

Correction of the manuscript CP-2023-15: Reconstructing 15,000 years of southern France temperatures from coupled pollen and molecular (brGDGT) markers (Canroute, Massif Central).

Léa d'Oliveira et al., July 2023

We thank the reviewers for their attentive reading and their accurate comments. We certainly appreciate the feedback they provided and have strived to improve our manuscript according to their suggestions. To summarize, we reduced the number of calibrations used for brGDGTs-based temperature reconstructions. We investigated the impact of pH variations as a confounding factor to brGDGT-based temperature reconstructions to propose a more robust climate interpretation. Following the first one, we also highlighted another period for which the detrital and brGDGTs proxies seem affected by a second response to local hydrological changes. We also provide a point-by-point account of our rebuttal, please see below.

Responses to the comments of Reviewer #1 (Anonymous Reviewer)

General comments

One concern is about the use of different modern pollen databases. It is intriguing that you select the Scandinavian calibration, given the study site location. Do you have a climatically explanation for that?

Response: Given the CAN02 sequence's location in southern France, the use of the Scandinavian and Temperate Europe (TEMPSCAND) calibration may appear odd. However, three separate meteorological currents influence Canroute peatland: oceanic from the west, Mediterranean from the south and mountainous from the north. Because of the very few Mediterranean taxa preserved in the Canroute pollen diagram and the triple meteorological influence, it is more appropriate to adopt the Scandinavian calibration, which combines pollen records from Scandinavia and Temperate Europe, so with a more corresponding vegetation. Concerning the EAPDB modern calibration, this dataset was finally not selected because, when compared to the TEMPSCAND modern calibration, even though both signals are close, the climate signal produced by the TEMPSCAND calibration shows less variability than the EAPDB one. This may be because the TEMPSCAND dataset is more regional than the EAPDB dataset, which seems to improve the reconstructed climate signal as regional datasets appear to be more reliable than global datasets (Dugerdil et al., 2021).

It is true that the MEDTEMP calibration gives slightly low R² values (0.91 for the BRT method), but the EAPDB calibration gives comparable R² values for the BRT method (0.92), although with higher RMSE values. In my view, this EAPDB calibration gives a reasonable MAAT profile and is comparable to the TEMPSCAND one. Why do you do not include it in the MAAT profile with the brGDGTs-MAAT reconstructions? Please add some discussion about that.

Response: Our goal is to select only one calibration, which might be global or regional, from the most accurate current pollen dataset. Calibrations using regional datasets tend to be more reliable than those using global datasets (Dugerdil et al., 2021), particularly for the WA-PLS. Although the EAPDB and the TEMPSCAND datasets are very similar, the TEMPSCAND current dataset is more regional, has less variability and has higher RMSE and R² values. As a result, only the TEMPSCAND calibration was used for comparison with the brGDGTs-MAAT signal.

Text modifications: The following adjustments have been made to the text to make the modern database selection at L 462-466 clear: “*The TEMPSCAND calibration produces a particularly close signal between the two methods, exhibits less variability, and has better R² and RMSE values, bolstering the reliability of the reconstructions based on this calibration database. Furthermore, calibrations employing regional datasets appear to be more reliable than those using global datasets (Dugerdil et*

al., 2021), and because the TEMPSCAND modern dataset is more regional than the EAPDB one, it will be taken into account in the subsequent discussion”.

In general, the climate variability during the Lateglacial has been characterized by warm conditions during the B-A (14.700-12.900 yr) and a cold YD period (12.900-11.700 yr) before the warm Holocene. This trend is likely showed by the MEDTEMP calibration but not really showed with the brGDGTs calibrations. The brGDGT-MAAT calibrations show similar trends to the EAPDB or TEMPSCAND calibrations. Do you have a hypothesis for that?

Response: The MEDTEMP calibration has been ruled out due to its lack of reliability when applied to the Canroute sequence. Temperature values reconstructed using the METDTEMP dataset during the Lateglacial period are excessively high, with values at least 2°C greater than the modern value. The absence of classic Lateglacial climate trends such as Bølling-Allerød and Younger Dryas in brGDGT reconstructions could be attributed to a poor record of the time caused by the low temporal resolution (low peat accumulation rate, as indicated by low PAR values). It could potentially be related to a not marked Lateglacial in this region, given Canroute is located at a low altitude, which is unusual for a sequence in this region. Similar to the reconstruction of Lateglacial brGDGTs from Lake Matese in Italy (Robles et al., 2023), the Younger Dryas is not well-marked. The “classic” Lateglacial trends are also obscured by the pollen-based signal, lending support to the brGDGTs reconstruction. Furthermore, bioturbation, which was initially not previously examined in the Younger Dryas record, may have an effect on sequence resolution at this time (Bradley et al., 2015).

Text modifications: Following the specific comment of Reviewer #2, bioturbation was added in the discussion at L 628 with the following sentence: *“The lack of typical Lateglacial events can be attributed to a low resolution of the record, possibly caused by bioturbation smoothing abrupt events (Bradley et al., 2015), a low accumulation rate (Fig. 3a) or a not very marked Lateglacial in the region”.*

Specific comments

Line 37: delete nevertheless and replace fluctuations by oscillations :

Line 38: at millennial timescale

Text modifications: Modified accordingly in L 36 for both comments with the following sentence *“The Holocene demonstrates regional climate oscillations at millennial and centennial timescales”.*

Line 38: indicated

Text modifications: Modified accordingly in L 37 as *“At a millennial scale, palaeoclimatological studies indicated the occurrence of a mid-Holocene thermic optimum”.*

Line 74: please add after palaeotemperatures depending on the type of the archive and the region

Text modifications: Precision was added according to recommendations at L 88 as *“Indices and calibrations have been developed to allow quantitative reconstruction of palaeotemperatures based on archive type and region”.*

Line 92: please add De Jonge et al., 2021 before Robles et al., 2022b

Please review references by Robles et al. along the text.

Robles et al., 2022a is now Robles et al., 2022

Robles et al., 2022b is now Robles et al., 2023

Response: References have been corrected according to recommendations on L 106.

Line 96: there is other compared pollen- and brGDGT-based studies, please add for instance Watson et al., 2018

Response: References were added to the sentence at L 117.

Line 107: Conroute peatland

Text modifications: Correction on “peatland” has been made at L 127 according to recommendations.

Line 137: Add the acronym (OMC), and then use it in line 139

Text modifications: Acronym added to the sentence at L 162.

Line 151: Did you monitor the *m/z* 1303 instead of 1302?

Response: After verification, GDGT-0 were indeed scanned for *m/z* 1302 and not 1303.

Text modifications: “*m/z* 1303” has been corrected by “*m/z* 1302” in the text at L 176.

Lines 160-161: I would denote mr and mrs as mr-1 and mr-2

Response: Due to a reducing in the number of calibrations used, one of the multi-regression calibrations has been removed.

Text modifications: The name of the mr calibration used has been simplified according to recommendations at L 186, 345, 356, 498, 507, 512, 514, 593 and 613.

Lines 161 and 162: Bayesian

Text modifications: “bayesian” corrected to “Bayesian” at L 187, 345 and 356.

Line 164: add the calibration error or RMSE for the Bayesian calibration

Text modifications: The RMSE has been added for the Bayesian calibration at L 187.

Line 261: isoGDGTs

Text modifications: “iso GDGTs” corrected to “isoGDGTs” at L 309.

Line 299: I would skip mr and mrs to refer multiple regressions, see my previous comment. You can refer as mr-1 and mr-2 and cite the corresponding reference (De Jonge et al., 2014b).

I find confusing the codes of the different soil and peat calibration used, and I would avoid the initial of the references, since they are indicated in Table 1 and along the text. Then I would replace some of them as:

Soil MBT’ (Peterse et al., 2012), Soil MBT (De Jonge et al., 2014b), Soil MBT’5Me-1 and Soil MBT’5Me-2 (Naafs et al., 2017b), Bog MBT’5Me (Naafs et al., 2017a), Index1 (De Jonge et al., 2014a), mr-1 and mr-2 (De Jonge et al., 2014b).

Text modifications: The names of each calibration used have been simplified according to recommendations and the number of calibrations has been reduced as follows:

- “Soil 5Me DJ.” (Peterse et al., 2012) has been removed.
- “Soil Index1 DJ.” (De Jonge et al., 2014a) modified to “Index1”.
- “Soil MBTp DJ.” (De Jonge et al., 2014a) modified to “Soil MBT’5Me”.
- “Mr DJ.” (De Jonge et al., 2014a) has been removed.
- “Mrs DJ.” (De Jonge et al., 2014a) modified to “mr”.
- “Soil N.” (Naafs et al., 2017a) has been removed.
- “Soil 5Me N.” (Naafs et al., 2017a) has been removed.
- “Bog N.” (Naafs et al., 2017a) was modified to “Bog MBT’5Me”.
- “Soil/peat Bayesian DCF.” (Dearing Crampton-Flood et al., 2020) was modified to “Soil Bayesian”.

Line 338: WA-PLS

Text modifications: Acronym “WAPLS” corrected “WA-PLS” at L 385.

Line 339: 1.9 °C

Text modifications: “1.9” corrected to “1.9° C” at L 396.

Line 548: the presence of the HTM in the Mediterranean region.

Text modifications: Sentence corrected as follow in the text “*the presence of the HTM in the Mediterranean region in many studies*” at L 660.

Line 570: a Late-Holocene

Text modifications: “late Holocene” corrected to “Late Holocene” at L 680.

Table 1: Please add the calibration error of each equation before the reference.

Response: RMSE of each calibration has been added in Table 1 according to recommendations.

Figure 4 Panel (a): Abundance (%), GDGT-0, GDGT-1, etc. Cren and Cren’ instead of GDGT4 and Crenach’, in consistence with the text, whereas it is referred as GDGT-0/Cren ratio.

Response: Crenarcheol isomer (Cren’ or Crenarch’) have not been identified at m/z 1292. m/z 1292 and m/z 1294 were detected and respectively correspond to Crenarch and GDGT-4 (Hopmans et al., 2016; Naafs et al., 2018).

Panel (b): you have identified some double prime isomers, please add some discussion about their significance in the manuscript. Are they detected along the whole record or just at some intervals, likely between 6.600-5.000 yr? Perhaps their distribution suggests one of the brGDGT-based calibration.

Response: We did not use their distribution in the selection of best calibrations from the CAN02 sequence because of their very low abundance at each occurrence, and the fact that, after the reducing of the number of calibrations used, only the Index1-based calibration included the double isomer compounds.

Text modifications: Double prime compounds have been added to the Introduction at L 76 as follows: “*Furthermore, studies showed the presence of methyl isomers at the C6 position (6-methyl isomer), which when excluded from the MBT index resulted in the better temperature correlated MBT’_{5Me} index*”

(Naafs et al., 2017a) and a novel C7 position (7-methyl isomer) that co-elute with the 5- and 6-methyl brGDGTs (Ding et al., 2016)”

To Methods in section 2.5.1 at L 177 as follow: “The high-performance liquid chromatography allowed for the separation of the 5-, 6- and 7-methyl brGDGTs isomers (Ding et al., 2016; Naafs et al., 2017a)”. The naming compound was changed to 7Me instead of double prime (e.g., IIa” modified to IIa_{7Me}).

In Results section 3.3.2 the sentence “Double isomers are present in very low abundances and are present only between 7,800 and 6,420 cal. BP, at two occurrences for the IIIa_{7Me} and one occurrence for the IIa_{7Me}.” has been added at L 314 to discuss the presence of the 7-methyl isomer in the sequence.

Rebuttal Figure 4:

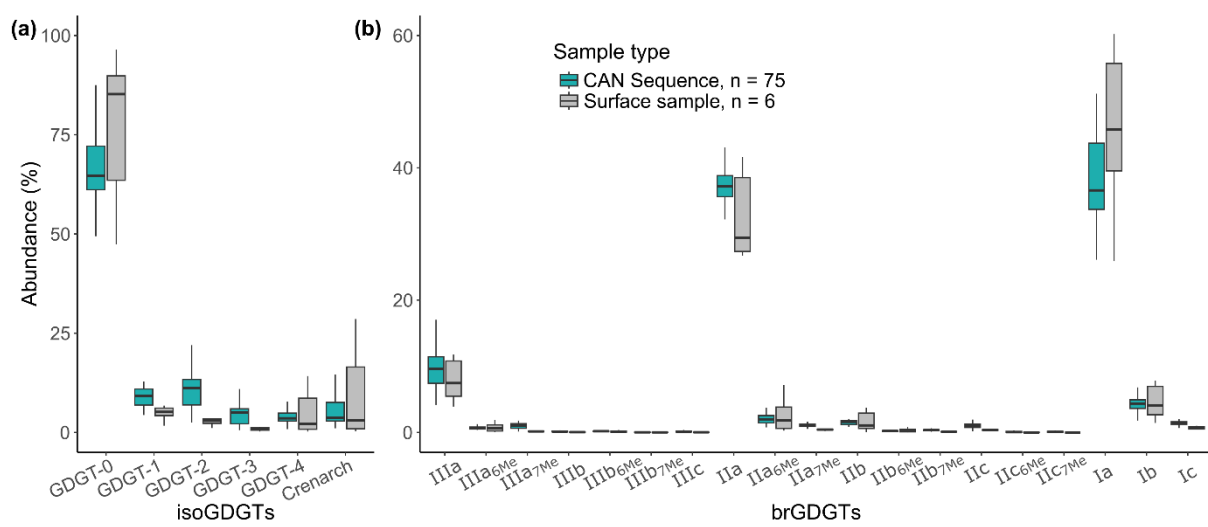


Figure 4: GDGT results: Fractional abundances (%) of (a) isoGDGT and (b) brGDGT compounds for the CAN02 sequence (n = 75, blue) and surface samples (n = 6, grey).

Figure 7: Please add different symbols for the different profile in each panel for better reading in a black and white printed version. In panel b, replace as WA-PLS

Text modifications: Different symbols for the different profiles were added.

Rebuttal Figure 7:

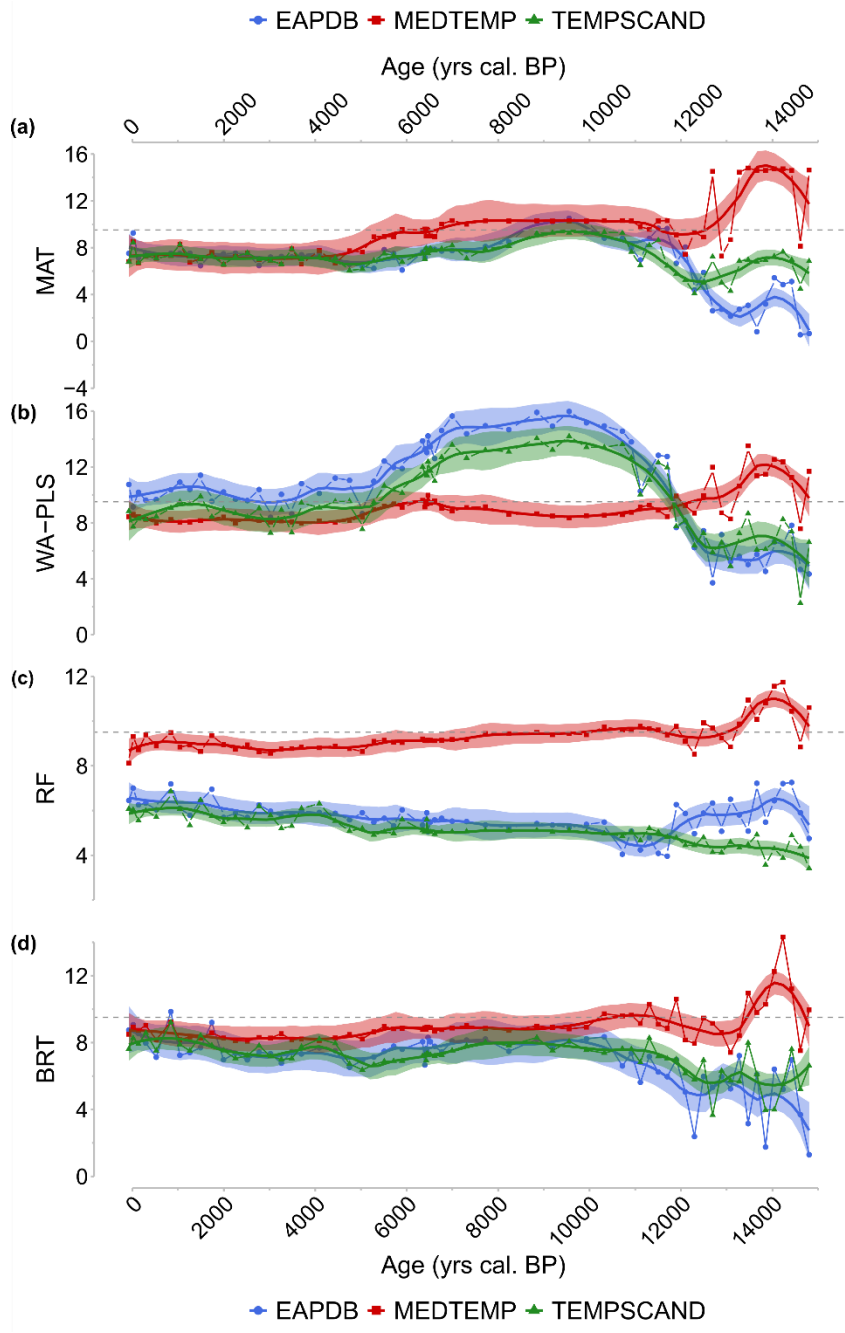


Figure 7: Climate signal from the three calibration sets EAPDB (blue), MEDTEMP (red) and TEMPSCAND (green) for the MAT (a), the WA-PLS (b), the RF (c) and the BRT (d) methods. Black dashes: current MAAT at Canroute.

Figure 8: Please rename the codes of soil and peat calibrations avoiding the initial of each reference, as Bog, Index1, Soil, mr-2, Soil MBT, mr-1, mr-2, etc. See my previous comment.

Text modifications: The names of each calibration used have been simplified in Figure 8. The modifications are detailed in a previous response addressing the same concern, please report to it for the updated names of each calibration retained.

Rebuttal Figure 8:

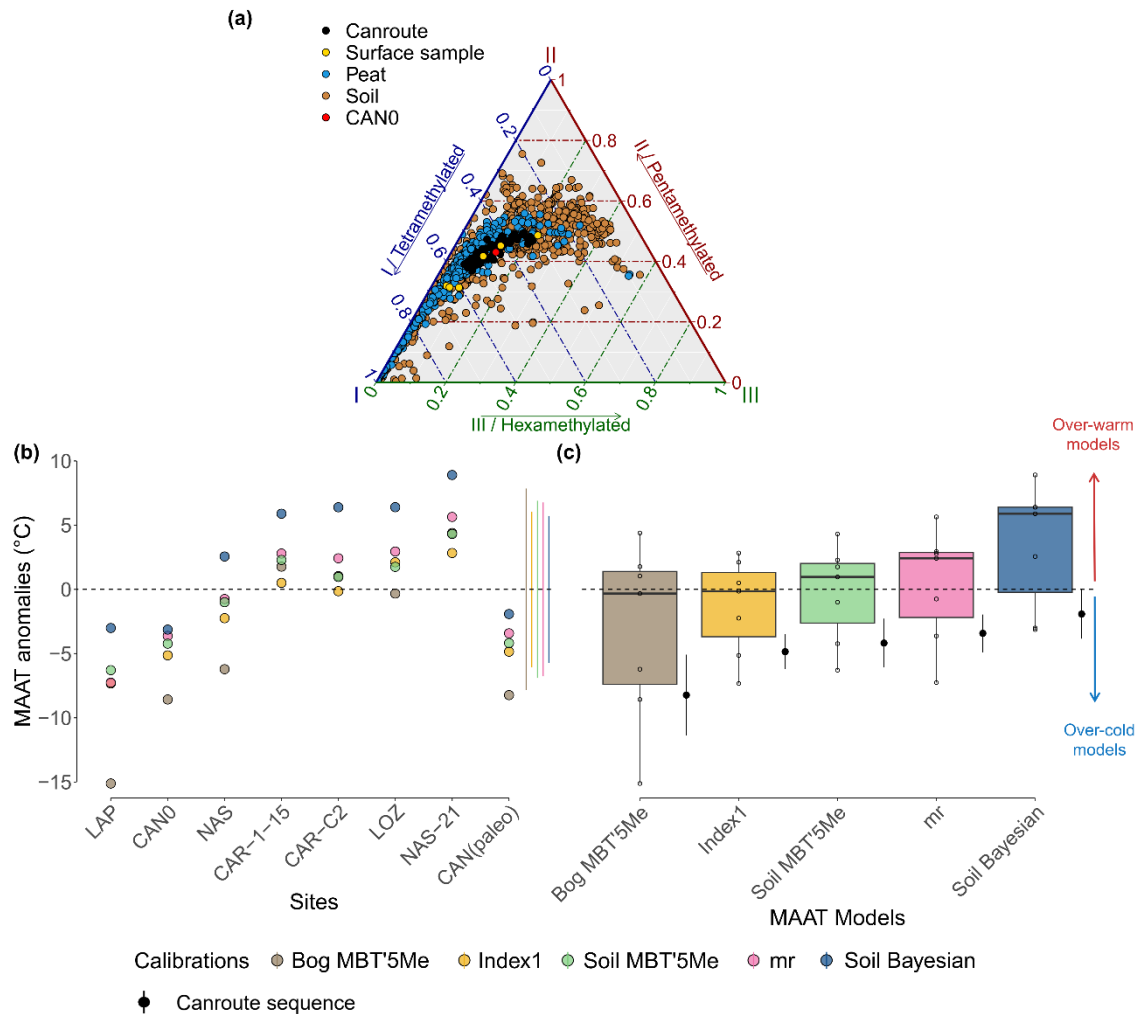


Figure 8: (a) Ternary plot of fractional abundances of tetra-, penta- and hexamethylated brGDGTs for CAN02 core samples (in black) and Massif Central surface samples (in yellow and red); and for global peat (Naafs et al., 2017b, in blue) and soil databases (Yang et al., 2014; Naafs et al., 2017a; Dearing Crampton-Flood et al., 2020, in brown). (b, c): Testing of soil and peat calibrations on surface samples and CAN02 core samples. (b): Reconstructed MAAT from each calibration expressed as anomalies with respect to the mean annual temperatures measured at the sites. The standard deviation of each calibration applied to the CAN02 sequence (palaeo) is represented by the lateral lines on the right side. (c): Boxplot representing the results of the calibrations applied to the surface samples ($n = 6$). Black points with error bars next to each calibration correspond to temperature anomalies of CAN02 core samples.

Figure 9: Please add different symbols for the different profile in each panel for better reading in a black and white printed version. Also, in panel (b), I would rename as Soil Bayesian, mr-2 and Index1 (see my previous comment). Accordingly in the figure caption, please rewrite as: Soil Bayesian (XX symbol and dark blue line; Dearing Crampton-Flood et al., 2020), mr-2 (XX symbol and light blue line; De Jonge et al., 2014b), and Index1 (XX symbol and red line; De Jonge et al., 2014b).

Response: The names of each calibration used have been simplified and symbols have been added to Figure 10 (Figure 9 became Figure 10 due to the insertion of a supplementary figure, which is discussed in depth in Reviewer #2's specific remarks).

Text modifications: The caption has been updated accordingly as follows: "CAN02 temperature reconstructions (MAAT, in °C) obtained from (a) pollen assemblages using BRT (yellow curve, triangle

shape) and MAT (blue curve, square shape) methods based on the TEMPSCAND calibration. The solid line corresponds to locally estimated scatterplot smoothing (loess) regression curves, the shaded area corresponds to its 95 % confidence interval, and (b) brGDGT signal for the three selected soil calibrations: Soil Bayesian (square symbol and dark blue line, Dearing Crampton-Flood et al., 2020), mr (triangle symbol and pink line, De Jonge et al., 2014b) and Index1 (round symbol and yellow line, De Jonge et al., 2014b). On the right, lateral lines represent calibration errors (RMSE). Finally, the black dashed line corresponds to Canroute's modern MAAT. The time highlighted in light grey reveal a large fall in tetramethylated brGDGT abundance (6,600–4,700 cal. BP) and a shift in accumulation rate. The era shown in dark grey highlights the significant decrease in IR (4,700–2,300 cal. BP).”

Rebuttal Figure 10:

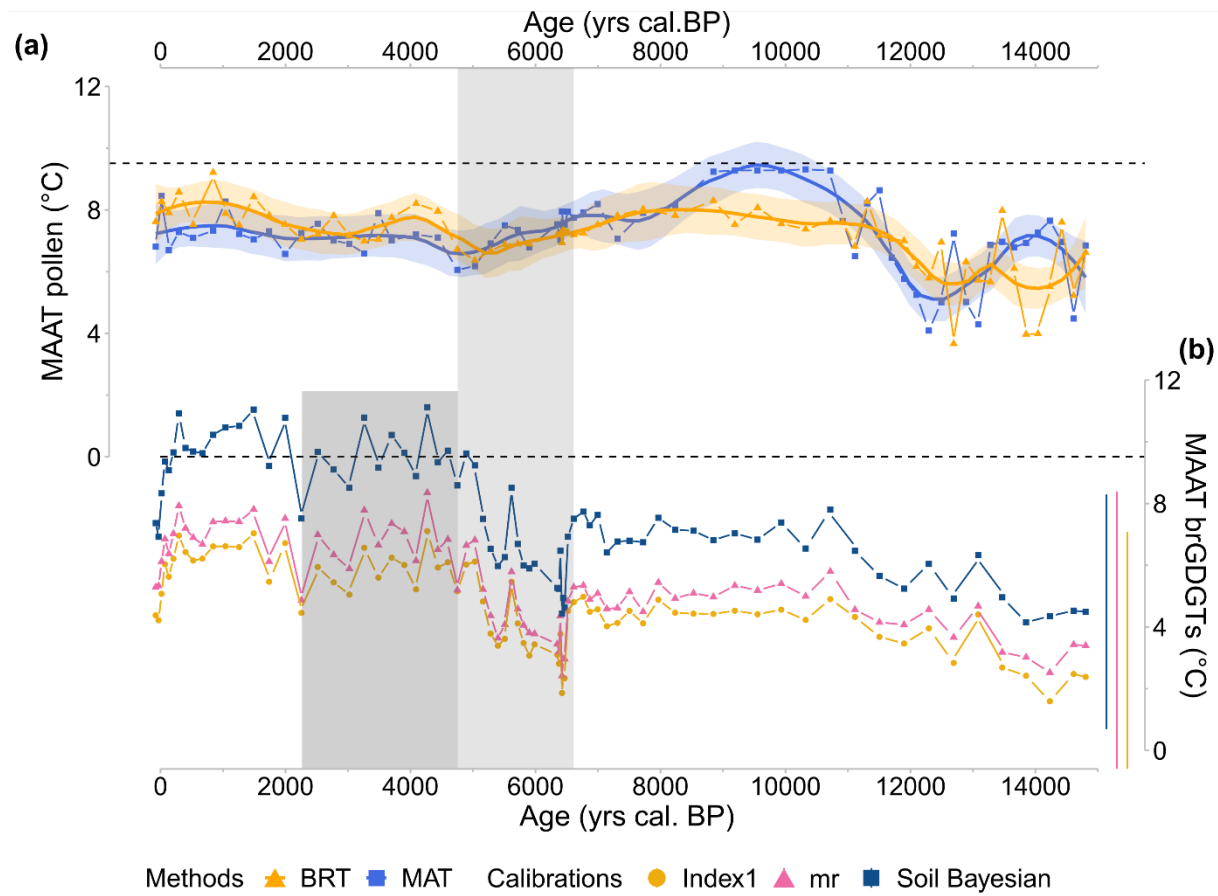


Figure 109: CAN02 temperature reconstructions (MAAT, in °C) obtained from (a) pollen assemblages obtained with using BRT (yellow triangles and curve) and MAT (blue squares and curve) methods based on the TEMPSCAND calibration. For both curves, the solid line corresponds to locally estimated scatterplot smoothing (loess) regression curves, the shaded area corresponds to its 95 % confidence interval, and (b) brGDGT signal for the three selected soil calibrations: Soil Bayesian DCF (dark blue squares and line, Dearing Crampton-Flood et al., 2020), Mrs DJ mr (triangle symbol and light blue pink line, De Jonge et al., 2014b) and Index1 DJ (yellow circles and red line, De Jonge et al., 2014b). On the right, lateral lines represent calibration errors (RMSE) are represented by lateral lines on the right side. Finally, the black dashed line corresponds to the modern MAAT at Canroute's modern MAAT. The time highlighted in light grey reveals a large fall in tetramethylated brGDGT abundance (6,600–4,700 cal. BP) and a shift in accumulation rate. The era shown in dark grey highlights the significant decrease in IR (4,700–2,300 cal. BP). The shaded period highlights the period of significant decrease in tetramethylated brGDGT abundance (6,600–5,000 cal. BP) and a shift in accumulation rate.

Responses to the comments of Reviewer #2 (Cindy De Jonge)

General comments:

Based on accumulation rate, XRF and pollen, there is a sequence of 3 different wetland/peat types. The presence of a mid-Holocene period with a low pH sphagnum peat has been recognized by the authors, but also the change into the late Holocene wetland is associated with a large change in the pH, as indicated by the CBT_{5ME} value. A change in soil/peat pH can have a significant impact on GDGT based temperature, with potential offsets in the range of 5-10 degrees Celsius (De Jonge et al., 2021, full reference see minor comments). The reconstructed pH (based on brGDGTs) should thus be discussed, to potentially constrain this impact. For this, the CBT' or IR can be calculated and used to reconstruct a pH variability. The ratios that can be used to constrain confounding factors (CI, CBT' , IR) should be reported before variations in GDGTs are interpreted as temperature (i.e. before or in section 3.3.3).

Response: We acknowledge the accuracy of this comment, and we have amended the content accordingly. The CBT' -index has been calculated to reconstruct pH values, while the IR and CI ratios were investigated to indicate potential bacterial population and brGDGT composition changes. Before interpreting GDGT-based temperatures, reconstructions of pH have been added to Fig. 3 and described in section 3.3.2, as have variations in IR, CI ratio and CBT' , which have been evaluated and discussed in section 4.3.3. The investigation of pH via the CBT' , IR and CI ratios, enabled us to highlight a second period, extending from 4,700 cal. BP to 2,300 cal. BP, during which detrital and brGDGTs data appear to show a second response to local hydrological changes reported from 6,600 to 4,700 cal. BP (light grey area highlighted in Figs. 2, 3, 4, 9). This second response's addition and modification precisions are detailed below.

Text modifications: The CBT' equation (De Jonge et al., 2014b) substituted the CBT equation (Weijers et al., 2007) in Table 1 in section 2.5.2. In addition, the IR and CI ratio equations were updated according to De Jonge et al. (2021), and the $pH_{CBT'}$ calibration equation (De Jonge et al., 2014b) was added.

The results of those proxies applied to the CAN02 sequences have been included to the text in section 3.3.2 (L 325-333) “*The CBT' index ranges from 1.58 to 0.98 ($sd = 0.05$; $n = 75$, Fig. 5b), and shows a slight continuous decrease over time. [...] The Isomer Ratio (IR) values range from 0.03 to 0.13 with the lowest values obtained between 4,700 and 2,300 cal. BP (Fig. 5d). Throughout the record, the Community Index (CI) values range from 0.30 to 0.55 and are less than 0.65 (Fig. 5e). The period from 15,000 to 4,700 cal. BP has mean CI values of 0.38, but the period from 4,700 to -80 cal. BP has higher mean CI values of 0.49. The pH varies from 4.6 to 5.6 and shows a little continuous decline over time (Fig. 5f)*”.

These results were then discussed in section 4.3.3 (L 530-552) for the interpretation of the climatic signal as follows: “*Past vegetation and detrital activity revealed the presence of three distinct local conditions in the peat, which can result in large pH fluctuations because plants influence soil and peat pH (De Jonge et al., 2021). Changes in pH can alter the fractional composition of brGDGTs and the bacterial community, influencing the MBT'_{5Me} -based temperature. CBT' and IR show the increase of 6-methyl brGDGTs as pH increases. IR and CBT' values in the CAN02 sequence are at their lowest from 5,000 to 2,300 cal. BP (Fig. 3b, Fig. 5d, dark grey shaded area), resulting in a modest decrease in pH values (Fig. 3e). There is no change in the MBT'_{5Me} during this time period, indicating that the change in brGDGTs composition has no effect on the MBT'_{5Me} employed for temperature quantification. [...] Temperature reconstructions can be affected by changes in the bacterial community of brGDGT producers (De Jonge et al., 2019), which can be studied using variations in CI values (De Jonge et al., 2021). Although the CI values in the CAN02 sequence, do not exceed the 0.65 thresholds established by De Jonge et al. (2021), a significant shift in value indicates a potential change in bacterial community composition from 6,600 to 4,700 cal. BP (Fig. 5e), implying that the temperature interpretation during this period should be done with caution.*”

On Figs 3, 4, 9 and 10, a dark grey area highlights the second response period displayed by detrital and brGDGTs records. A new section (section 4.4 *Temporality of proxies' resilience to environmental changes*) was added to the discussion to investigate the apparent double response of detrital and brGDGTs proxies. The following text has been added: “*There are two distinct periods for which proxy records are impacted by environmental influences, notably a hydrological change in the peatland (Figs. 3, 6, shaded areas). The record of vegetation (Fig. 6), detrital signal (Fig. 3) and brGDGT compounds (Fig. 5) all show a first response to a loss of water runoff on the wetland surface between 6,600-4,700 cal. BP, the result of either a reduced water supply from a change in the river system or the natural rise of peatland surface that gradually isolated peat-forming vegetation from groundwater. Two of the three proxy (detrital signal and brGDGT compounds) exhibit a second response to the hydrological shift between 4,700 and 3,000 for the detrital signal and 2,300 cal. BP for the brGDGT compounds (Figs. 3, 5, D1, dark grey area). To explain the timing in the proxies' responses to environmental changes, a distinct resilience, depending on the proxy, might thus be postulated. (Walker 2004) defines resilience as the system's ability to absorb disturbance and reorganize while experiencing change in order to retain essentially the same function, structure, identity, and feedback. In this study, vegetation appears to be more resilient than the detrital signal and brGDGT compounds, returning to equilibrium faster. Both the sedimentological and brGDGT signals demonstrate a synchronous reaction to vegetation, as well as a second response after the vegetation has returned to equilibrium. Furthermore, brGDGTs appear to have less resilience than sedimentological signals because the return to equilibrium is not recorded before 2,300 cal. BP, whereas the detrital signal is recorded before 3,000 cal. BP. However, it is unknown if this second response to environmental changes affects brGDGTs distribution and bacterial community composition*”.

The authors have employed a wide variety of brGDGT proxies, including several that have been considered outdated or less relevant in literature (the same manuscripts that propose the proxies). Afterwards, the selection of the ‘best’ temperature reconstructions seems to rely on which temperature trend matches better with expected temperature variability. I ask the authors to reduce would the number of ratios used and to not to exclude temperature reconstructions based on whether they match expected variability. The comparison, of offsets with current MAAT all falls within the calibration error, and can not be used as a real argument for selection between calibrations.

Response: The number of calibrations has been reduced from 9 to 5, and their selection is justified in section 2.5.2. We eliminated the calibration based on the MBT' proxy (Peterse et al., 2012), the two based on the MBT'_{5Me} proposed by Naafs et al. (2017b) as they do not improve the calibration based on the MBT'_{5Me} proposed by De Jonge et al. (2014a) and one of the multi regression-based calibrations proposed by De Jonge et al. (2014a). The selection of the best calibration is a difficult and determinant issue that cannot rely solely on the statistical parameters produced by the calibration itself (i.e. the R² and RMSE values for each calibration), especially since these statistical parameters do not take into account the Canroute sequences' local peculiarities. To discuss the impact of calibrations on Canroute MAAT-reconstructions, we propose discussing the scattering of surface samples-reconstructed MAAT from each calibration (the lower the scatter, the better the calibration) and the standard deviation value of each calibration when applied to the CAN02 sequence. For example, the bog calibration based on MBT'_{5Me} (Naafs et al., 2017a) demonstrates high variability for the CAN02 sequence and most surface samples, allowing us to discard it from the final temperature interpretation.

Text modifications: At L 188, the calibration selection has been justified as follows in the text “*Due to the removal of the pH-dependent 6-methyl brGDGTs, MBT'_{5Me} and Index1-based calibrations allow to overcome the substantial correlation between MBT and soil pH (De Jonge et al., 2014b). Multiple regression connects the MAAT with the fractional abundances of tetra- and penta-methylated brGDGTs and show a little accuracy improvement over MBT'_{5Me}-based calibration (De Jonge et al., 2014b). The intuitive reasoning of the relationship between MBT'_{5Me} and MAAT can be respected using Bayesian-based calibration (i.e., brGDGTs-producing bacteria respond to temperature changes, not the other way around) (Dearing Crampton-Flood et al., 2020). The Bayesian calibration employed in this study refers to Dearing Crampton-Flood et al. (2020)'s threshold-based calibration, which calibrates the MBT'_{5Me} index to the average temperature of all months with an average temperature above freezing.*

Changes in peat pH can have a significant impact on brGDGTs-based temperature, hence pH reconstruction based on brGDGTs, has been examined (De Jonge et al., 2021). The CBT'-based calibration was utilized (De Jonge et al., 2014b)"

The PCAs seem to be based on non-standardized counts (for XRF) and abundances (for GDGTs). Please consider recalculating these based on standardized relative abundances to show compositional variability.

Response: The PCA for brGDGTs was previously based on component fractional abundances, i.e., on standardized relative data. PCA for XRF values has been reapplied to standardized counts (Ti count standardisation) and replaced in Fig. 3 (see the Specific comment section for the rebuttal Figure 3).

Text modifications: In section 2.7, the following sentence has been added at L 258 *"Additionally, element counts have been normalized over the Ti element (Davies et al., 2015)"*.

Specific comments

L 14: Latitude of data, I suggest to replace by latitude of record, or even latitude of the lake?

Text modifications: "Latitude of data" corrected by "latitude of record" at L 14.

L 17, add the word 'change' after 'vegetation and climate'.

Text modifications: "Change" added after "vegetation and climate" at L 17.

L 39. Specify that this is a temperature optimum.

Text modifications: The sentence has been corrected as follows in the text at L 38: *"the occurrence of a mid-Holocene thermic optimum called the "Holocene thermal maximum" (HTM)"*.

L 51. Mediterranean basin? Is this the region you would refer to describe the general area of the peat core?

Text modifications: "Mediterranean basin" has been changed to "southern Europe" at L 41. Canroute, located in the south of the Massif Central (France) belongs to the southern part of Europe.

L 53. What do the authors mean with 'site effect'? Also the impact of erosion on the interpretation of the terrestrial archives is mentioned too briefly and therefore not clear.

Text modifications: "Side effects" has been replaced by "elevation" at L 52 and the following sentence has been added to clarify our meaning: *"Erosion has an impact on the detrital contribution to terrestrial archives; its dynamics are linked to, but not solely to, climate changes. Land clearing, for example, can increase detrital activity and so impact the terrestrial record (van Andel et al., 1990)"*.

L 62. Indicate that this is not a complete representation of the diversity and amount of lacustrine studies where GDGTs are used for paleoclimate reconstruction.

Text modifications: Corrected in the text at L 62 with the following sentence *"However, few research based on GDGTs have been conducted on the continental realm thus far, with the majority focused on lacustrine environments (c.f., Sun et al., 2011; Sinninghe Damsté, 2016; Russell et al., 2018)"*.

L 63. The temperature dependency of isoGDGTs is not a subject of this paper. Please remove it from the introduction. Instead, the GDGT0/cren ratio is used in the paper, but not introduced. Include the literature on this ratio in the introduction.

Also, the CI ratio (based on brGDGTs) is used but not introduced. Include the literature on this ratio in the introduction.

Text modifications: Modified accordingly at Ls 79-88 as follow “*De Jonge et al. (2019) revealed that brGDGTs had a varied relationship with temperature and pH in "warm" and "cold" bacterial communities, demonstrating that those correlations are also reliant on the bacterial population. A "community index" (CI ratio, De Jonge et al., 2019) has been defined to assess whether there is a change between the bacterial community and temperature or pH. De Jonge et al. (2019) determined a threshold value of 0.64 to separate the two groups of bacterial communities. This figure shows that, if the CI ratio is exceeded, a shift in the bacterial communities might be predicted, perhaps affecting the relationship between brGDGTs and temperature or pH (De Jonge et al., 2021). Furthermore, edaphic factors such as anoxic/oxic conditions have an impact on GDGT production and bacterial communities (Weber et al., 2018). Because crenarchaeol and GDGT-0 can be derived from Group I Crenarchaeota, the GDGT-0/Cren ratio can be used to investigate the presence of methanogenic archaea that thrive in anoxic conditions in sediments, whereas methanogenic Archaea synthesize GDGT-0, but no crenarchaeol (Blaga et al., 2009). The lower the ratio, the lower the anoxic conditions”.*

L 71. Include the recent studies from Halamka et al. (2023) and Chen et al. (2022) here, that have shown GDGT production in a bacterial culture. Zeng et al. (2021) and Sahonero et al. (2021) are better references to support the statement that the producers of brGDGTs are still a subject of investigation.

1. Zeng, Z. et al. Identification of a protein responsible for the synthesis of archaeal membrane-spanning GDGT lipids. *Nat Commun* 13, 1545 (2022).
2. Sahonero-Canavesi, D. X. et al. Disentangling the lipid divide: Identification of key enzymes for the biosynthesis of membrane-spanning and ether lipids in Bacteria. *Science Advances* 8, eabq8652 (2022).
3. Halamka, T. A. et al. Production of diverse brGDGTs by *Acidobacterium Solibacter usitatus* in response to temperature, pH, and O₂ provides a culturing perspective on brGDGT proxies and biosynthesis. *Geobiology* 21, 102–118 (2023).
4. Chen, Y. et al. The production of diverse brGDGTs by an *Acidobacterium* providing a physiological basis for paleoclimate proxies. *Geochimica et Cosmochimica Acta* 337, 155–165 (2022).

Text modifications: References added at Ls 71-72.

L 72. More recently, Naafs et al. (2021) also support that the structure of the membrane lipids determines membrane fluidity.

1. Naafs, B. D. A., Oliveira, A. S. F. & Mulholland, A. J. Molecular dynamics simulations support the hypothesis that the brGDGT paleothermometer is based on homeoviscous adaptation. *Geochimica et Cosmochimica Acta* 312, 44–56 (2021).

Text modifications: References added at L 74.

L 78. Dearing Crampton Flood does not discuss lake sediments (but instead soils and peats, as they don't find a difference between the temperature dependency of these groups). Include a correct reference(s) here (see suggestions below).

1. Raberg, J. H. et al. Revised fractional abundances and warm-season temperatures substantially improve brGDGT calibrations in lake sediments. *Biogeosciences* 18, 3579–3603 (2021).

2. *Martinez Sosa, P. et al. A global Bayesian temperature calibration for lacustrine brGDGTs. (2020).*
3. *Russell, J. M., Hopmans, E. C., Loomis, S. E., Liang, J. & Sinninghe Damsté, J. S. Distributions of 5- and 6-methyl branched glycerol dialkyl glycerol tetraethers (brGDGTs) in East African lake sediment: Effects of temperature, pH, and new lacustrine paleotemperature calibrations. Organic Geochemistry 117, 56–69 (2018).*

Text modifications: References added at L 93.

L 81. ‘specificity of the brGDGT proxy’ is too vague, consider removing as the confounding factors of brGDGT temperature dependency are explained in more detail below.

Text modifications: Sentence removed from L 97.

L 83: For brGDGTs in soils specifically the impact of pH change is a demonstrated confounding factor. This should be mentioned here with a reference, suggestion given below.

1. *De Jonge, C. et al. The influence of soil chemistry on branched tetraether lipids in mid- and high latitude soils: implications for brGDGT- based paleothermometry. Geochimica et Cosmochimica Acta (2021) doi :10.1016/j.gca.2021.06.037.*

Text modifications: reference and pH as a confounding factor were added at L 99 as follows “*other factors, including as human activities, biological processes, edaphic conditions, pH shift, and so on, can influence their distribution and/or abundance (Sugita et al., 2006; Huguet et al. 2010; Martin et al., 2020; De Jonge et al., 2021; Ponel et al., 2022)*”.

L 85-94. This part can be restructured, it goes back and forth between GDGTs and pollen. Perhaps the authors can add here that the residual error in the most recent brGDGT calibrations is still large, which is part of the reason why brGDGTs have not been used often in Holocene temperature reconstructions, as the expected temperature range is small, compared to the error in the calibration.

Text modifications: Paragraph restructured, and text modified at Ls 105-113 as “*Production of molecular biomarkers, such as brGDGTs, may also differ depending on the source, edaphic parameters (e.g., anoxic/oxic conditions), soil type and vegetation (Weber et al., 2018; De Jonge et al., 2021; Robles et al., 2022). Human activities, like deforestation and agriculture, can disturb the natural record of the vegetation-climate interaction, resulting in a biased quantitative reconstruction of climatic parameters from pollen data (Seppä and Bennett, 2003; Birks and Seppä, 2004). Furthermore, several studies document anthropogenic impacts on bacterial communities, demonstrating that reconstructions based on brGDGTs might be disrupted by human intervention in specific contexts, such as watersheds (Martin et al., 2019). In addition, due to the still significant calibration errors ($\pm 3.8^\circ\text{C}$ to $\pm 5.5^\circ\text{C}$), brGDGTs-based paleoclimate quantification should be interpreted with caution for periods with small temperature changes ($< 2^\circ\text{C}$), as it is the case for the Late Holocene (last 2,000 years) (Naafs et al., 2019).*”

L 96. Can the authors comment on whether these studies with combined pollen and GDGTs allowed to reach a more robust interpretation? This would further support the approach used here.

Text modifications: Comment added at L 114 as “*Although some studies [...] allowing to reach complementary and more robust interpretations*”.

L 109. Can oceanic affinity be rephrased? Would this be typical for coastal environments? Or do the authors mean something else?

Response: Canroute is influenced by three distinct climate regimes: oceanic, mountainous and Mediterranean. Although the Canroute peatland is not near the shore, and hence not associated with

coastal environments, the oceanic climate can exist further inland if there are no topographical obstacles such as mountains nearby to impede air masses, as is the case along the Aquitaine basin. Canroute peatland is thus subject to a western Atlantic Ocean effect, which influences the vegetation present in the peatland.

Text modifications: “Oceanic affinity” has been rephrased to “associated with the western Atlantic Ocean influence” at L 129.

L 110. Atlantic Ocean influence from the east instead?

Response: Given Canroute peatland’s location (Southern Massif Central), we consider the CAN02 sequence exposed to Atlantic air masses flowing from the country’s west coast. That is why, at Canroute, we consider the oceanic influence coming from the west.

Text modifications: To clarify our meaning, the sentence at L 131 was modified to “*The influence of the Atlantic Ocean from the west due to air masses arriving from the country’s west coast, which are not prevented by any topographical obstacles in the Aquitaine basin*”.

L 128. Is peat material influenced by a reservoir age/ hard water offset when performing 14C measurements? Please add if any corrections were performed.

Response: Peat material is not expected to be influenced by reservoir age or hard water offset.

Text modifications: The information has been added to the manuscript with the following sentence at L 155 “*No reservoir effects corrections were performed on the 14C measurements*”.

L 138. A first calcination. Was there a second heating step? IF not, remove ‘first’.

Text modifications: “First” has been removed as per recommendations at L 163.

L 151. Was 1303 scanned for GDGT-0? Why is this (I had the impression that 1302 was more commonly used)? Are the GDGTs 1-3 used in the manuscript? If not, you can remove their masses here.

Response: After verification, GDGT-0 were indeed scanned for m/z 1302 and not 1303.

Text modifications: “ m/z 1303” has been corrected by “ m/z 1302” in the text at L 176.

L 153, can the authors add the number of compound ($n=xx$), when they say ‘all GDGTs’? Usually fractional abundances are reported calculated either relative isoprenoid or branched GDGTs.

Text modifications: Precision on the number of compounds have been added in the manuscript at L 179 with the following sentence “*the proportion of the compound to the sum of all iso- or brGDGTs ($n = 6$ and 19 respectively)*”.

Table 1: please write explicitly when 5 and 6 methyl compounds are added in the ratios. (Fi CBT, is this ratio based on $Ib + Iib_{5ME} + Iib_{6ME}$? Also, is I in fact $Ia + Ib + Ic$? Write out in full for clarity. The a, b and c suffixes should not be in subscript.

Text modifications: Precision was added when 5 of 6 methyl compounds were used (5Me or 6Me respectively in the equations).

Modifications in Table 1:

- MBT_{5Me}: I modified to Ia + Ib + Ic
- Index1: IIa_{6Me} and IIa_{7Me} modified to IIa_{6-7Me}. The same modification has been made for IIIa_{6Me} and IIIa_{7Me}.
- IR_{6Me} has been modified to IR: IIc and IIIc were removed from the equation according to De Jonge et al. (2021).
- CI ratio: IIa and IIIa modified to IIa_{5Me} and IIIa_{5Me}.

L 171. Please add magnification of the microscope.

Text modifications: Magnification of the microscope was added at L 203 as “*the pollen was identified [...] under a light microscope at a standard magnification of × 400*”.

L 173. Please make the complete dataset be available as supplementary materials.

Response: Complete dataset will be available with the latest version of the paper.

Section 2.5.2. It is not clear what the selection of GDGT based ratios is based on. For instance the MBT' with CBT correction does not make much sense, as it introduces error that has been resolved with the updated chromatographic method used here. I suggest removing this calibration and consider narrowing down further the suite of calibrations used. At the least, the use of each selected calibration should be motivated in section 2.5.2, along the Ls of section 2.6.2.

Response: We admit that the choice of calibrations has not been fully explained and that some of the proxies used were not ideal among the most recently developed calibrations. As a result, calibration based on MBT' and CBT has been eliminated. Furthermore, two MBT'_{5Me} calibrations were removed.

Text modification: The selection of the 5 remaining calibrations has been motivated in section 2.5.2. as follows (L 188-197) “*Due to the removal of the pH-dependent 6-methyl brGDGTs, MBT'_{5Me} and Index1-based calibrations allow to overcome the substantial correlation between MBT and soil pH (De Jonge et al., 2014b). Multiple regression connects the MAAT with the fractional abundances of tetra- and penta-methylated brGDGTs and shows a little accuracy improvement over MBT'_{5Me}-based calibration (De Jonge et al., 2014b). The intuitive reasoning of the relationship between MBT'_{5Me} and MAAT can be respected using Bayesian-based calibration (i.e., brGDGTs-producing bacteria respond to temperature changes, not the other way around) (Dearing Crampton-Flood et al., 2020). The Bayesian calibration employed in this study refers to Dearing Crampton-Flood et al. (2020)'s threshold-based calibration, which calibrates the MBT'_{5Me} index to the average temperature of all months with an average temperature above freezing. Changes in peat pH can have a significant impact on brGDGTs-based temperature, hence pH reconstruction based on brGDGTs, has been examined (De Jonge et al., 2021). The CBT'-based calibration was utilized (De Jonge et al., 2014b)*”.

L 208. It would be interesting to see selected other temperature reconstructions, for instance seasonal reconstructions, based on the current discussion on the seasonality of the HTM.

Response: Based on the current debate on the seasonality of the HTM, we agree that investigating Canroute seasonal characteristics is worthwhile. The study of the Mean Temperature of the Warmest month (MTWA) at Canroute is still ongoing and has not been included in the scope of this publication. The seasonality of the Canroute sequence may be the subject of a separate study or integrated into another. As a result, the seasonal reconstruction at Canroute is not covered in this paper.

Also, precipitation changes should be plotted, as the change between peat and lake (or lake depth) will also influence the distribution of brGDGTs.

Response: Precipitation changes inferred from Canroute pollen data, noted MAP in the manuscript, have been added to the discussion and compared to brGDGT-based pH reconstruction in section 4.3.3

but independent precipitation proxies are required. A new figure (Fig. 9, see below) has been added to the manuscript to compare the brGDGT-based pH reconstruction and the pollen-based MAP reconstruction.

Text modifications: The following paragraph was added to the discussion in section 4.3.3 (L 536-547). “Soil/peat pH is also related to global climate patterns via precipitation, meaning that changes in precipitation dynamics over time might cause pH variations (De Jonge et al., 2021). Pollen-based precipitation changes (MAP) at Canroute can be confronted to brGDGTs-based pH reconstruction to try to differentiate the effects of climate and bacterial communities on pH variation (Fig. 9). The same methods and calibration as MAAT were utilized for the MAP signal, namely the BRT and MAT methods with the TEMPSCAND calibration. The MAP and pH signals do not appear to correspond well, as the wettest periods (from 11,500-8,500 cal. BP and 4,500 cal. BP onwards) are not associated with a noticeable decrease in pH (Fig. 9). This shows that precipitation dynamics have little effect on pH in Canroute peatland. Precipitations, which are normally acidic can cause a low pH in ombrotrophic peatland (water supplied primarily by precipitation) (Sennès, 2004). Canroute, on the other hand, is a soligenous peatland, and because most of its water supply originates from streams and springs (Julve, 1994), its local vegetation is less affected by precipitation dynamics. This shows that pH variations are produced mostly by local vegetation and detrital changes, which are influenced by local hydrological conditions.”

Rebuttal Figure 9:

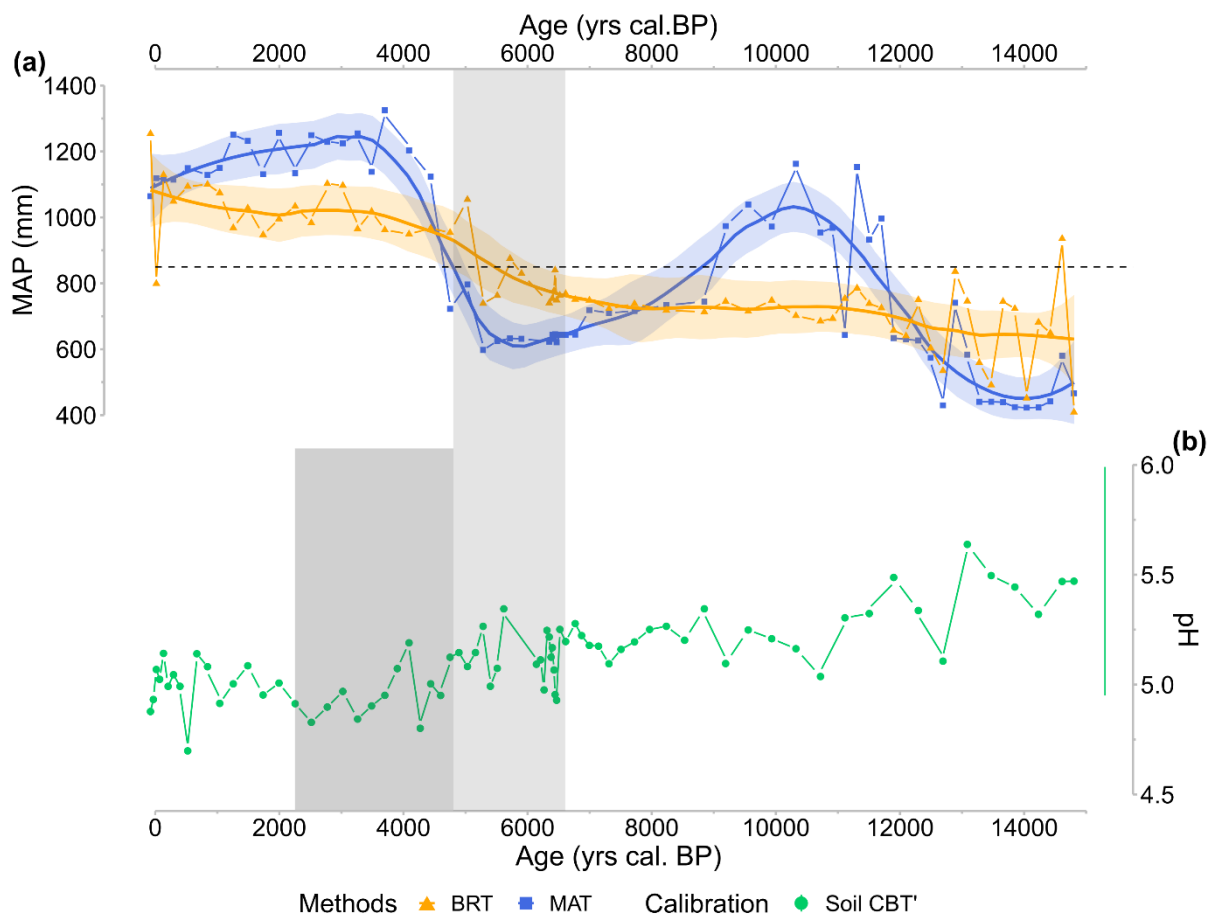


Figure 9: CAN02 (a) pollen-inferred reconstructions of the annual precipitation (MAP, in mm) obtained with BRT (yellow squares and curve) and MAT (blue triangles and curve) methods based on the TEMPSCAND calibration. The solid line corresponds to locally estimated scatterplot smoothing (loess) regression curves, the shaded area corresponds to its 95 % confidence interval. (b) CBT'-based pH

reconstruction (green circles and line, De Jonge et al., 2021). On the right, the lateral line represents calibration error (RMSE). The time highlighted in light grey reveals a large fall in tetramethylated brGDGT abundance and a shift in accumulation rate (6,600–4,700 cal. BP) and a shift in accumulation rate. The era shown in dark grey highlights the decrease in CBT' and IR values (4,700–2,300 cal. BP).

L 221. This statistical treatment is not so common and needs to be explained. What is the effect?

Text modifications: This statistical procedure is now described in the manuscript on L 255 as “Missing value imputation reduces the loss of information caused by missing values, lowering the ability to discern patterns (Dray and Josse, 2014). Regularized imputation entails filling in missing values with values selected from a Gaussian distribution, with mean and standard deviation estimated from observed values (Josse and Husson, 2016)”.

The PCA based on XRF shows (unexpectedly?) that all elements plot positively on PC1. What standardization was done on the XRF values before analysis using a PCA? Performing this analysis on the standardized counts can result in visualizing the real variance in elemental composition.

Response: There was no standardization before utilizing a PCA in the prior edition of the manuscript. We recognize the requirement to standardize XRF values before applying a PCA, thus we standardized our data on the Ti element for both XRF interpretation and PCA computation (Davies et al., 2015).

Text modifications: on L 258, the following sentence has been added “Additionally, element counts have been normalized over the Ti element (Davies et al., 2015)”. Moreover, the PC2 has been added to Fig. 3 to improve understanding of PCA results.

Rebuttal Figure 3:

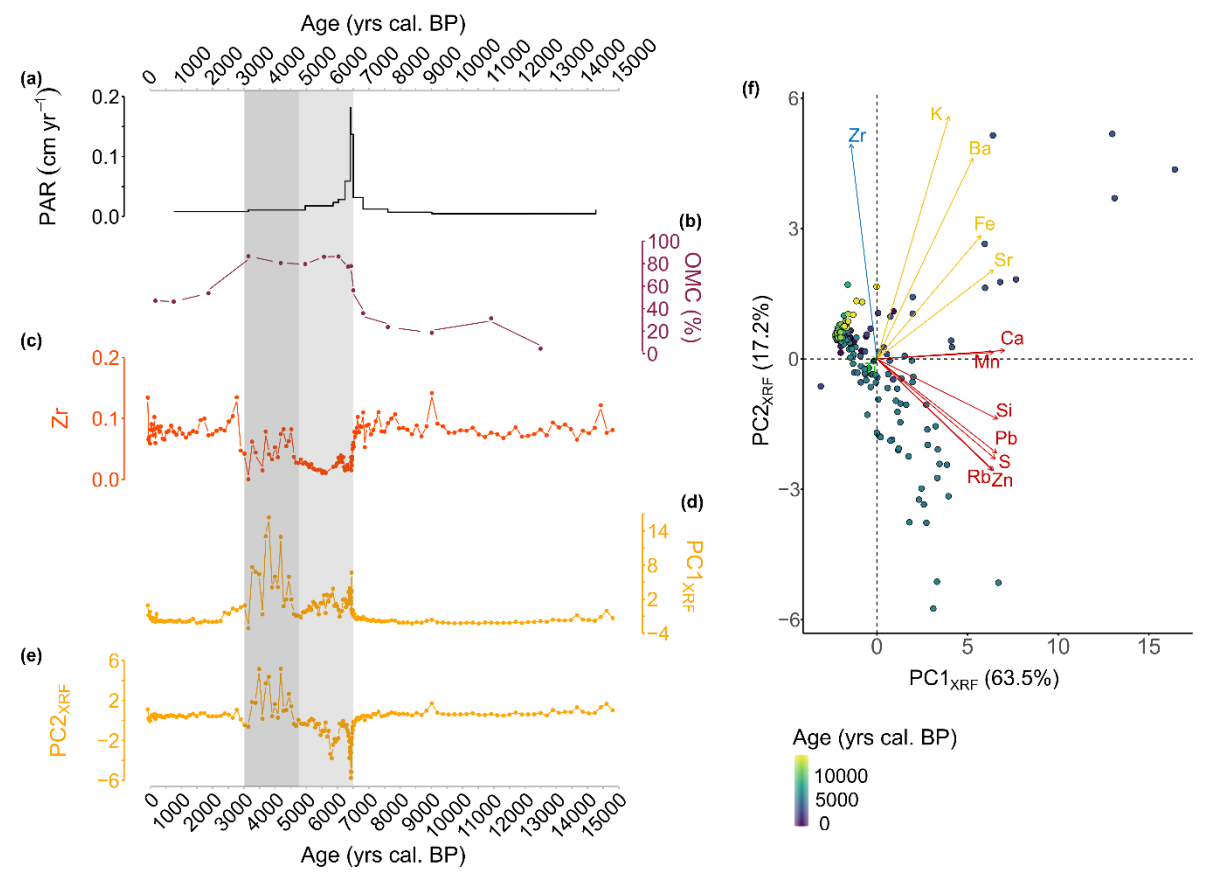


Figure 3: Geochemical data from the CAN02 sequence. (a): Peat accumulation rate (PAR) calculated from the age-depth model (cm yr^{-1}). (b): Organic matter content (OMC, %) derived from loss on ignition (LOI). First dimension (PC1_{XRF}) extracted from the principal component analysis (PCA) made on XRF data. (c) Zr relative counts, standardized on Ti element. (d): Organic matter content (OMC, %) derived from loss on ignition (LOI). First dimension (PC1_{XRF}) extracted from the principal component analysis (PCA) made on the XRF data. (e): Second dimension (PC2_{XRF}) extracted from the principal component analysis (PCA) made on the XRF data. (f): Principal component analysis (PCA) of the XRF signal. The principal components are grouped into three ~~four~~ clusters. Samples are coloured according to the age gradient (yrs cal. BP). One (a) to (e) shaded periods corresponds to the period between 6,600 and ~~5,000~~ 4,700 cal. BP when the accumulation rate increases and detrital activity decreases (light grey period) and between 4,700 and 3,000 cal. BP when detrital activity increases and detrital input dynamics changes (dark grey period).

L250. Fig. 4 caption. “Zr element” is not enough information. Are counts plotted? Relative counts?

Text modifications: Corrected to “Zr relative counts” at L 294.

Fig. 4. Is the compound plotted crenarchaeol or crenarchaeol isomer? These are generally plotted as separate compounds. Don’t write 1-4 as subscript.

Response: After verification, the compound plotted is Crenarchaeol, Crenarchaeol isomer was not identified in our study.

Text modifications: Correction and precision have been added to clarify.

The double prime (fi IIa’’) compounds are not mentioned before. Include in introduction and methods.

Response: Responses and changes are listed following the specific comment of Reviewer #1.

L 281-285. Please rephrase, the meaning of this sentence is not clear.

Text modifications: Sentence rephrased at Ls 334-340 as “PCA on *brGDGT* relative abundances reveals that $\text{PC1}_{\text{brGDGTs}}$ and $\text{PC2}_{\text{brGDGTs}}$ account for more than 58 % of the variance in *brGDGT* compounds ($\text{PC1}_{\text{brGDGTs}}$ 45.6 %, $\text{PC2}_{\text{brGDGTs}}$ 13.1 %, Fig. C1d, e). The cluster analysis revealed three clusters, delimited by depth (cluster 1: 15,000-5,400 cal. BP; cluster 2: 5,400-2,300 cal. BP; cluster 3: 2,300-0 cal. BP), and demonstrated that the lower half of the sequence (cluster 1) is positively correlated with most *brGDGT* compounds (except Ia, Ib and Ic), while the upper half (cluster 2 and 3) is negatively correlated. That is, the lower half of the sequence (15,000-5,400 cal. BP) has a high abundance of most *brGDGT* compounds, whereas the upper half (especially 5,400-2,300 cal. BP) has a high abundance of primarily Ia.”

L 282. It took me some time to find this figure in the Appendix. Fig. Dd is not self-explanatory, please correct.

Text modifications: Corrected as Fig. C1d as the Appendix Fig. C has been removed and as per CP guidelines.

Fig. 5e. The interpretation of this panel is not helped by the many temperature reconstructions plotted. Reduce the number (fi all calibrations based on MBT’5ME will show the same temperature trend), or summarize the variability by plotting a 95% confidence range based on all reconstructed temperatures?

Response: The number of calibrations has been reduced to 5.

Rebuttal Figure 5:

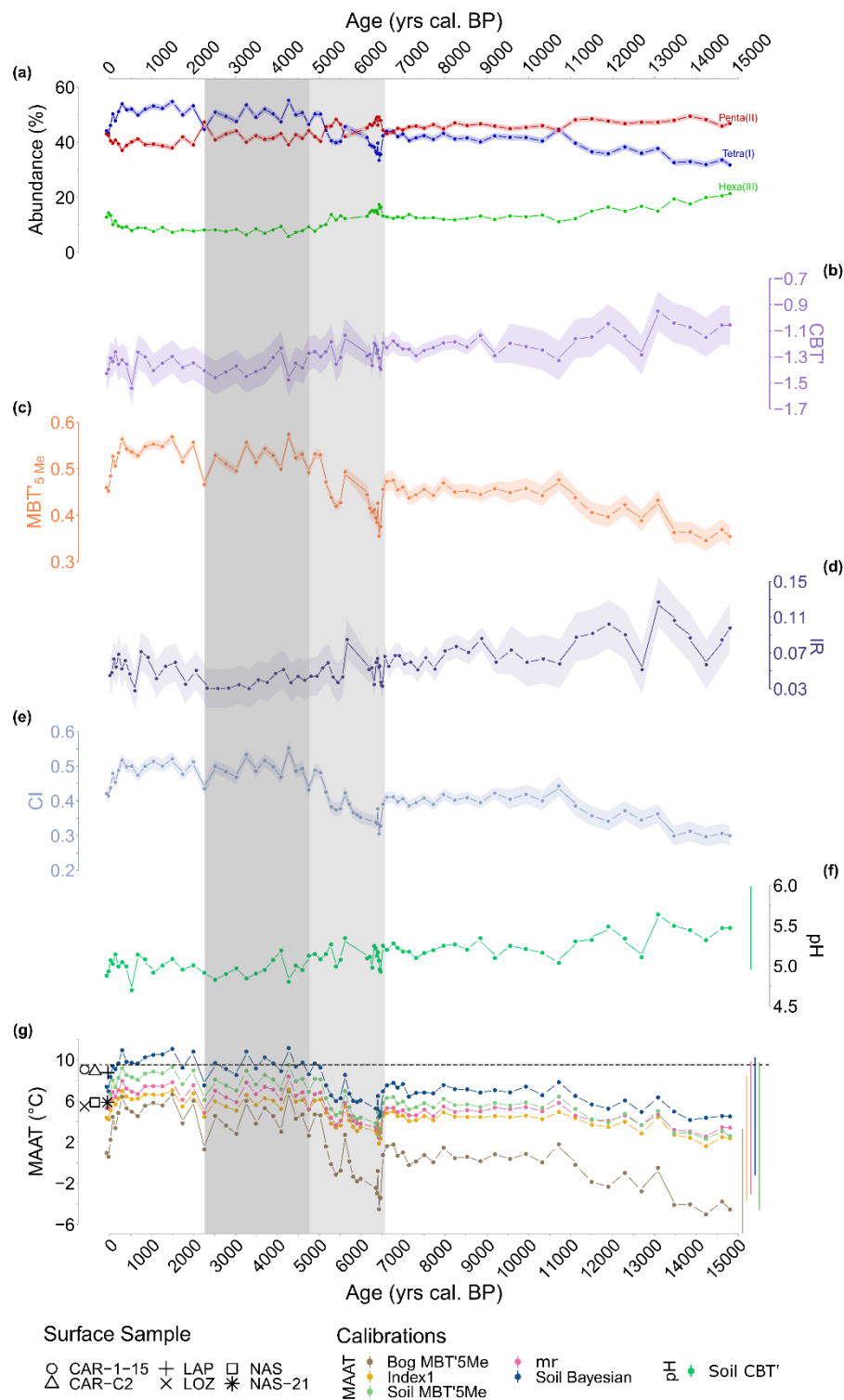


Figure 5: (a): Relative abundances of tetra-, penta- and hexamethylated brGDGTs in the CAN02 sequence. (b): Index of the degree of cyclisation (CBT'_{6Me}). (c): Index of the degree of methylation (MBT'_{5Me}). (d): Isomer Ratio (IR) through time. (e): Bacterial Community Index (CI) through time. (f): pH reconstruction based on global calibration of De Jonge et al., (2014a, Soil CBT'). (g): Annual mean temperature (MAAT) reconstructions based on global calibrations of De Jonge et al., (2014a) (Soil, b) (Index1 DJ, Soil MBT'5Me, Soil MBT_p-DJ, Mr DJ, Soil 5Me-DJ), mr, Naafs et al., (2017a) (Bog

N₁₅-MBT'5Me) and Dearing Crampton-Flood et al., (2020) (Soil Bayesian *DCF*). Calibration errors are represented by the lateral lines on the right side. Shaded periods highlight the significant decrease in abundance of tetramethylated brGDGTs and a shift in accumulation rate from 6,600 to 4,700 cal. BP (light grey area) and the decrease in IR from 4,700 to 2,300 cal. BP (dark grey area). Symbols: modern MAATs of surface samples. Black dashed line: current ~~MAAAT~~-calculated MAAT at Canroute.

L 361. The accumulation rates here are very low. Is it possible that the YD is not recorded in the peat record because of lack of accumulation?

Response: The response and text modification applied in the manuscript are detailed following the general comment of Reviewer #1, please refer to it for this comment.

L 365. It is not clear why the expanse of oak compared to hazelnut would have resulted in the observed decrease in detrital material. Is there any reference for this?

Response: Hazelnut woodland is associated with a more open character than oaks woodland. An open environment is more subject to mechanic soil erosion than a closed one. Therefore, the expanse of oak trees will induce a closing of the environment, thus a decrease in mechanic soil erosion which in turn will affect the detrital signal (Mohammad et Adam, 2010).

Text modifications: In section 4.1 (L 425-429) the following modification had been applied to clarify our meaning concerning the decrease in detrital material with the woodland-type vegetation change: *“The beginning of the Holocene is marked by a strong dominance of Corylus avellana that constituted woodland, whose open character can be associated with a dominant mechanic erosion of the soil (Mohammed et Adams, 2010), allowing the strong detrital activity revealed by XRF until 9,000 cal. BP. After this date, the progressive decrease in detrital activity may be attributed to the slow expansion of deciduous oaks, which replaced hazelnut open woodland across southern France and reduced the mechanic erosion.”*

L 402: ‘show more reliable reconstructed MAAT anomalies’, what is this based on? Compared to the current MAAT? Compared to the pollen record? Compared to what is expected from literature? Needs to be explained. The same goes for the other sites (next few lines).

Response: “Show more reliable reconstructed MAAT anomalies” is based on the comparison with the current MAAT value regarding each location (i.e., surface samples or Canroute).

Text modifications:

- L 497: *“For these three samples [...] show more reliable reconstructed MAAT anomalies compared to the current MAAT of their respective location (Fig. 8b)”*.
- L 500: *“For the samples from the Caroux site [...] appear to be the most reliable compared to their current MAAT (Fig. 8b)”*.
- L 502: *“For the Canroute surface sample (CAN0) [...] provides temperature values closer to the present temperatures at Canroute (Fig. 8b)”*.
- L 504: *“Soil calibrations based on IndexI, MBT'5Me, multi-regression and Bayesian calibration [...] low scatter and a current MAAT close to the observed climatic conditions at Canroute (Fig. 8c)”*.

L 420. A change in the IIIa/IIa ratio from 0.12 to 0.46 is a large shift, the relative abundance of IIa in this ratio shows a fourfold increase. No change in the BIT index doesn't mean anything in this context, right? Unless this can be substantiated, please remove.

Response: Because it is irrelevant in this paper context, the BIT-index has been removed from the manuscript. The significance of the IIIa/IIa ratio has been clarified in the manuscript.

Text modifications: at L 524, the following sentence has been added to clarify the significance of the IIIa/IIa ratio. *“Furthermore, the brGDGTs index IIIa/IIa, which investigates brGDGTs sources (Xiao et al., 2016), exhibits a significant shift in its values throughout the sequence (0.12 to 0.46), demonstrating the effect of environmental change on brGDGT composition”.*

L 423. In addition to the CI index, the impact of pH needs to be discussed here as well! The calculation of these ratios should precede the discussion of the MBT⁵ME as a temperature proxy in the results and discussion as well.

Response: Reconstruction of pH has been added to Fig. 5 (see above for the rebuttal Figure 5) and described in section 3.3.2, together with the IR, CI ratio and CBT⁷ variations, and discussed in section 4.3.3 before the interpretation of GDGT-based temperatures.

Text modifications: In section 3.3.2, pH reconstruction, IR, CI ratio and CBT⁷ variations have been described as follows in the text (L 325-333) *“The CBT⁷ index ranges from -1.58 to -0.98 (sd = 0.05; n = 75, Fig. 5b), and shows a slight continuous decrease over time. [...] The Isomer Ratio (IR) values range from 0.03 to 0.13 with the lowest values obtained between 4,700 and 2,300 cal. BP (Fig. 5d). Throughout the record, the Community Index (CI) values range from 0.30 to 0.55 and are less than 0.65 (Fig. 5e). The period from 15,000 to 4,700 cal. BP has mean CI values of 0.38, but the period from 4,700 to -80 cal. BP has higher mean CI values of 0.49. The pH varies from 4.6 to 5.6 and shows a little continuous decline over time (Fig. 5f).”*

L 425. The link between this sentence and the next is not clear. Bacteria and archaea have different environmental drivers. Unless the first sentence means that oxic/anoxic conditions have been shown to impact GDGT producer communities, the link with GDGT₀/cren implies a link between archaea and GDGT producing bacteria that’s arguably not there?

Response: The first sentence’s intended meaning was that oxic/anoxic conditions have an impact on GDGT-producing communities. The GDGT₀/Cren is utilized in this context to highlight anoxic conditions, which might alter the brGDGT-based temperature reconstruction, and are associated to the development of methanogenic archaea that thrive in anoxic environments.

Text modifications: To clarify our meaning, we modified and added precision at Ls 518-522 in the following sentence: *“Furthermore, edaphic factors such as anoxic/oxic conditions have an impact on GDGT production and bacterial communities (Weber et al., 2018). Because crenarchaeol and GDGT-0 can be derived from Group I Crenarchaeota, the GDGT-0/Cren ratio can be used to investigate the presence of methanogenic archaea that thrive in anoxic conditions in sediments, whereas methanogenic Archaea synthesize GDGT-0, but no crenarchaeol (Blaga et al., 2009). The lower the ratio, the lower the anoxic conditions.”*

L 433-442. It is not clear why the authors discuss the seasonality in precipitation here. It seems like there is no indication that this peat environment would be characterized by a lack of moisture? Also, the seasonality of temperature can be mentioned in the methods and materials section, and should not be mentioned this late in the discussion?

Response: Indeed, a hypothetical shortage of moisture in a peatland in this area (southern Massif Central and impacted by Mediterranean climate) may not be immediately apparent. Our reasoning is based on the fact that, despite being a soligenous peatland (water supplied primarily by springs and streams), the region’s high seasonality in precipitation (variation of monthly precipitation) can affect local hydrological dynamics, which in turn affects brGDGT production in the peatland. As a result, it becomes critical to study the possibility of seasonality in precipitation for the Canroute peatland.

Text modifications: Temperature seasonality (TS) and precipitation seasonality (SoP) have been added in methods and materials section at L 132 (section 2.1 Study area) as follows “*These influences result in an average annual temperature of 9.5°C, a temperature seasonality (TS, standard deviation of the monthly mean temperatures) of 0.5° C (WorldClim 2.0, Fick and Hijmans, 2017), higher summer temperatures, and an average annual rainfall of ca. 895 mm with a slightly drier summer period (Fig. 1c, Table A, CRU TS version 4.06, Harris et al., 2020) and a precipitation seasonality (SoP, standard deviation of the monthly precipitation) of 21 (WorldClim 2.0, Fick and Hijmans, 2017).*”

L 458. Why was the MEDTEMP database then considered in the first place? Consider removing this calibration from the paper.

Response: As we mention for brGDGT-based MAAT calibration in the publication, one of the goals of our study is to assess the role of the modern pollen dataset on climate reconstruction and to determine whether global or regional calibrations provide more credible climate trends and values. The MEDTEMP modern database was eliminated due to its contrasting trends with the EAPDB and TEMPSCAND modern databases, as well as the paucity of Mediterranean taxa in the Canroute pollen record. However, we believe it is critical to verify and debate this calibration because regional calibrations are currently infrequently used on pollen sequences.

L 490. What time period do the authors mean when referring to ‘Mid-Holocene cooling’ here? This is not observed in brGDGTs when the grey area is removed. There is stable temperatures between 12ka and ~7 ka (unless Mid-Holocene falls between 7 and 12ka, but then it should be specified in the sentence), and a warmer period since 5ka. Mind that this is also occurring at a different CBT5ME and that thus the effect of pH needs to be constrained first!

Response: Mid-Holocene cooling refers to the cooling that occurs after the Holocene thermal maximum (HTM), which has been well documented in northern Europe and marine proxies. The HTM and subsequent cooling are observed at Canroute, but not from the brGDGTs-based signal or the BRT pollen-based signal. The HTM and the onset of the subsequent cooling are noticed before the shaded area using the MAT pollen-based signal. Finally, without taking into account the shaded time period, colder temperatures are seen at Canroute, from 15,000 to 6,500 cal. BP (relative to present mean annual temperatures), followed by a warming since 5,000 cal. BP. Because local hydrological changes appear to alter bacterial communities, we can conclude that climate variations based on brGDGTs before and after the shaded time period cannot be compared, and we should instead examine relative climate variations rather than absolute climate differences.

Text modifications: at L 597, precision has been added to clarify the meaning of the Mid-Holocene cooling trend as follows: “*After the thermic optimum, the onset of a cooling trend until 6,000 cal. BP*”.

L 514. Please be specific, which events are meant here? What is there expected duration?

Response: Here Lateglacial events refer to abrupt events such as Bølling-Allerød and Younger Dryas.

Text modifications: Precision was added in the text at L 627 as follows: “*Typical millennial Lateglacial events, such as the Bølling-Allerød and Younger Dryas, cannot, however, be seen on both proxies since the brGDGT signal does not reflect such abrupt events (Fig. 11a)*”.

L 515. Would the authors argue that the peat sequence lacks the required resolution to see these events? Not perse based on the number of samples, but rather on the impact of bioturbation on the wetland type archive.

Response: The response and text modification applied in the manuscript are detailed following the general comment of Reviewer #1. To sum up, the bioturbation, not considered in the previous version of this manuscript, has been added in section 4.5.2 to discuss the record of abrupt Lateglacial events.

L 539. If both these records are based on the MBT, calibration can not cause a disagreement between the trends.

Text modifications: Sentence corrected at L 653 by the removal of “and calibration (soil vs. lake)”.

L 540. This sentence is not clear about the ‘cooling trend’. Which cooling trend is supported?

Response: The ‘cooling trend’ refers to the decrease in temperature, following the Holocene Thermal Maximum, which is typically observed in the sequences where the HTM is recorded (e.g., northern Europe, marine cores ...).

Text modifications: To clarify our meaning, the sentence at L 653 has been modified as “*On the other side, summer temperature proxies support the cooling trend that typically follows the HTM (Herzschuh et al., 2022; Heiri et al., 2003; Samartin et al., 2017; Jalali et al., 2016).*”

Appendix: Fig. C does not seem necessary.

Response: The Appendix Fig. C has been removed.

New references added

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