of total series length and a frequency response of 50%), to the raw TRW utilizing the R library dplR (Bunn, 2008). "

Line 95 states two patterns are different. How are they different?

#### We change the sentence to:

"The correlations between the TRW and  $\delta^{13}$ C have many age classes with no significant r including all values class. But the pattern for the spline-detrended (Fig. 1 i) values do not show negative r values in contrast to Fig. 1 c, f."

Line 123 states "the correlation between the TRW and the two water isotopes of all the trees, not divided in cambial age classes, were non-significant." This is inconsistent with Figure 1 panels a, b, d, e, and f, which all show significant correlations between TRW and water isotopes where all age classes are combined.

Thank you. We change the sentence to:

"An additional result was that the correlation values between the TRW and the two water isotopes of all the trees, not divided in cambial age classes, were higher in cembran pine than in larch-"

Lines 124-126. Good point.

## Thank you

Technical Corrections. Line 16. Replace "year" with "years".

# Corrected

Line 29 refers to "the two species" but the species are not introduced until the following line.

## We added this information

Lines 32-33. Replace "along the cambial age" with "as a function of cambial age".

## Done

Line 34. Insert "have" before "analysed".

#### Done

Line 46. Replace "spanning" with "combining" and insert "from individual trees" after "tree-rings". Lines 75-79 simply explain the contents of Figure 1. This information belongs in the figure caption, not the Results Section.

#### Done

Line 82. Replace "did neither change" with "changed neither"

# Done

Line 84. Replace "Similar is the pathway for correlations between TRW and d18O (Fig. 1 b,e) with positive" with "Similarly, TRW and d18O (Fig. 1 b,e) show positive".

## Done

Line 86. Replace "with respect to" with "than" Line 128. Replace "are" with "is", delete "of", and replace "works" with "work".

# Done

Line 130. Replace "age" with "trees". Line 134. Replace "was" with "has been".

# Done

Line 138. Insert "to" after "leading".

# Done

Line 143. Insert "the" before "growth"

## Done

Line 147. Replace "The correlation, between dD and d18O, changed during the juve-nile phase in both phases with parallel trends . . . " with "That the correlation between dD and d18O changed during the juvenile phase in both species . . . ".

### Done

Line 151. Replace "described before" with "already known".

Done