

Clim. Past Discuss., author comment AC2
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Reply on RC2

Anna Wieland et al.

Author comment on "Climate signals in stable carbon and hydrogen isotopes of lignin methoxy groups from southern German beech trees" by Anna Wieland et al., Clim. Past Discuss., <https://doi.org/10.5194/cp-2021-135-AC2>, 2022

We thank Referee #2 for the positive evaluation and the helpful comments. All comments and requested changes were taken into account. Please note that comments by the referee are in italics and that in the authors' answer the mentioned line numbers refer to the version of the revised manuscript including track changes.

General comments

As reviewer 1 states, tree-ring lignin methoxy $\delta^{13}\text{C}$ and $\delta^2\text{H}$ is an interesting and new methodology, and it may offer additional information about past climate compared to traditional tree-ring widths, density and cellulose isotope ratios, and therefore readers of this journal may find this manuscript worthwhile. However, discussion of this manuscript misses some important points readers may wish to know. As a non-specialist of tree-ring lignin methoxy groups, I wanted to read more about the comparison between traditional tree-ring cellulose isotope ratios and tree-ring isotopes of lignin methoxy groups, however, such comparison was not sufficiently described in the manuscript.

Authors: We have added further information regarding isotopic signatures of cellulose and lignin methoxy groups in trees. For more details, please refer to the answers to the specific comments below. Please note that the development and application of lignin methoxy groups as a paleoclimate proxy is a relatively young research field and so far, there exist only a limited number of studies, which have measured and compared isotopic values of methoxy groups and cellulose of the same tree rings.

I think majority of the readers of this manuscript will be those who study tree-ring isotopes and therefore I think authors should add more information about the difference between the two. For example, compared to cellulose deposition, lignin deposition to cell walls happens at the latter part of the growing season and duration of lignin deposition to cell walls is longer than that for cellulose, which may explain the correlations observed in this study (Ln 16-19). Authors should discuss more about the physiological background of the correlations observed in this study.

Authors: As mentioned in our response before, we have added more information to the introduction. To deal with this issue in much greater detail in the discussion section, it would have been necessary to compare the isotopic ratios from lignin methoxy groups with those derived from cellulose of the same trees at Hohenpeißenberg. Due to labour, time, and financial aspects, we were not able to also measure the isotopic ratios of

cellulose from these trees. Thus, it makes not much sense to compare the results of lignin methoxy groups from Hohenpeißenberg with previous studies, where also cellulose has been measured. However, for a detailed discussion regarding the comparison of stable isotope values of methoxy groups with cellulose or bulk measurements we refer the referee and the readers to the studies by Mischel et al. (2015) and Gori et al. (2013). Concerning the specific question (Ln 16.-19), please refer to the answer below (specific comments). We have added further information regarding the observed differences between the carbon isotope composition of cellulose, whole wood, and lignin methoxy groups to the revised manuscript (from line 51).

In addition, we included a new schematic diagram to the revised manuscript (Figure 12) to illustrate to readers the novel application of $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values of lignin methoxy groups for temperature reconstructions. Stable carbon isotope values reflect mainly the regional summer temperatures, whilst $\delta^2\text{H}_{\text{LM}}$ values show large-scale 'shifted' annual temperatures. The combination of the two isotopic approaches provides information on different temporal and spatial scales. To the best of our knowledge, this is not possible with 'traditional' stable isotope tree ring analyses such as from cellulose.

Specific comments

Ln 16-19 "The calibration of $\delta^{13}\text{C}_{\text{LM}}$ chronologies against instrumental data reveals highest correlations with regional summer ($r = 0.68$) and mean annual temperatures ($r = 0.66$), as well as previous-year September to current-year August temperatures ($r = 0.61$)". What about EPS? Did you or any previous studies compared traditional tree-ring isotope ratios and tree-ring isotopes of lignin methoxy groups?

Authors: For the 1916-2015 period, the calculated EPS value of the $\delta^{13}\text{C}_{\text{LM}}$ chronology is 0.71. The study by Anhäuser et al. (2020) analysed the $\delta^2\text{H}_{\text{LM}}$ values of the same tree ring sample set and recorded an EPS value of 0.91, indicating a sufficient sample size to establish a representative $\delta^2\text{H}_{\text{LM}}$ chronology at Hohenpeißenberg. It should be noted that the study by Anhäuser et al. (2020) included 9 different cores. F1 was represented by three cores and F2-F4 by only two cores. To eliminate a higher quantifier of F1, all $\delta^{13}\text{C}_{\text{LM}}$ chronologies of the four trees are now represented by two cores.

In several studies the threshold of ≥ 0.85 for the EPS value is controversially discussed (for instance: Wigley et al., 1984; Briffa and Jones 1990; Mérian et al., 2013). Buras (2017) concluded that the application of the EPS does not necessarily give us a valid information whether tree ring data can be used for climate reconstructions. However, in this study we used eight cores from four different trees and as we described in the manuscript (line 474, 543), that ideally more replicates would be required for $\delta^{13}\text{C}_{\text{LM}}$ studies to produce a temporally robust mean $\delta^{13}\text{C}_{\text{LM}}$ chronology. In this case, a larger number of replicates would also lead to an increased EPS value.

The EPS value has been added and discussed in the revised manuscript from line 227.

The study by Mischel et al. (2015) compared the oxygen, hydrogen, and carbon isotopic composition of whole wood, alpha-cellulose, and lignin methoxy groups. In this study, EPS analysis showed that four trees were sufficient for $\delta^{13}\text{C}$ measurements of whole wood, and three to four trees were adequate for $\delta^{13}\text{C}$ measurements of cellulose. For $\delta^{13}\text{C}_{\text{LM}}$ measurements, the sample set should be increased to seven replicates. Nevertheless, with this study we could show that $\delta^{13}\text{C}_{\text{LM}}$ values have a great potential for reconstructing temperature changes if tree ring series are not influenced by inconsistencies within the series.

In addition, the study by Gori et al. (2013) analysed a similar approach and compared hydrogen, oxygen, and carbon isotope compounds of whole wood, cellulose, and lignin methoxy groups. This study used tree samples from three different elevation sites in the south-eastern Alps (900 m, 1300 m, and 1900 m) and showed that the carbon, oxygen, and hydrogen isotopic ratios of whole wood and cellulose were highly correlated while the carbon and hydrogen isotopic composition of lignin methoxy groups correlate to a lesser

extent with the other components. Similar findings were documented in the study by Mischel et al. (2015). They analysed trees from a low elevation environment (Altenkirchen, Germany, about 300 m). Here, the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of whole wood and cellulose were also highly correlated ($r = 0.89$; $r = 0.96$) while the correlations between the carbon isotope values of whole wood and lignin methoxy groups or cellulose and lignin methoxy groups showed the same but lower correlation coefficient of $r = 0.72$. The two studies suggest that stable carbon and hydrogen isotope values of methoxy groups contain a different climate signal and seem to be influenced by different environmental and biochemical factors. The extraction of cellulose, however, may not be necessary as the isotopic compounds of cellulose and whole wood receive the same climate signal. The isotope measurement of lignin methoxy groups, on the other hand, might be a beneficial proxy for climate reconstructions in a different temporal and spatial context. This conclusion is supported by the study of McCarroll et al. (2003) which implicated that the key to amplifying the climate signal lies in combining independent proxies that are not similar. In this context, Gori et al. (2013) showed that the best prediction model for reconstructing temperature changes is obtained when the hydrogen and carbon isotope compounds of whole wood and methoxy groups are combined. We have added further information regarding the observed differences between carbon and hydrogen isotopic composition of cellulose, whole wood, and lignin methoxy groups to the revised manuscript (from line 51). However, for detailed discussion regarding comparison of stable isotope values of methoxy groups with cellulose or bulk measurements, we refer the referee and the readers to the studies by Mischel et al. (2015) and Gori et al. (2013).

Ln 24 "large-scale temperatures" Why $\delta^2\text{H}_{\text{LM}}$ reflects larger scale temperature than $\delta^{13}\text{C}_{\text{LM}}$? Can this be explained partly by the fact that hydrogen isotope ratios of precipitation reflects larger scale temperature of this area?

Authors: The $\delta^2\text{H}_{\text{LM}}$ values are directly connected to the $\delta^2\text{H}$ values of the precipitation. For detailed information about the biosynthetic pathway which is responsible for $\delta^2\text{H}$ fractionation between precipitation and lignin methoxy groups in tree rings please refer to the study by Greule et al. (2021). Therefore, the referee is right, that the stable water isotopes of precipitation of this area are considered to indicate large-scale atmospheric phenomena rather than variations in local or regional climate states. Further information has been added to the revised manuscript from line 82.

Ln 31-32 "Weather and climate parameters affect the physiological process within these tree rings."

As far as hydrogen isotope ratios are concerned, weather and climate (temperature) also affects hydrogen isotope ratios of precipitation, which eventually affects tree ring $\delta^2\text{H}$. This is not physiological, but a hydrological process.

Authors: The sentence has been revised to read: 'Weather and climate parameters affect direct or indirect the physiological process within these tree rings.' (Line 31)

Ln 54-55 "Therefore, the temperature dominated signal in $\delta^2\text{H}$ precipitation (Dansgaard, 1964) is reflected in $\delta^2\text{H}_{\text{LM}}$ values as has been demonstrated for mid-latitude sites (Anhäuser et al., 2017a; Greule et al., 2021)." Is $\delta^2\text{H}$ precipitation not affected by the amount of precipitation (of a single precipitation event)? Please explain.

Authors: Yes, the isotopic composition of precipitation is also influenced by the amount of precipitation. The amount effect describes lower $\delta^2\text{H}_{\text{precip}}$ values in rainy months and higher $\delta^2\text{H}_{\text{precip}}$ values in months of less rainfall. This effect occurs mainly in tropical regions as well as in high alpine mountain ranges. However, in mid- and high latitudes, the temperature effect is the dominating factor controlling the isotopic composition of precipitation (latitude effect). In this context, several studies suggested that $\delta^2\text{H}_{\text{LM}}$ values

reflect $\delta^2\text{H}$ values of the tree's source water as significant relationships between $\delta^2\text{H}_{\text{LM}}$ values and mean annual $\delta^2\text{H}_{\text{precip}}$ values have been observed implying that $\delta^2\text{H}$ values of the tree's source water reflect an annual integral of site-specific $\delta^2\text{H}_{\text{precip}}$ values. We added more information to the revised manuscript (line 76).

*Ln 57-60 "Wang et al. (2020) found significant correlations between $\delta^2\text{H}_{\text{LM}}$ values and April-August temperatures ($r = 0.58$ to 0.7) for two coniferous species (*Larix gmelinii*, larch and *Pinus sylvestris* var. *mongolica*, pine) from a permafrost forest in northeastern China." Lignin deposition takes place over a longer period of time than cellulose deposition. Therefore, I expect tree-ring lignin to reflect temperatures over a longer period of months compared to tree-ring cellulose from the same tree. April-August seems longer than those for tree rings, which usually shows correlations to summer (June-July) climatic variables in my impression.*

If such comparison is possible, then please add more explanation here about the difference between cellulose and lignin.

Authors: The studies of Mischel et al. (2015) and Gori et al. (2013) suggested that the isotopic composition of methoxy groups reflects different environmental and biochemical factors than the isotopic composition of cellulose or whole wood. As the referee mentioned one possible explanation could be that the lignin deposition occurs over a longer time period than cellulose deposition. In contrast to this assumption, Mischel et al. (2015) found the highest correlation between $\delta^2\text{H}$ values of lignin methoxy groups and maximum temperatures at the beginning of the growing season, while $\delta^{18}\text{O}$ values of cellulose from the same sample set showed the highest correlation with the maximum temperatures from March to October. Gori et al. (2013) found the highest correlation between $\delta^2\text{H}$ values of cellulose and seasonal and mean annual temperatures where these correlations are strongly dependent on the altitude of the sample sites. This study applies a temperature proxy from a low elevation environment. The study by Wang et al. (2020) analysed trees from a permafrost forest and most of the 'traditional' isotope ratio analysing studies used trees from growing boundaries, at high latitude or on high elevation sites. Sample site specific factors like snowmelt, monsoon events, growing period, or different growing limitation factors massively influence the temperature sensitivity. It is, therefore, difficult to compare the temperature sensitivity between the proxies from different studies.

In addition, if tree ring lignin reflects temperatures over a longer period of months compared to tree ring cellulose, the $\delta^{13}\text{C}_{\text{LM}}$ analysis in this study should also reflect temperatures over a longer period. Indeed, our results showed that the $\delta^{13}\text{C}_{\text{LM}}$ values are highly correlated with summer temperatures (June, July, and August) and thus similar to the results of the cellulose-based analysis.

Consequently, we assume that varying site-specific factors make it difficult to compare the temperature sensitivity of cellulose, whole wood, or lignin methoxy groups. It will be necessary to measure the isotope ratios of cellulose and lignin methoxy groups from the same sample set in further studies to understand the impact of cellulose and lignin deposition.

Lines 63-64 "The carbon of each annual tree ring has its origin in the atmospheric CO_2 " What about the origins of hydrogen of each annual tree ring? Please explain.

Authors: Recently, Greule et al. (2021) provided detailed information about the biosynthetic pathway responsible for $\delta^2\text{H}$ fractionation between precipitation and lignin methoxy groups in tree rings. In this study, it is considered that precipitation accumulates to soil water and further to xylem water with no substantial isotope fractionation. This is supported by a recent study of Chen et al. (PNAS, 2020). Isotopic fractionation occurs in mainly two biosynthetic processes, the transfer reaction of serine to the CH_2 -unit (ranging from 0 to -50 mUr) and the hydrogen atom transfer by certain flavoproteins with a depletion of -580 mUr down to -780 mUr (for further information, please refer to the

study by Greule et al. (2021)). Based on the mentioned constraints, $\delta^2\text{H}_{\text{LM}}$ values are directly linked to the source water and likely not influenced by further isotope fractionation such as leaf water enrichments.

See also the response to referee #1, regarding a similar comment.

Ln 137-138 "The maximum differences between two individual cores of the same tree ranged from 1.54 for F1 to 3.26 mUr for F2" Did you calculate expressed population signal for $\delta^{13}\text{C}_{\text{LM}}$? If so, how was it compared to tree rings from the same trees? Is $\delta^{13}\text{C}_{\text{LM}}$ more coherent than $\delta^{13}\text{C}$ of tree rings?

Authors: The mean EPS value of the $\delta^{13}\text{C}_{\text{LM}}$ chronology was 0.71. Correlations between the $\delta^{13}\text{C}_{\text{LM}}$ values of two cores of one tree were 0.73 for F1 (EPS: 0.85), 0.54 for F2 (EPS: 0.701), 0.6 for F3 (EPS: 0.75), and 0.31 for F4 (EPS: 0.47). The EPS signal of the mean $\delta^{13}\text{C}_{\text{LM}}$ chronology is thus similar to the two series of one tree.

Ln 418-419 "However, our results also indicate that temperature reconstructions based on stable isotope ratios of tree ring lignin methoxy groups are sensitive to low inter-series correlations." Does this mean inter-series correlations for lignin methoxy groups were lower than those for tree-ring cellulose? Please write more about the comparison between carbon and hydrogen isotope ratios of tree-ring lignin methoxy groups and tree-ring cellulose, because it is an important information for readers to judge if analysis of tree-ring lignin methoxy groups are worth trying.

Authors: We assume that sample site-specific factors are responsible for our low inter series correlations between 1940 to 1965. This study used two cores from only four trees. Irregularities in only one or two trees would massively influence our inter series signal. The study site Hohenpeißenberg is massively influenced by human activities. Factors such as tree clearing, nitrate, or water availability may conceal the climate influence factor (further limiting factors were discussed in section 4.2 of the manuscript). As these factors are strongly dependent on the sampling location it is difficult to compare the R_{bar} values of this study with other studies. The referee is certainly right, it would be interesting to compare the inter series correlation of the isotopic ratios from cellulose and lignin methoxy groups from the same sample set. Due to labour, time and financial aspects, we were not able to also measure the isotopic ratios of cellulose at this site so far. However, there are several benefits regarding stable isotope measurements of methoxy groups if compared with other isotope proxies such as cellulose. These are:

- Bulk wood samples can be used
- No time-consuming extraction procedures of single wood structural compounds
- Removal of water from the samples is not necessary
- For hydrogen isotope measurements no hydrogen exchange of covalent bound methoxy hydrogen atoms with surrounding water has been reported

For detailed discussions of these issues, we would like to refer the referee to the studies by Anhäuser et al. (2020), Greule et al. (2021, 2009, 2008), and Keppler et al. (2007). For a detailed comparison of isotope values of cellulose and methoxy groups, we would like to refer the referee again to the studies by Mischel et al. (2015) and Gori et al. (2013). These researchers show that isotopic ratios of cellulose and whole wood are highly correlated, whilst the isotopic ratios of lignin methoxy groups seem to be also controlled by different environmental and physiological factors. Measuring $\delta^{13}\text{C}_{\text{LM}}$ and $\delta^2\text{H}_{\text{LM}}$ values therefore provide us a more time-saving method to analyse additional past climate changes in a different spatial and temporal context.

Further information was added to the revised manuscript from line 51.

Figure 2 Why does moving R_{bar} suddenly decreases around 1940? Is it possible that one of your samples are wrongly dated after 1940?

Authors: We assumed that site specific factors influence the robustness of the mean inter series correlation (See chapter 4.2). The TRW measurements show no indication that one of the samples were wrongly dated after 1940.

Figure 5 If you calculate temperature from Hohenpeissenberg with temperature all over Europe, then I think you will find similar results, because temperature shows similarity over wider regions compared to precipitation. So I think this is self-evident. What do you think?

Authors: Interesting point! The local temperature at Hohenpeißenberg is highly correlated to the temperature changes mostly all over Europe. So, $\delta^{13}\text{C}_{\text{LM}}$ values also show a good correlation with large-scale temperature changes. However, having a closer look at Figure 5 shows us that $\delta^{13}\text{C}_{\text{LM}}$ values are most strongly correlated with local temperature variations. If we now compare this with the $\delta^2\text{H}_{\text{LM}}$ chronology, we can see that the $\delta^2\text{H}_{\text{LM}}$ values are mainly controlled by large-scale atmospheric phenomena. Anhäuser et al. (2020) found that atmospheric circulation such as the NAO index, meridional atmospheric transport, or the potential mixing of air masses influence the isotope signal in precipitation and thus directly the $\delta^2\text{H}_{\text{LM}}$ values. Therefore, we would like to keep Figure 5 in the revised manuscript.

Technical corrections

Ln 13 "skilful" sounds strange. What about "less successful"?

Authors: Change applied.

Ln 37 "For a more detailed overview of its applications in paleoclimate research, readers can refer to following studies"

Authors: Change applied.

Ln 41 Change " a-cellulose" to "α-cellulose" or "alpha-cellulose".

Authors: Change applied.

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