Associate editor comments:

Summary

First of all, let me thank both reviewers again for their review and you for your reply. I don't want to be too difficult but a slightly more cleaned up version of the annotated manuscript would have been nice. This multi-coloured mash up, I assume with different colours coming from different co-authors, with French comments in the side-line is not great to read. I also have some other comments. I think new proxies always come with their issues, nothing to be ashamed off. I also think old or more established proxies also have their issues, that is one of the reasons new proxies are always welcome. Multiple proxies for the same parameter and based on different compounds, organisms and analytical techniques are always great to have and use together. Therefore, I do not think you need to sell this proxy based on hydroxy fatty acids by making it very clear the more established proxy has a relatively large RMSE. It is not like your proposed proxy has no issues itself. Personally, I would have framed this, line 88 to 95, a bit different. My guess is that would have also helped with the response. The multi proxy approach is great and wonderful, but for that you need multiple proxies. Something along these lines would have been great. The "despite improvements it is still not great" statement is not necessary and completely ignore the fact that all proxies have their good and bad sides. As long as your proxy is not approaching perfection, universally applicable with extremely small errors just be careful.

I like the multiple models used. I also like the explanation of why one works better under certain conditions etc. It also clearly indicates that this is in the trail and error phase. As the authors mention, more soils need to be analysed both for the calibrations as well as applications. The application here is nice and it helps explain some of the pros and cons of the different models, but it is also fairly limited and definitely not the dataset that ends all discussion on the applicability of these models. Be careful on how much emphasis you want to put on the example

We would like to thank once again the reviewers and especially the associate editor for his positive and constructive comments. We would like to apologize for the previous unclean version of the annotated manuscript. This was corrected in the new revised version.

A detailed list of changes and arguments answering to the different comments is provided below. The line numbers are those of the annotated version of the manuscript.

We totally agree with the editor that the different molecular proxies are complementary and should be presented as such. The introduction was modified to take this comment into account:
"Even though brGDGT proxies were largely investigated over the last 10 years (e.g. De Jonge et al., 2014; Dearing Crampton-Flood et al., 2020) and were applied to various paleorecords (e.g, Coffinet et al., 2018; Wang et al., 2020), new molecular proxies, independent of and complementary to brGDGTs, are needed to improve the reliability of temperature reconstructions in terrestrial settings." Line 91
We also agree with the editor that the paleoapplication presented in this paper is only a first test of the applicability of the global calibrations proposed in the manuscript. Additional studies are needed to improve the models and further test their potential. A sentence was added at the end of the discussion to highlight this point:

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"Additional paleoapplications are also required to further test and validate the applicability of the global MAAT and pH calibrations based on 3-OH FAs presented in this study." Line 884

Minor comments:

Line 114-115: suggesting that regional relations may be more adapted to apply RAN15 as a temperature proxy in soils. I assume you mean something like "suggesting that regional or local RAN15 calibrations maybe more appropriate to apply".

This sentence was modified as follows: "...suggesting that regional or local RAN_{15} relations may be more appropriate to apply for temperature reconstruction in terrestrial settings." Line 114

Line 146-150: The sentence that starts with "In addition" and ends with "settings". This has become a very long and difficult to follow sentence.

This sentence was amended as follows: "In addition to linear regressions, non-parametric, machine learning models were used to improve the global relationships between 3-OH FA distribution and MAAT/pH. These models present the advantage of taking into account non-linear environmental influences, in line with the intrinsic complexity of the environmental settings." Line 140

Line 155-157: again without any indication on how great the 3-OH FA proxy might be the authors are very negative about a more established proxy. This is not necessary and only puts readers off.

The negative part of the sentence was removed, as it was, indeed, not necessary.
"As brGDGTs are the only microbial organic proxies which can be used for temperature and pH reconstructions in terrestrial settings so far, they can serve as a reference proxy to understand the temperature and pH dependency of 3-OH FAs analyzed in the same dataset. 3-OH FAs and brGDGTs have thus been concomitantly analyzed to assess their reliability and complementarity as independent temperature and pH proxies." Line 146

Line 386: never start a sentence with But, ok, only very rarely start a sentence with But. Why not however? Think about rewriting up until line 431. Quite difficult to follow.

Following this comment, theses sentences were rephrased:
"To overcome this limitation of the k-NN method, data selection was performed randomly on the dataset with a stratification modality according to the MAAT or the pH. This approach allows to limit the impact of extreme values as detailed below." Line 327

Line 530: order of magnitude, from 0.1 to 1? Sounds very dramatic order of magnitude, varied between 0 and 1 is much less dramatic.

This sentence was amended as follows: "The RIAN index varied between 0.1 and 0.8 among the eight elevation transects (Table 1)." Line 401

Line 662: low Ph ranges, I think you mean narrow or small.

This sentence was corrected as follows: "...supporting the hypothesis that narrow pH ranges limit the potential of obtaining linear relationships" Line 485

Line 794: are pooled very narrowly?

In order to be clearer, this sentence was modified as follows: "...where the samples from this region are pooled separately from the rest of the dataset." L.575

Line 844: "and sources of these compounds" I can guess what you try to say, but I think it is best if it is written more clearly.

This sentence was corrected as follows: "This represents a limitation, as the relative distribution of bacterial lipids can be concomitantly influenced by several environmental parameters (e.g. Véquaud et al., 2021) and can also depend on the diversity of the bacteria producing these compounds (Parker et al., 1982; Bhat and Carlson, 1992; Zelles, 1999)." Line 605

Line 845-847: Who is using bacterial relative abundances. No one measured bacterial relative abundances for this manuscript, right?

Indeed, there was a missing word in this sentence. This was amended: "In contrast, using bacterial lipid relative abundances rather than a single index in the relationships with environmental variables appears less restrictive, and more representative of the environmental complexity." Line 610

Line 937: All or the C10 to C18? If I understood it correctly there were more.

In order to be clearer, this sentence was corrected as follows: "All the 3-OH FA homologues of Gram-negative bacteria origin (i.e. with chain lengths between C_{10} and C_{18}; Wilkinson et al., 1988) were included in the models whatever their abundance to keep the maximum variability and take into account the specificity and complexity of each altitudinal transect." Line 639

It should be noted that 3-OH FAs are widely distributed in microorganisms with chain lengths up to 26 C and can be produced by e.g. yeasts, fungi, and Gram-positive bacteria in addition to Gram-negative ones. Nevertheless, in the present paper we only consider 3-OH FAs with 10 to 18 C, typical for Gram-negative bacteria (Wilkinson et al., 1988).

Line 953: whole suite limited to C10 to C18?

This was rephrased: "This model, which takes into account the Gram-negative bacterial 3-OH FAs (C_{10}-C_{18}; Wilkinson et al., 1988), presents..." L. 657

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Line 1181-1182: along some, but not all, of the altitudinal transects … At least I guess that's what you want to say.

This sentence was modified accordingly to this comment: "This may explain why linear relationships between the RAN_{15}/RAN_{17} and MAAT could be established along some, but not all, of the altitudinal transects investigated until now…" Line 674

Line 1345-1346: by working on the microbial level, please explain?

In this section, it is hypothesised that pH can influence the biosynthesis of new 3-OH FAs, and that this mechanism influences all 3-OH FAs between C_{10} and C_{18}. But this hypothesis requires the study of the membrane adaptation mechanisms of the 3-OH FAs source bacteria.
Thus, in order to be clearer, this sentence was corrected as follows:
"These results suggest that soil Gram-negative bacteria may respond to pH variations by modifying the whole distribution of associated 3-OH FAs (C_{10}-C_{18}). This would need to be further confirmed by e.g. investigating the influence of pH variations on pure strains of Gram-negative bacteria isolated from soils. Line 724

Line 1813: is it really much smaller than the 6.5 to 19.7 of the k-NN method?

In order to be more rigorous, the sentence was corrected as follows "Finally, the random forest model yielded MAAT estimates between 10.6 and 19.3°C, i.e. a smaller estimation range than the k-NN algorithm and multiple regression model (Supp. Fig. 4)." Line 790

Line 217-2019; I don't this is needed in this way. The proxies can strengthen each other, use that. I mean the information is correct, I would just frame it a less "confrontational".

The confrontational part of this sentence was removed, i.e ". This RMSE is also much lower than the one related to the latest global MAAT-brGDGT calibrations (> 4 °C; De Jonge et al., 2014; Naafs et al., 2017; Dearing Crampton-Flood et al., 2020), even though the latter are based on a larger number of soil samples than the global 3-OH FA model proposed in the present study."

In your reference database, could you please change J.S.S. Damsté into J.S. Sinninghe Damsté.

This was corrected.