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Constraints on hopanes and brGDGTs as pH proxies in peat

Valentine Schaaff¹, Vincent Grossi¹, Matthew Makou¹, Yannick Garcin², Pierre Deschamps²,
David Sebag³, Benjamin Ngounou Ngatcha⁴, Guillemette Ménot¹

¹ Université de Lyon, ENS Lyon, UCBL, CNRS, UMR 5276 LGL-TPE, 69364 Lyon, France

² Aix Marseille University, CNRS, IRD, INRAE, CEREGE, Aix-en-Provence, France

³ IFP Energies Nouvelles, Earth Sciences and Environmental Technologies Division, 1 et 4
Avenue de Bois-Préau, 92852 Rueil-Malmaison, France

⁴ Department of Earth Sciences, Faculty of Sciences, University of Ngaoundéré, Ngaoundéré,
Cameroon

Corresponding author: Valentine Schaaff valentine.schaaff@ens-lyon.fr

39 **Abstract:** pH is one of the major parameters governing peat functioning, and pH variations in modern
40 peatlands affect carbon and methane production and consumption. Paleo-pH reconstructions have
41 been limited thus far, but they could help to better understand peat functioning in variety of settings
42 and also serve as an indirect proxy for climatic and environmental variations such as precipitation.
43 Bacterial hopanes and branched Glycerol Dialkyl Glycerol Tetraethers (brGDGTs) were investigated
44 in a 10-ka peat record from North-East Cameroon (NGaoundaba, Western Central Africa). Recently
45 developed pH proxies using the hopane $\beta\beta/(\alpha\beta+\beta\beta)$ ratio and the brGDGT cyclization ratio (CBT_{peat})
46 were applied and compared with previously published bulk organic data from the same core.
47 Different hypotheses are usually proposed to explain the high abundance of the “thermally mature”
48 C_{31} $\alpha\beta$ hopane in peats: acid-catalyzed isomerization of $\beta\beta$ to $\alpha\beta$ isomers with or without biological
49 mediation or the direct input of C_{31} $\alpha\beta$ hopanes by bacteria. In the NGaoundaba peat deposit, the
50 opposite variation in the $\beta\beta/(\alpha\beta+\beta\beta)$ ratio of C_{30} and C_{31} hopanes and the carbon isotopic
51 composition of C_{31} $\beta\beta$ and $\alpha\beta$ hopanes suggests that an eventual transformation of C_{31} $\beta\beta$ to $\alpha\beta$
52 isomers is not possible without biological mediation. A linear correlation between hopane- and
53 brGDGT-reconstructed pH is observed, suggesting a similar response to environmental changes for
54 these two independent proxies. A large increase in reconstructed pH values coincides with changes
55 in vegetation and precipitation at the end of the African Humid Period (AHP). Lower pH values are
56 observed during the AHP, coinciding with a period of increased precipitation and consistent with
57 bulk organic data and slightly higher C_{31} $\beta\beta$ hopane $\delta^{13}C$ values compared to middle and late
58 Holocene. Bulk organic data indicate an interruption of the AHP by a drier intermission that coincides
59 with a decrease in reconstructed pH values, probably reflecting a decrease in evapotranspiration.
60 Variations in pH values could be interpreted in terms of preservation of peat organic matter and
61 might reflect past changes in methane cycling in the investigated peatland. The present study
62 reinforces the idea that reconstruction of pH in peat deposits represents a promising proxy of
63 environmental change, enabling a better understanding of changes in peat functioning over large
64 timescales and various locations.

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67 **Keywords:** Lipid biomarkers, hopane isomer ratios, ^{13}C composition, pH reconstruction, African
68 Humid Period

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1. Introduction

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93 Peat deposits are important carbon sinks that are becoming increasingly important for the scientific
94 community because they are sensitive to anthropogenic climate change (e.g., Belyea and Malmer,
95 2004; Leifeld and Menichetti, 2018; Loisel et al., 2021). Continental records can be scarce and
96 discontinuous but peat deposits have great potential to improve our understanding of past climate
97 variations, especially using proxies based on organic biomarkers (e.g., Naafs et al., 2019) due to the
98 high content and good preservation of organic matter in peat deposits (Moore, 1989). Developing new
99 proxies based on lipid biomarkers for paleoclimatic and paleoenvironmental reconstructions is limited
100 by our understanding of the origin and diagenesis of these compounds in sediments (e.g., Castañeda
101 and Schouten, 2011; Naafs et al., 2019) and by the lack of records in some areas, especially in the
102 tropics. Recent advances in peat and biomarker proxies are expanding our ability to reconstruct past
103 climates and environments (Naafs et al., 2019 and references therein). Several peat-calibrated proxies
104 based on different classes of compounds, such as hopanoids and branched glycerol dialkyl glycerol
105 tetraethers (brGDGTs) have recently been developed (Naafs et al., 2017; Inglis et al., 2018; Zhang et
106 al., 2020), extending the possibilities of paleoclimatic reconstructions based on peat studies. Peat pH
107 has a strong influence on microbial communities (e.g., Girkin et al., 2020), peat organic matter
108 preservation (Moore, 1989) or methane production and consumption (Dunfield et al., 1993). Paleo pH
109 reconstructions could constrain the evolution of peat formation and carbon/methane cycling on
110 geological timescales. In addition, pH variations have been suggested to reflect past changes in
111 vegetation or precipitation and paleo-pH reconstructions may therefore represent new indirect
112 proxies of these paleoclimatic parameters.

113 Hopanoids are pentacyclic triterpenoids commonly found in diverse geological settings, where they
114 generally occur as hydrocarbons (i.e., geohopanoids) (e.g., Quirk et al., 1984; Ries-Kautt and Albrecht,
115 1989; Innes et al., 1997; Farrimond et al., 2003). These compounds are produced by a wide range of
116 bacteria, although estimates of the frequency of hopanoid biosynthesis among bacteria are still
117 unclear (Rohmer et al., 1984; Pearson et al., 2007). The role of biological hopanoids is proposed to be
118 similar to that of eukaryotic sterols, which regulate membrane fluidity and permeability (e.g., Ourisson
119 et al., 1987), as biohopanoids have been shown to play role in bacterial outer membrane ordering
120 (Sáenz et al., 2015). A role in protection against pathogens or pH homeostasis has also been reported
121 (Welander et al., 2009; Schmerk et al., 2011). Hopanoids are promising biomarkers for the
122 reconstruction of methane cycling in peats based on their carbon isotopic signature (Hayes et al., 1990;
123 Pancost et al., 2007; Inglis et al., 2019) and their use as proxy for pH variations in peats based on
124 specific ratios of hopane isomers has also been suggested (Huang et al., 2015; Inglis et al., 2018).
125 Isomerization of biologically-derived $17\beta,21\beta(\text{H})$ -hopanoids (hereafter abbreviated $\beta\beta$) into the more
126 thermally stable $17\beta,21\alpha(\text{H})$ ($\beta\alpha$) and $17\alpha,21\beta(\text{H})$ ($\alpha\beta$) forms, as well as their isomerization at the C22
127 position, are well known to occur during the thermal maturation of sediments (Ensminger et al., 1974;
128 Mackenzie et al., 1980; Mackenzie and McKenzie, 1983). Accordingly, isomer ratios have been used to
129 describe the thermal maturity of sediments (e.g., Farrimond et al., 1996). In peat deposits, high
130 proportions of the $\alpha\beta$ stereoisomer have been reported despite the low thermal maturation of these
131 sediments (e.g., Quirk et al., 1984; He et al., 2015; Huang et al., 2015; Inglis et al., 2018) and the
132 abundance of C_{31} $\alpha\beta$ hopanes has even been used to trace input of acidic peat into marine sediment
133 (Smittenberg et al., 2004; Inglis et al., 2022). A direct input of $\alpha\beta$ hopanes by indigenous bacteria could
134 explain their high abundance (Rosa-Putra et al., 2001). It has also been suggested that these
135 compounds are formed by microbially mediated isomerization (Ries-Kautt and Albrecht, 1989) or by
136 acid-catalyzed isomerization without microbial mediation (Pancost et al., 2003; Inglis et al., 2018). This
137 isomerization reaction may also be influenced by temperature and redox conditions (Huang et al.,
138 2015), but a lack of correlation between isomerization of geohopanoids and temperature has been
139 demonstrated in global peat database while a significant correlation with peat pH was observed (Inglis

140 et al., 2018). To date, the cause of the high abundance of $\alpha\beta$ hopanes in peat is still unclear and
141 debated.

142 Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are prokaryotic membrane-spanning lipids.
143 Unlike isoprenoid GDGTs produced by Archaea, brGDGTs are of bacterial origin (Weijers et al., 2006).
144 BrGDGTs were first identified in a Dutch peat bog (Sinninghe Damsté et al., 2000) and since then, a
145 wide range of brGDGTs with various number of methylation and cyclopentyl moieties have been found
146 in natural environments (Schouten et al., 2000b, 2013). *Acidobacteria* have been proposed as the main
147 producers of brGDGTs since they are abundant in soils and peats and produce isodiabolic acid, a
148 potential building block of brGDGTs (Weijers et al., 2009; Sinninghe Damsté et al., 2011, 2014, 2018).
149 Recent culture studies have confirmed that Acidobacteria are likely the main source of brGDGTs (e.g.,
150 Chen et al., 2022; Halamka et al., 2023), but other producers than *Acidobacteria* cannot be excluded
151 (Weber et al., 2018). Biosynthetic pathway of brGDGTs based on the condensation of two branched
152 dialkylglycerols instead of the reduction of tetraesters of glycerol based on isodiabolic acid has also
153 been suggested (Weijers et al., 2006; Halamka et al., 2023). Environmental parameters exert significant
154 control over brGDGTs distribution, with the cyclization ratio (CBT) primarily dependent on pH and the
155 methylation ratio (MBT) primarily influenced by temperature (Weijers et al., 2007; De Jonge et al.,
156 2014). Numerous empirical calibrations for temperature and pH have been developed over the past
157 decades as a result of technical improvement and the need for environment-specific (lake, marine,
158 peat, and soil) global and local calibrations (e.g., Peterse et al., 2012; Loomis et al., 2012; Naafs et al.,
159 2017; Dearing Crampton-Flood et al., 2020; Martínez-Sosa et al., 2021).

160 A statistically significant correlation between brGDGT-based and hopane-based reconstructed pH
161 values was observed in a global peat database, whereas striking similarities between the two pH
162 reconstructions were obtained in an early Paleogene lignite deposit from Schöningen (Germany) (Inglis
163 et al., 2018). However, applications of these pH calibrations have remained limited until now (Zhang
164 et al., 2020). Peat brGDGT-based pH reconstructions using a previous soil calibration from Weijers et
165 al. (2007) showed large pH variations associated with major changes in peat-forming vegetation
166 (Weijers et al., 2011; Nichols et al., 2014) or with changes in humification associated with changes in
167 precipitation (Zheng et al., 2015; Wang et al., 2017).

168 In this study, we further investigate the distribution of hopanes and brGDGTs in peat samples and their
169 respective use as tracers of pH variations. The results are based on a 10-ka record from the
170 Ngaoundaba peat deposit located on the Adamawa Plateau (North East Cameroon, West Africa),
171 already constrained by a study on bulk organic parameters (Schaaff et al., 2023). By comparing various
172 parameters for hopane and brGDGT distributions (relative abundances, specific ratios, and carbon
173 isotopic data for C_{31} hopanes), we propose new constraints on the origin of hopanes distribution in
174 peat. The comparison of hopane- and brGDGT-based pH reconstructions further allows us to assess
175 the applicability of these reconstructions to Holocene peat records and their potential paleoclimatic
176 and paleoenvironmental interpretations.

177

178 **2. Material and methods**

179

180 **2.1. Study site**

181

181 The Ngaoundaba peat deposit is located in a volcanic crater on the Adamawa Plateau in Northeast
182 Cameroon (N7.135° E13.690°, 1175 m above sea level). The vegetation at the Ngaoundaba peat
183 deposit is dominated by sedges (*Cyperus spp*) while the site lies in the Soudano-Guinean shrubs
184 savannas zone (Letouzey, 1958). Present day climate is characterized by two contrasting seasons: a 7-
185 month rainy season from April to October, and a 5-month dry season from November to March. The
186 mean annual air temperature (MAAT) is 22.2°C and the mean annual precipitation (MAP) is 1482 mm
187 (CRU database, Harris et al., 2014). The average monthly temperature ranges from around 20.6 in
188 December to 24.5 °C in March (CRU database, Harris et al., 2014).

189

190 **2.2. Sediment core, ^{14}C dating and bulk organic analyses**

191

192 A 6-meter-long core (named NGBA19) was collected in February 2019 using a Russian peat corer. The
193 core is composed of dark brown peat without visible sandy or clayey zones. The core was then sub-
194 sampled at 2.5-cm intervals. Organic geochemical analyses were performed on 73 samples.

195 The age model of the Ngaoundaba peatcore is based on 30 radiocarbon dates carried out at the LMC14
196 Laboratory (Saclay, France). Details on the dating and calibration of the age model are given in Schaaff
197 et al. (2023).

198 Bulk organic analyses are presented in detail in Schaaff et al. (2023) To summarize, samples were
199 analyzed for Total Organic Carbon (TOC) and $\delta^{13}\text{C}$ composition of TOC (hereafter $\delta^{13}\text{C}_{\text{bulk}}$) using an
200 elemental analyzer (Vario ISOTOPE Select, Elementar) coupled to a thermal conductivity detector and
201 an isotope ratio mass spectrometer (Vision, Elementar). A working standard (IVA sediment) was
202 analyzed every 10 samples and used to normalize the mass spectrometer signals.

203

2.3. Organic geochemical analyses

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2.3.1. Sample preparation

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206

207 All samples were freeze-dried, ground and homogenized. Extraction was performed two times for 0.3g
208 of each sample with 10mL of dichloromethane (DCM)/methanol (3:1, v/v) using a MARS 6 CEM
209 microwave extraction system. Following each extraction, the supernatant was filtered through a
210 sintered Teflon filter. Synthetic C_{46} GDGT was added to the combined extracts of each sample as an
211 internal standard (Huguet et al., 2006). The total lipid extract was separated by column
212 chromatography on silica gel into three fractions. The hydrocarbon fraction containing hopanes was
213 eluted first with 8mL of hexane. A second fraction was eluted with 5mL of hexane/DCM (1:1, v/v).
214 BrGDGTs were eluted in a third fraction with 10mL of DCM/methanol (1:1, v/v).

215

2.3.2. Hopane identification and analyses

216

217

218 Copper curls activated with hydrogen chloride (HCl) were added to the hydrocarbon fractions to
219 remove elemental sulfur. After a first analysis using gas chromatography-flame ionization detector
220 (GC-FID) (HP 6890A), gas chromatography-mass spectrometry (GC-MS) analyses (Agilent 6890N GC
221 coupled to an Agilent 5975C MSD and Agilent 7890B GC coupled to an Agilent 5977B MSD) were
222 performed for identification and quantification of the different compounds. Both GC-MS instruments
223 provided comparable data. The GC-MS was equipped with a HP5 MS UI column (30 m x 0.25 mm, film
224 thickness 0.25 μm). The oven temperature was programmed from 60°C (0.5 min hold) to 130°C at
225 20°C/min and then rises to 320°C at 4°C/min. This final temperature was held for 20 min. The carrier
226 gas flow was constant at 1mL/min and the mass spectrometer was used at 70eV with a m/z range from
227 50 to 700. Hopanes were identified based on their retention time and mass spectrum. The
228 identification of hopane isomers is consistent with published literature (Naafs et al., 2019). Semi-
229 quantification was performed using the total ion chromatograms (TIC) by comparison with an external
230 standard solution containing cholestane. The hopanes concentrations were normalized to TOC.

231 Prior to carbon isotopic measurements, the hydrocarbon fractions were separated into a saturated
232 and an unsaturated fraction by column chromatography using silica gel impregnated with silver nitrate
233 (AgNO_3 , 10 % wt). Saturated fractions containing hopanes were eluted with 3 to 4 mL of *n*-heptane.
234 Unsaturated fractions were eluted with 3 to 4 mL of ethyl acetate. Carbon isotopic compositions were
235 measured at the LGL-TPE (Université Claude Bernard Lyon 1) using an Agilent 7890B GC coupled to an
236 Isoprime visION Isotope Ratio Mass Spectrometer via an Isoprime GC-5 combustion interface
237 (Elementar). The GC-IRMS was equipped with a VF-1ms (60 m x 0.25 mm, film thickness 0.25 μm). The
238 GC-5 furnace was operated at 850 °C. An autotune and tests of stability and linearity of the signal were
239 performed daily using the software ionOS. Stable carbon isotopic compositions (expressed as $\delta^{13}\text{C}$
240 values) were calibrated using a reference CO_2 gas reported to VPDB scale. Two Mix hydrocarbon B4
241 standards (A Schimmelmann, Indiana University) were measured every 4 to 5 samples and used to
242 correct the $\delta^{13}\text{C}$ values. Samples were analyzed in duplicates when the quantity of samples allowed it

243 (see supplementary material for more details). The average standard deviation on the B4 standard is
244 0.37 %.

245 **2.3.3. brGDGT analyses**

247
248 Fractions containing brGDGTs were filtered using 0.45 µm PTFE filters. BrGDGT analyses were
249 performed using High Performance Liquid Chromatography Mass Spectrometry (HPLC-APCI-MS,
250 Agilent 1200) at the LGL-TPE (ENS de Lyon). Separation was achieved using two silica columns in series
251 (ACQUITY UPLC BEH HILIC, WATERS) following the procedure of Hopmans et al. (2016) slightly modified
252 (i.e. using hexane/isopropanol (98.2:1.8, v/v)). Peaks corresponding to brGDGTs were manually
253 integrated and quantified using the peak area of C₄₆. BrGDGT concentrations were normalized to TOC.
254 The selected *m/z* values are 743.9, 1050.2, 1048.2, 1046.2, 1036.2, 1034.2, 1032.2, 1022.2, 1020.2 and
255 1018.2.

256 **2.3.4. Hopane- and brGDGT-based ratios and pH reconstructions**

257
258 The degree of hopane isomerization was calculated using the $\beta\beta/(\alpha\beta+\beta\beta)$ and $22S/(22S+22R)$ indices
259 developed by Mackenzie et al. (1980). The $\beta\beta/(\alpha\beta+\beta\beta)$ ratio was calculated for both C₃₀ and C₃₁
260 hopanes. The C₃₁ $\alpha\beta$ hopane was considered as the sum of the 22S and 22R isomers. The hopane-based
261 pH reconstruction uses the C₃₁ $\beta\beta/(\alpha\beta+\beta\beta)$ index and was calculated using the following equation
262 proposed by Inglis et al. (2018) for peats:
263
264

$$265 \quad pH = 5.22 * \frac{\beta\beta}{\alpha\beta + \beta\beta} + 3.11 \quad (n = 94, r^2 = 0.64, RMSE = 1.4)$$

266
267 The brGDGT-based pH reconstruction is based on the CBT_{peat} index using the following equation
268 developed by Naafs et al. (2017) for peats:

$$269 \quad CBT_{peat} = \log\left(\frac{Ib + IIa' + IIb + IIb' + IIIa'}{Ia + IIa + IIIa}\right)$$
$$270 \quad pH = 2.49 * CBT_{peat} + 8.07 \quad (n=51, r^2=0.58, RMSE=0.8)$$

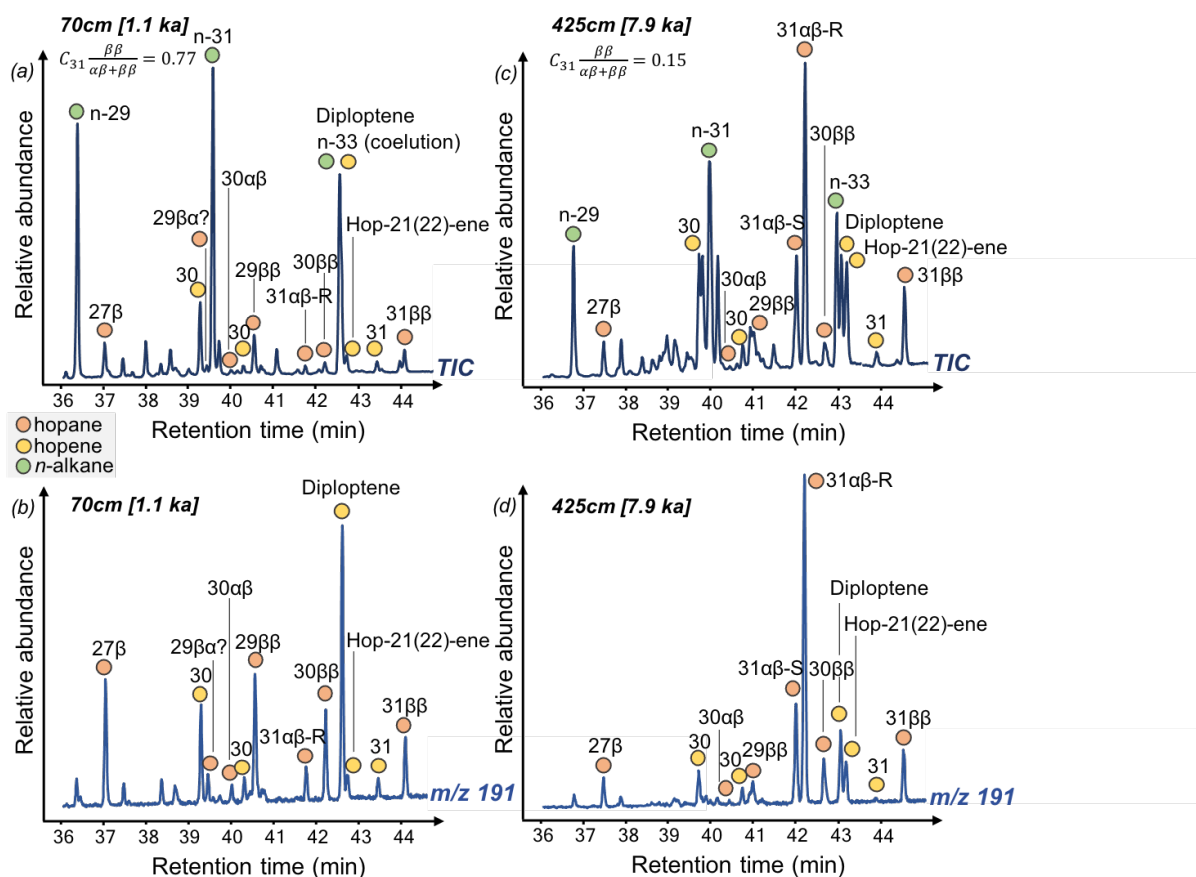
271
272 Compound structures (with numbering) are presented in Fig. S.1.

273 **3. Results**

274 **3.1. Relative abundance and concentration of biomarkers**

275 **3.1.1. Hopanes**

276
277 Diverse hopanes and hopenes were detected in the Ngaoundaba peat records. Hopanes range from
278 C₂₇ to C₃₁ and are accompanied by C₃₀ and C₃₁ hopenes (Fig. 1). C₃₁ hopanes are present in three
279 different configurations: $\beta\beta$, $\alpha\beta$ -R and $\alpha\beta$ -S isomers (Fig. 1). The C₃₁ $\alpha\beta$ -S hopane is present only
280 between 8.4 ka cal BP and 6.0 ka cal BP, and its concentration ranges from 0 to 32.0 µg/g_{TOC} (Fig. 2 A).
281 The concentration of C₃₁ $\alpha\beta$ -R hopane ranges from 0 to 44.4 µg/g_{TOC} (Fig. 2 A), reaching its highest
282 concentration (above 10 µg/g_{TOC}) between 9.7 and 9.4 ka cal BP and between 8.5 to 7.3 ka cal BP, and
283 its lowest (below 5.7 µg/g_{TOC}) from 5.8 ka cal BP to present. The concentration of the C₃₁ $\beta\beta$ hopane
284 ranges from 1.2 to 12.1 µg/g_{TOC} (Fig. 2 B) and shows similar variations as the C₃₁ $\alpha\beta$ -R hopane. Except
285 for a brief period in the late Holocene, the C₃₁ $\beta\beta$ hopane is less abundant than the C₃₁ $\alpha\beta$ -R isomer.
286 C₃₀ hopanes are dominated by the $\beta\beta$ isomers in most samples (Fig. 1). The concentration of C₃₀ $\beta\beta$
287 hopane ranges from 0.1 to 3.5 µg/g_{TOC}, while the concentration of the C₃₀ $\alpha\beta$ isomer ranges from 0 to
288 1.8 µg/g_{TOC}. The concentration of C₃₀ $\beta\beta$ hopane presents its maximum from the bottom of the core to
289 9.4 ka cal BP and from 8.1 ka cal BP to 5.8 ka cal BP (Fig. S.3).
290
291



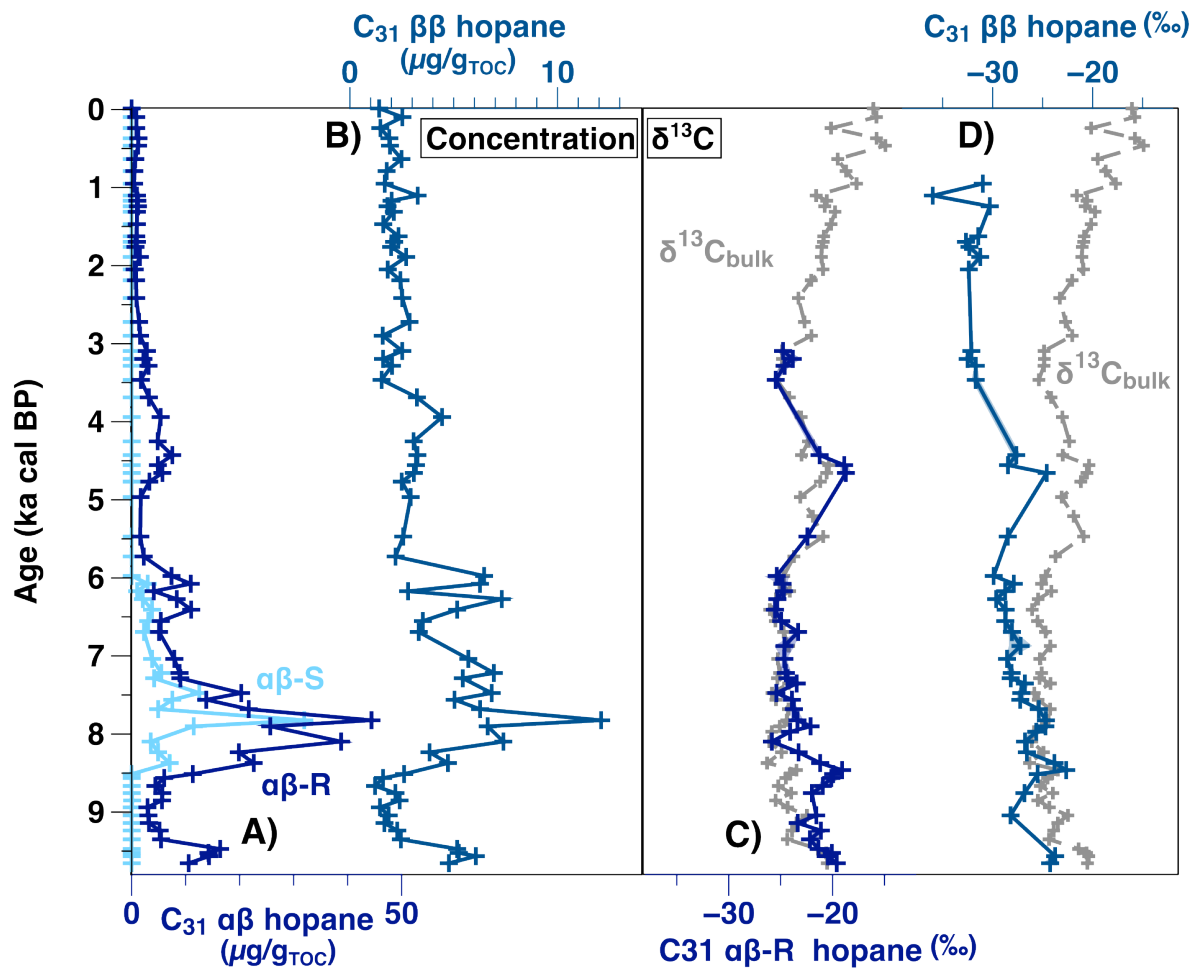
292
 293 **Fig. 1: GC-MS partial chromatograms of the hydrocarbon fractions of two samples with distinct**
 294 **hopanoid distribution: 70 cm-depth Total Ion Chromatogram (TIC) (a) and m/z 191 (b) and 425 cm-**
 295 **depth TIC (c) and m/z 191 (d). Ages of the samples are in ka cal BP. These chromatograms present**
 296 **the hydrocarbon fraction prior to separation of saturated and unsaturated compounds.**
 297

298 **3.1.2. brGDGTs**
 299

300 The brGDGT assemblage is dominated by brGDGT-Ia, which represents between 57.7 and 84.6 % (see
 301 supplementary material, Table S.1 and Fig. S.2.). The concentration of the brGDGT-Ia ranges from 45.4
 302 to 194.0 $\mu\text{g/g}_{\text{TOC}}$. The relative abundance and concentration of brGDGTs decrease with increasing
 303 number of methylation (Ia to IIIa) and degree of cyclization (Ia to Ic) (see Table S.1 and Fig. S.3 for more
 304 detail). The 6-methyl brGDGTs (IIa', IIb' and IIIa') exhibit lower relative abundances and concentrations
 305 than their 5-methyl counterparts.
 306

307 **3.2. Carbon isotope composition of C_{31} $\beta\beta$ and $\alpha\beta$ -R hopanes**
 308

309 The $\delta^{13}\text{C}$ composition of the C_{31} $\alpha\beta$ -R and C_{31} $\beta\beta$ hopanes could be measured for 42 and 43 out of 73
 310 samples, respectively, most of them in duplicates. The $\delta^{13}\text{C}$ of the C_{31} $\alpha\beta$ -R hopane ranges from around
 311 -25.8 to -18.7 ‰ with a mean standard deviation of 0.24 ‰ (min: 0, max: 0.9) (Fig. 2 C). The most ^{13}C -
 312 depleted values of the C_{31} $\alpha\beta$ isomer are recorded between 8.1 and 5.9 ka cal BP and between 3.5 and
 313 3.1 ka cal BP with all values but one below -23.3‰. ^{13}C -enriched values range from -18.7 to -23.4 ‰
 314 at 9.7, 8.5 and 4.6 ka cal BP (Fig. 2 C). The $\delta^{13}\text{C}$ composition of the C_{31} $\beta\beta$ hopane appears slightly ^{13}C -
 315 depleted when compared to the $\alpha\beta$ isomer, ranging from around -36.0 to -22.6 ‰ with a mean
 316 standard deviation of 0.2 ‰ (min: 0, max: 0.8) (Fig. 2 D). The $\delta^{13}\text{C}$ value of the C_{31} $\beta\beta$ hopane presents
 317 a global decreasing trend towards present day with periods of short increase around 8.5 ka cal BP and
 318 at 4.7 ka cal BP (Fig. 2 D). The two C_{31} hopane isomers present similar $\delta^{13}\text{C}$ values until ca. 7 ka cal BP,
 319 after which the two $\delta^{13}\text{C}$ records diverge (Fig.2 C and D).



321
 322 **Fig. 2: Concentration of (A) C_{31} $\alpha\beta$ R and $\alpha\beta$ S and (B) $\beta\beta$ hopanes ($\mu\text{g}/\text{g}_{\text{TOC}}$) and carbon isotopic data**
 323 **for (C) C_{31} $\alpha\beta$ R and (D) $\beta\beta$ hopanes compared to the carbon isotopic data of bulk sediment from**
 324 **Schaaff et al. (2023) along the NGaoundaba peat core. The uncertainties for $\delta^{13}\text{C}_{\text{bulk}}$ values are**
 325 **smaller than the size of the data point, those for $\delta^{13}\text{C}_{\text{hopane}}$ values are shown on the figure but are, in**
 326 **most cases, smaller than the size of the data point.**

327

328

3.3. Hopane stereoisomers and CBT ratios

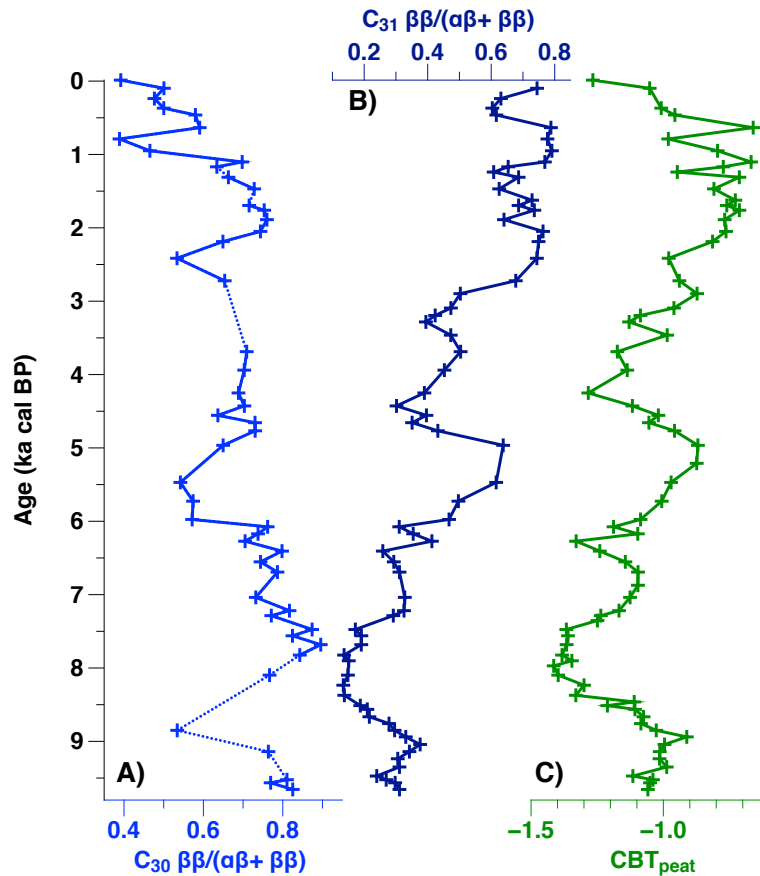
329

330 The $\beta\beta/(\alpha\beta+\beta\beta)$ indices of C_{31} and C_{30} hopanes were obtained for 67 and 49 samples out of 73,
 331 respectively. The C_{31} 22S/(22S+22R) index was only obtained for samples between 8.9 and 6 ka cal BP
 332 due to the lack of the 22S isomer in samples younger than 6 ka cal BP (Fig. S.4).

333 The C_{30} $\beta\beta/(\alpha\beta+\beta\beta)$ index ranges from 0.4 to 0.9 (Fig. 3 A). This index is high (around 0.7-0.8) from 9.7
 334 to 6.0 ka except for an isolated sample with a value around 0.5 at 8.8 ka cal BP. The ratio decreases to
 335 values around 0.5 between 6.0 and 5.4 ka cal BP and increases again to values around 0.7 between 5.0
 336 and 1.0 ka cal BP. From 1.0 ka cal BP upward, the C_{30} $\beta\beta/(\alpha\beta+\beta\beta)$ index shows its lowest values with
 337 minima around 0.8 ka cal BP and in the topmost sample corresponding to the post bomb period (Fig.
 338 3 A).

339 The C_{31} $\beta\beta/(\alpha\beta+\beta\beta)$ index ranges from 0.1 to 0.8 (Fig. 3 B). This index is low until 6.0 ka cal BP with
 340 values below 0.4 and presents its lowest values (below 0.2) between 8.5 and 7.4 ka cal BP. The ratio
 341 then increases until ca. 4.9 ka cal BP, and after a period of values below 0.5 between 4.9 and 2.8 ka cal
 342 BP, the late Holocene is characterized by values above 0.6. Short time-scale variations are recorded
 343 between 2.0 and 1.1 ka cal BP (Fig. 3 B).

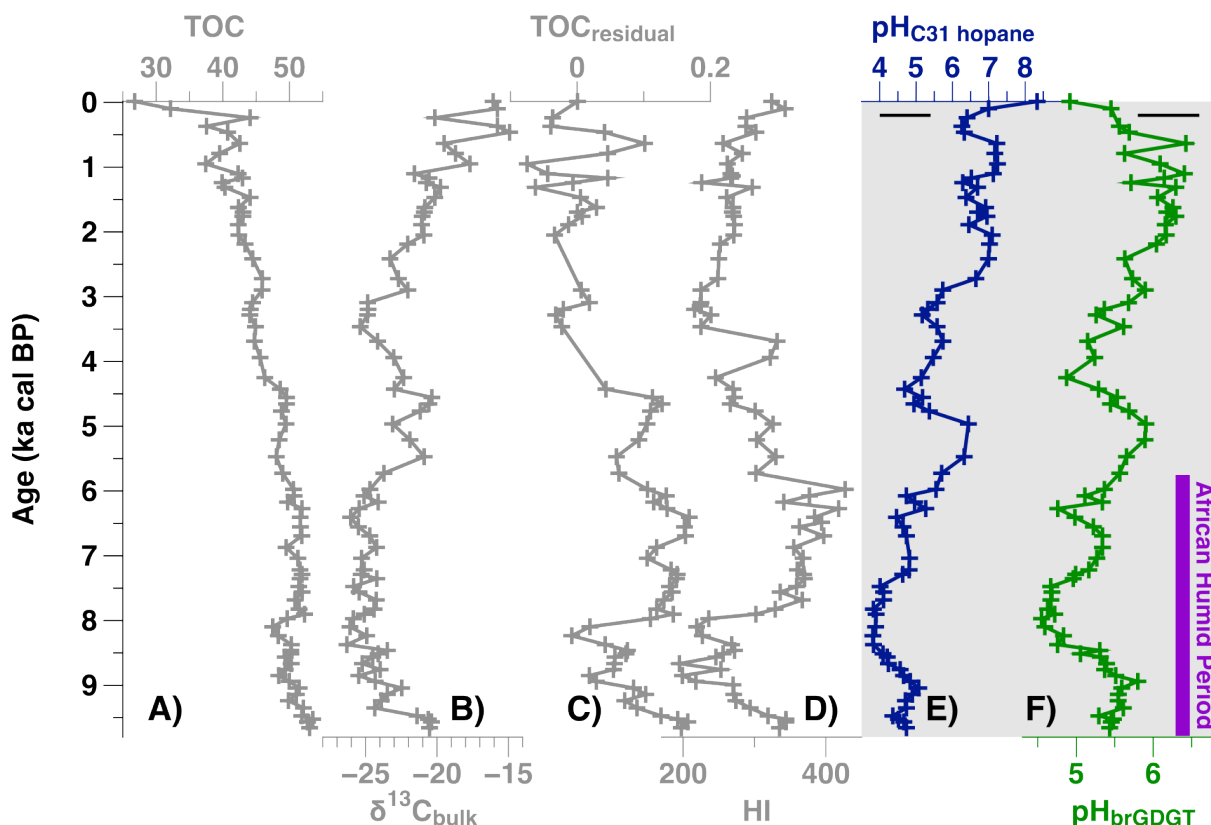
344 The CBT_{peat} index ranges from -1.4 to -0.7 (Fig. 3 C). This index oscillates around -1 from 9.7 to 8.9 ka cal BP, and then progressively decreases to reach its lowest values between 8.4 and 7.4 ka cal BP. The
 345 CBT_{peat} index increases between 7.4 and 4.9 ka cal BP, with a short decrease centered at 6.3 ka cal BP.
 346 It then decreases from 4.9 to 4.0 ka cal BP and increases again roughly between 4.0 to 0.9 ka cal BP
 347 with short time-scale variations. The CBT_{peat} index then decreases until the present day (Fig. 3 C).
 348



349 **Fig. 3: Downcore evolution of hopane- and GDGT- indices with time: $C_{30} \beta\beta/(\alpha\beta+\beta\beta)$ (A), C_{31}**
 350 **$\beta\beta/(\alpha\beta+\beta\beta)$ (B) and CBT_{peat} (C). The dotted line shows the gaps in the data and the isolated values**
 351 **compared to the other indices.**
 352

3.4. pH reconstructions

353
 354
 355 BrGDGT-based reconstructed pH values range from 4.5 to 6.5, while hopane-based reconstructed pH
 356 values range from 3.8 to 8.3 (Fig. 4 E and F). From 9.7 to 9 ka cal BP, pH reconstructions record stable
 357 values around 4.8 and 5.5 for hopane-based and brGDGT-based reconstructions, respectively. Both pH
 358 reconstructions then decrease and present their lowest values between 8.4 and 7.4 ka cal BP. Values
 359 remain low until 6.2 ka cal BP. During the middle and late Holocene, pH globally increases with smaller
 360 scale variations visible in both pH records. In particular, both pH reconstructions show a constant
 361 decrease between 4.8 and 3.3 ka cal BP and more stable values after 3.3 ka cal BP. In contrast to the
 362 rest of the core, the shallowest samples show inverse trends for both pH reconstructions, with an
 363 increasing trend in the hopane-based pH and a decreasing trend in the GDGT-based pH (Fig. 4 E and
 364 F).
 365



366
 367 **Fig. 4: Downcore evolution of bulk organic data: TOC (A), $\delta^{13}\text{C}_{\text{bulk}}$ (B), $\text{TOC}_{\text{residual}}$ (C) and HI (D)** (from
 368 Schaaff et al., 2023) and pH reconstructions: C_{31} hopane-based pH (E), brGDGT-based pH (F). The
 369 RMSE associated with each pH calibration is indicated at the top of each panel. The $\text{TOC}_{\text{residual}}$ curve
 370 was obtained by using the TOC values and a simple logarithmic decomposition model to remove the
 371 part of the signal associated with the decomposition of the organic matter (Schaaff et al., 2023). The
 372 duration of the African Humid Period at the NGaoundaba peat deposit was defined based on
 373 transitions observed in bulk organic data and is consistent with published literature (Schaaff et al.,
 374 2023).

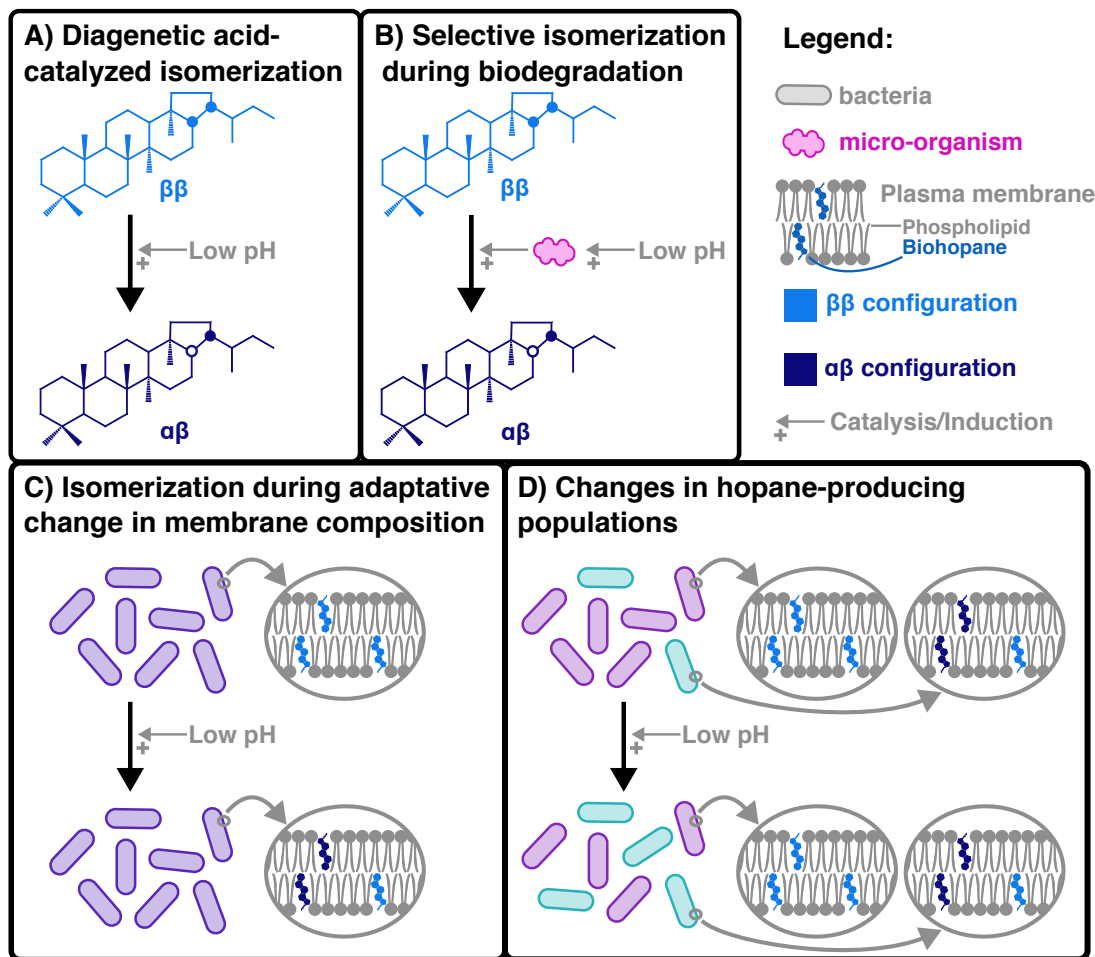
377 4. Discussion

378 4.1. Constraints on the origin of hopanes in peat

379
 380 To explain the high abundance of C_{31} $\alpha\beta$ isomer in peats (e.g., Quirk et al., 1984; Ries-Kautt and
 381 Albrecht, 1989; this study) as well as the correlation between the C_{31} $\beta\beta/(\alpha\beta+\beta\beta)$ ratio and pH (Inglis
 382 et al., 2018), several hypotheses have been proposed and are illustrated in figure 5. Using hopanes
 383 data from the NGaoundaba peat deposit and comparison with previously published data, this section
 384 evaluates these hypotheses.

385 The first hypothesis proposed to explain the high abundance of the “thermally mature” $\alpha\beta$ hopane
 386 isomers in peats characterized by low pH values relies on the abiotic isomerization of the carbon
 387 skeleton at the C17 position of the $\beta\beta$ isomers catalyzed by the low pH of the system (Fig. 5 A) (Pancost
 388 et al., 2003). This hypothesis is consistent with the low abundance of the C_{31} $\alpha\beta$ hopane in peat
 389 characterized by neutral pH (Dehmer, 1995; Inglis et al., 2018) but still lacks a clear demonstration,
 390 notably from laboratory experiments. Furthermore, several observations in this study argue against
 391 such a direct acid-catalyzed isomerization of $\beta\beta$ hopanes. First, assuming that hopanes are produced
 392 exclusively as $\beta\beta$ isomers, the opposite variations in the $\beta\beta/(\alpha\beta+\beta\beta)$ ratios of C_{30} and C_{31} hopanes
 393 observed throughout the core would not support a purely acid-catalyzed isomerization since C_{30}
 394 hopane and C_{31} homohopane should be affected similarly by such an abiotic process (Fig. 3 A and B
 395 and Fig. S.5). C_{30} and C_{31} hopanes are most likely produced by different sources, with C_{31} hopanes

396 primarily produced by heterotrophs and C₃₀ hopanes possibly produced by a broader range of sources,
 397 including methanotrophs (Inglis et al., 2019). If the isomerization from ββ to αβ hopanes is acid-
 398 catalyzed, C₃₀ and C₃₁ ββ/(αβ+ββ) ratios should follow similar trends regardless of the initial
 399 concentration of ββ isomers produced. Second, isomerizations by intramolecular rearrangement have
 400 no or limited isotope effect (Hightower and Hall, 1967), which is consistent with C₃₁ ββ and αβ hopanes
 401 having similar δ¹³C values during early diagenesis in oil shale (Sinninghe Damsté et al., 1995). The δ¹³C
 402 values of C₃₁ ββ and αβ hopanes present similar core profiles but differ by up to 5 to 10 ‰ at both the
 403 NGaoundaba peat deposit (Fig. 2 C and D) and the Dajiuhu peatland (Huang et al., 2018, see
 404 supplementary material), arguing against a direct acid-catalyzed isomerization of hopanes sharing the
 405 same biological source organism(s). The NGaoundaba peat deposit has a significant difference of up to
 406 10 ‰ compared to the Dajiuhu peatland (Huang et al., 2018) and the global peat database (Inglis et
 407 al., 2019), which rarely exceeds 5 ‰. The δ¹³C values of C₃₁ ββ and αβ hopanes differ mainly after ca.
 408 7 ka cal BP (Fig. 2 C and D), suggesting a change in the microbial community after 7 ka cal BP leading
 409 to different sources of C₃₁ ββ and αβ hopanes. This change in bacterial community could be driven by
 410 changes in physico-chemical conditions, such as the progressive increase in pH after 7 ka cal BP (Fig. 4
 411 E and F).
 412



413
 414 **Fig. 5: Different hypotheses are proposed to explain the high concentrations of the C₃₁ αβ hopane**
 415 **in peats and the correlation between the C₃₁ ββ/(αβ+ββ) ratio and pH. (A) The biologically produced**
 416 **ββ is isomerized abiotically into the C₃₁ αβ hopane catalyzed by the low pH of the peat. (B) The C₃₁**
 417 **ββ hopane is isomerized into the C₃₁ αβ hopane during the biodegradation by microorganisms. The**
 418 **development of the microorganisms involved in this degradation is likely pH-sensitive. (C) Both ββ**
 419 **and αβ configurations are biologically produced. Changes in membrane composition occur as an**
 420 **adaptation to changing pH. (D) Both ββ and αβ configurations are biologically produced and each**

421 **species of bacteria has its own biohopane composition. Changes in the microbial community in**
422 **response to pH variations are responsible for the observed distribution of $\beta\beta$ and $\alpha\beta$ geohopanes.**
423 **For simplification, bacterial membranes are represented here as a simple bilayer composed of**
424 **phospholipids and biohopanes.**

425
426 Such findings point to either a biological transformation of $\beta\beta$ hopanes into $\alpha\beta$ isomers during
427 biotransformation (Fig. 5 B) (Ries-Kautt and Albrecht, 1989), or a direct bacterial input of hopanes,
428 with different isomeric configurations (Fig. 5 C or D). Hopanes has been shown to undergo selective
429 biodegradation, such as during the demethylation at C25 of hopanes, which occurs preferentially in
430 low molecular-weight hopanes and favors different epimers (22S and 22R) depending on molecular
431 weight (Requejo and Halpern, 1989; Peters et al., 1996). Hopanes with different shapes, dimensions
432 and volumes, as well as different molecular weight and isomerization may affect their biodegradation
433 (Peters et al., 1996), but more research is needed to estimate these effects in sediment of low thermal
434 maturity. The different evolution of C_{30} and C_{31} $\beta\beta/(\alpha\beta+\beta\beta)$ hopanes ratios could be attributed to
435 selective biodegradation. It has been shown that some bacteria are capable of producing biohopanoids
436 epimers at C-22 (Peiseler and Rohmer, 1992) or $\beta\beta$, $\beta\alpha$ and $\alpha\beta$ biohopanoids although this has only
437 been demonstrated in only one strain of *Frankia* sp. so far (Rosa-Putra et al., 2001). On the other hand,
438 bacteria are able to alter their membrane composition in response to changes in environmental
439 parameters, enabling them to maintain acceptable membrane fluidity and cell activity (Fig. 5 C) (e.g.,
440 Russell and Fukunaga, 1990; Vinçon-Laugier et al., 2017). Changes in microbial community structure
441 as well as membrane lipid composition are used to explain the observed changes in MBT and CBT
442 indices and in temperature and pH calibrations based on brGDGTs (e.g., Weijers et al., 2007; Huguet
443 et al., 2014; De Jonge et al., 2021; Naafs et al., 2021; Halamka et al., 2023). A similar adaptation may
444 exist in biohopanoid-producing bacteria, with greater production of the more stable $\alpha\beta$ -isomers under
445 more acidic conditions (Fig. 5 C). However, as far as we know, the impact of pH variations on the
446 biohopanoid composition of bacterial membranes is not known and functionalized biohopanoids with
447 the $\alpha\beta$ configuration have only been reported in *Frankia* spp (Rosa-Putra et al., 2001). Further studies
448 showing the occurrence of $\alpha\beta$ hopanoids in living organisms are clearly needed. In addition, very few
449 data are currently available on the stereochemistry of biohopanoids such as bacteriohopanepolyols
450 (BHPs) in modern peat samples (e.g., Talbot et al., 2016) which could provide an indirect evidence of
451 the origin of $\alpha\beta$ hopanoids.

452 Carbon isotopic data of microbial-derived biomarkers provide unique information about the origin of
453 these compounds (e.g., Schouten et al., 2000a; Pancost et al., 2003; Schwartz-Narbonne et al., 2023).
454 Overall, the range of $\delta^{13}C$ values of C_{31} $\beta\beta$ and $\alpha\beta$ hopanes in the NGaoundaba peat deposit (Fig. 2 C
455 and D) is consistent with values reported in other studies (e.g., Pancost et al., 2003; Xie et al., 2004;
456 McClymont et al., 2008; Huang et al., 2018; Inglis et al., 2019). The $\delta^{13}C$ values of C_{31} $\beta\beta$ and $\alpha\beta$ hopanes
457 follow similar variation as the $\delta^{13}C_{bulk}$, in line with previous studies suggesting a heterotrophic origin
458 for these compounds without a major input from methanotrophic bacteria (Pancost et al., 2003; Inglis
459 et al., 2019). The $\delta^{13}C$ values of C_{31} $\alpha\beta$ hopane are nearly identical to those from the C_{bulk} at the bottom
460 of the core and from 8.1 to 6.0 ka cal BP but present a positive shift compared to the $\delta^{13}C_{bulk}$ from 9.3
461 to 8.2 ka cal BP. As suggested by Pancost and Damsté (2003), this shift towards higher values may
462 suggest preferential bacterial consumption of labile ^{13}C -enriched carbon during this period. However,
463 C_{31} $\beta\beta$ hopane appears to be between 0.9 and 9.6 ‰ more depleted in ^{13}C than the C_{31} $\alpha\beta$ isomer all
464 along the core (Fig. 2 D). Consumption of refractory ^{12}C -enriched carbon or depleted carbon sources
465 such as methane by the producer(s) of the $\beta\beta$ isomer cannot be excluded. Data from the Dajiuhe
466 peatland (Huang et al., 2018, see supplementary material) also show more depleted $\delta^{13}C$ values for C_{31}
467 $\beta\beta$ hopane compared with those from C_{31} $\alpha\beta$ hopane. The difference between the $\delta^{13}C$ values of C_{31}
468 $\beta\beta$ and $\alpha\beta$ hopanes thus supports distinct producers of these two hopane isomers that use different
469 carbon sources (Fig. 5 D). Similarly, a source decoupling between C_{30} and C_{31} hopanes, as proposed by
470 Inglis et al. (2019), may explain the opposite variations observed for the two hopane ratios at the
471 NGaoundaba peat deposit (Fig. 3).

472 Microbial biodegradation has been shown to have diverse effect on the carbon isotopic fractionation
473 of different lipids possibly associated with difference in the degradation pathway (Sun et al., 2004).
474 The differences between the carbon isotopic values of the $\beta\beta$ and $\alpha\beta$ isomers of hopanes might be
475 explained by heavy isotope effects in enzyme-catalyzed reactions (Heipieper et al., 2004; O'Leary,
476 1980), such as those involved during biodegradation of organic matter (Fig. 5 B). In enzyme-catalyzed
477 reactions, the magnitude of the isotope effect depends on the structure of the transitional state
478 related to the change in bonding to the isotopic atom (O'Leary, 1980). No estimation of the isotope
479 effect exists for hopane isomerization at present. However, a higher rate of isomerization from $\beta\beta$ to
480 $\alpha\beta$ may be expected to drive heavier $\beta\beta$ hopane $\delta^{13}\text{C}$ than that of $\alpha\beta$ hopane. This has been observed
481 for other reactions and compounds (Heipieper et al., 2004; Pan et al., 2014). At the NGaoundaba peat
482 deposit, low values of the C_{31} hopane $\beta\beta/(\alpha\beta+\beta\beta)$ ratio correlate with a period of more negative $\delta^{13}\text{C}$
483 values of $\alpha\beta$ hopane, reducing the difference between $\alpha\beta$ and $\beta\beta$ hopane $\delta^{13}\text{C}$ values (Fig. 2 C and D
484 and Fig. S.9). Thus, each scenario (B), (C) and (D) is not exclusive, and a combination of several of these
485 scenarios is possible.

487 4.2. Biomarker-based pH reconstructions

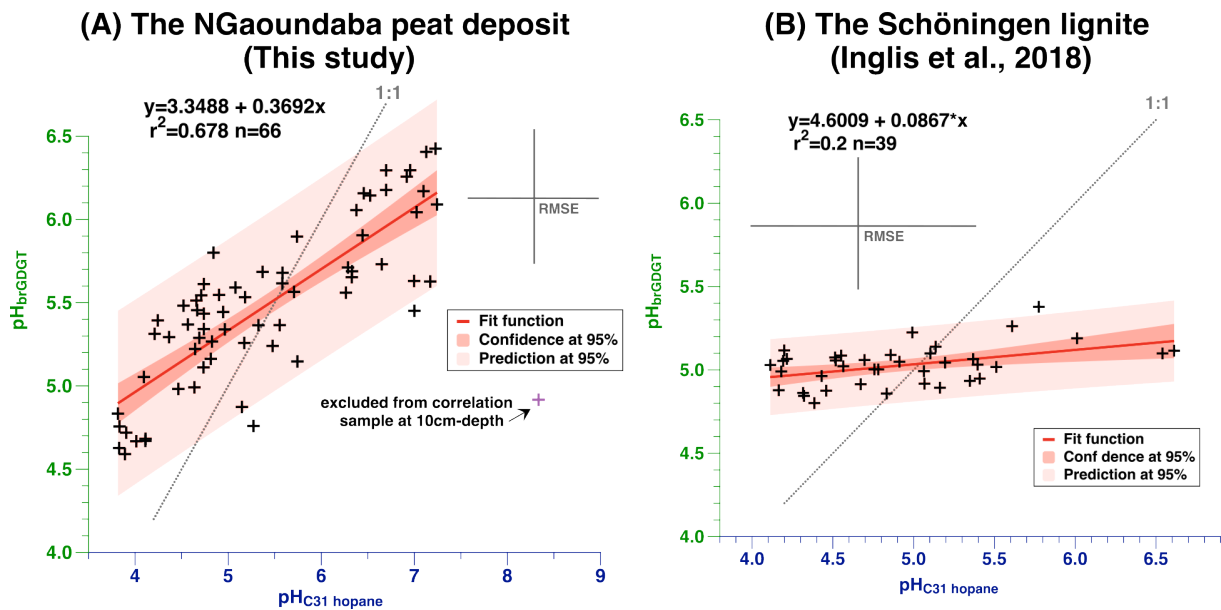
488 4.2.1. Comparison between hopane- and brGDGT-based pH reconstructions

489
490 Using the brGDGT and hopane molecular data, we reconstructed two pH records using previously
491 published empirical calibrations (Naafs et al., 2017; Inglis et al., 2018). The two calibrations are
492 respectively based on the C_{31} hopane $\beta\beta/(\alpha\beta+\beta\beta)$ ratio and on the brGDGT CBT_{peat} ratio. These two
493 ratios are a priori independent as they are based on two distinct classes of molecules known so far to
494 be produced by at least somewhat different populations of bacteria.

495 A statistically significant positive correlation between the two pH reconstructions ($r^2= 0.678$, $n=66$, p -
496 value $\ll 0.01$) is observed for the NGaoundaba peat record (Fig. 6 A), with only one outlier data point
497 corresponding to the uppermost sample (at 10 cm). The latter can be attributed to different production
498 or degradation depths between hopanes and brGDGTs. Consistent with this hypothesis, either one or
499 both of the hopane isomers are absent from several surface peat sediments (see supplementary
500 material in Inglis et al., 2018). In the NGaoundaba peat record, the C_{31} $\alpha\beta$ hopanes are absent in the
501 topmost sample, leading to a $\beta\beta/(\alpha\beta+\beta\beta)$ ratio equal to 1. Consequently, a maximum pH value is
502 obtained with the calibration from Inglis et al. (2018). Similarly, brGDGTs also show strong variations
503 in the more recent samples, with the topmost sample having the lowest concentrations in all brGDGTs
504 studied. The correlation between the two pH proxies suggests a similar response of these two
505 independent proxies to environmental changes and thus supports their use for paleo-pH
506 reconstructions despite different absolute reconstructed pH values. The range of pH values is larger
507 for hopane-based pH than for brGDGT-based pH and therefore the correlation between the two pH
508 reconstruction deviates from a 1:1 line (Fig. 4 E, F and Fig. 6 A).

509 As for the NGaoundaba peat deposit, similar variations between hopane-based and brGDGTs-based
510 pH reconstructions were previously observed in the Schöningen lignite dating from early Paleogene
511 (~55 Ma) (Inglis et al., 2015, 2018). We thus compared our results with those from the Schöningen
512 lignite (Inglis et al., 2015, 2018) (Fig 6 A and B, respectively). The correlation between the two sets of
513 pH values appears weaker for the lignite than that found for the NGaoundaba peat deposit. While the
514 pH range reconstructed using brGDGTs is very narrow (less than 0.6 pH unit), the pH range
515 reconstructed using C_{31} hopane isomers is much larger (around 2.6 pH unit), resulting in a correlation
516 that strongly deviates from the 1:1 line. Because the Schöningen lignite samples are older than the
517 NGaoundaba peat deposit samples, this result could suggest a diagenetic influence on hopane-based
518 pH reconstruction, with an increased deviation from the 1 :1 line with time. To test this hypothesis on
519 the hopane-based reconstructed pH values from the NGaoundaba peat deposit, we calculated the
520 shortest distance to the 1:1 line for each data point. The absence of correlation between this distance
521 and the sample age (Fig. S.6) indicates that the deviation from the 1:1 line is unrelated to the age of

522 the sample, at least for the time period covered in the present peat (ca. 10 ka cal BP) (Fig. S.6) and that
 523 diagenesis is likely not responsible for this deviation.
 524



525
 526 **Fig. 6: Correlation between C₃₁ hopane- and brGDGT-reconstructed pH values for the NGaoundaba**
 527 **peat deposit (This study) (A) and for the Schöninggen lignite (Inglis et al., 2015, 2018) (B).** The dotted
 528 **line represents the 1:1 line. The RMSE associated with each pH calibration is represented in light**
 529 **grey. For the NGaoundaba peat deposit, the uppermost sample (10cm-depth) has been excluded**
 530 **from the correlation.**
 531

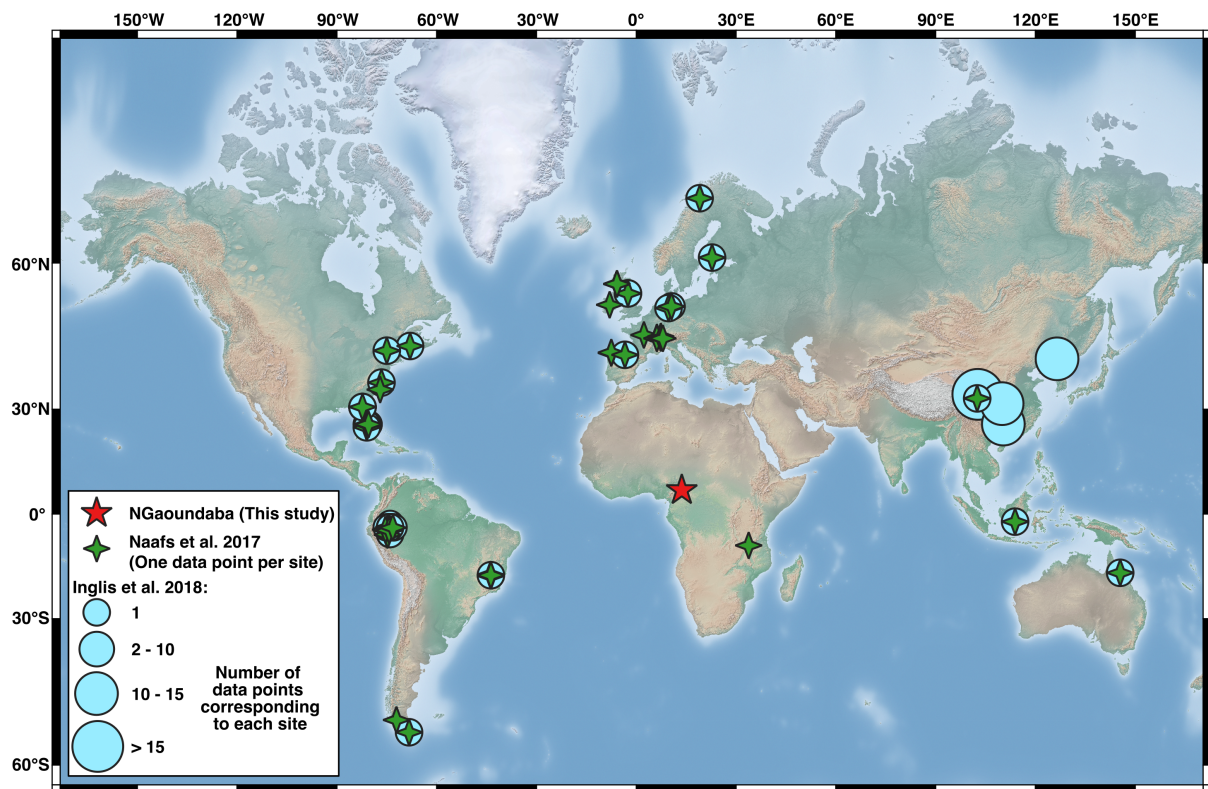
4.2.2. Bias in absolute pH values due to existing calibration datasets

532
 533 Unlike temperature, which can be estimated using climatic models, the pH values used in empirical
 534 calibrations must be measured directly *in situ*. Such data are lacking for some sites in the global peat
 535 database, resulting in a restricted pH calibration database (51 sites for pH out of the 96 used for
 536 temperature) (Fig. 7 - Green cross) (Naafs et al., 2017). Several studies have pointed out the lack of
 537 studies based on organic compounds in tropical peatlands and its impact on our understanding of
 538 brGDGT distributions and temperature/pH empirical calibrations (Weijers et al., 2011; Naafs et al.,
 539 2017). Among low latitude sites, African sites are particularly scarce in previous calibrations, with only
 540 1 out of 51 sites for the brGDGT pH calibration and none out of 94 sites for the hopane pH calibration
 541 (Naafs et al., 2017; Inglis et al., 2018). This poor representation of low latitude peat deposits may
 542 significantly bias the global empirical calibrations as the specificities of these low-latitude records, such
 543 as precipitation regime (amount and seasonality), vegetation types, and temperature variations are
 544 not taken into account. Vegetation types influence peat microbial activity in both temperate and
 545 tropical peatlands (e.g., Fisk et al., 2003; Andersen et al., 2013; Girkin et al., 2020). For both hopane
 546 and brGDGT pH calibrations, vegetation diversity is well represented among the sampled peats (Naafs
 547 et al., 2017; Inglis et al., 2018), meaning that biases associated with differences in vegetation (Liang et
 548 al., 2019) are likely included in calibration errors. However, in the hopane-based pH calibration
 549 proposed by Inglis et al. (2018), 63 data points out of 94 come from four Chinese peats (Fig. 7 - Blue
 550 Circles). The over-representation of the four sites located in a restricted geographical area may bias
 551 the global pH calibration and could partly explain the larger range of pH values reconstructed using
 552 hopanes compared to brGDGTs (see Huang *et al.* (2015) for details on the environmental and climatic
 553 characteristics of these four sites).
 554

555 Naafs et al. (2017) demonstrated that, in general, the overall brGDGT distribution is not significantly
 556 different between the upper 15 cm (acrotelm, likely oxic) and the peat sediment underneath (catotelm,
 557 likely anoxic), but that some high-latitude peatlands show strong differences in brGDGT distribution

558 between both layers, likely associated with the seasonal bias of brGDGT production (Naafs et al., 2017).
 559 To address this problem, Naafs et al. (2017) retained all data from the upper 1 m without
 560 differentiating the acrotelm and the catotelm, and averaged all samples for each peatland, except for
 561 high latitude peats, where the top 20 cm were excluded. The same problem may be encountered with
 562 hopanes. While some studies show strong variations in hopane distributions (Huang et al., 2015) and
 563 concentrations (Farrimond and Flanagan, 1996; He et al., 2015) within the first meter of peat deposits,
 564 a rapid isomerization is suggested in several studies (Inglis et al., 2018; Quirk et al., 1984). The use of
 565 both surface and averaged samples from the upper first meter could therefore bias the calibrations
 566 and explain the differences between the ranges of pH values obtained from hopane and brGDGT
 567 calibrations.

568 The CBT_{peat} and $C_{31} \beta\beta/(\alpha\beta+\beta\beta)$ values obtained for the first meter of the NGaoundaba peat deposit,
 569 along with the pH measured in the peat, were compared with the Global Peat Database, which was
 570 used to calibrate brGDGT- and hopane-based pH reconstructions (Fig. S.7). Despite the scarcity of
 571 African peat deposits in the global calibration dataset, values from the NGaoundaba site are consistent
 572 with the global calibrations, allowing interpretations derived from brGDGT and hopane distributions,
 573 as well as the carbon isotopic composition of hopanes in our study site to be generalized to other peat
 574 deposits worldwide.



575
 576 **Fig. 7: Map with the location of all peats used for global pH calibrations by Naafs et al. (2017) (green**
 577 **cross) and Inglis et al. (2018) (blue circles). The size of the blue circles is proportional to the number**
 578 **of data points for each site used in the calibration. The location of the NGaoundaba peat deposit is**
 579 **indicated by the red star.**

581 4.3. Local bias affecting pH reconstructions

582 4.3.1. Influence of microbial activity in lower peat layers

583
 584 The microbial community structure has been shown to vary significantly over the first meter of peat,
 585 due to significant changes in peat properties and environmental variables such as pH, redox conditions,
 586 moisture content, or nutrient availability (e.g., Williams and Crawford, 1983; Ausec et al., 2009;

587 Jackson et al., 2009; Andersen et al., 2013; Too et al., 2018; Girkin et al., 2020). The upper oxic layer
588 (acrotelm) has a relatively high decomposition rate of organic matter compared to the lower anoxic
589 layer (catotelm) (Clymo, 1984). The upper oxic layer is dominated by aerobic decomposition mediated
590 by fungal and/or bacterial populations (Winsborough and Basiliko, 2010; Tripathi et al., 2016).
591 Microbial diversity and activity are highest at the surface (Jackson et al., 2009; Too et al., 2018), but a
592 high relative abundance of certain phyla such as *Acidobacteria* has been reported in deeper layers (i.e.,
593 around 45cm-depth) (Too et al., 2018) and microbial activity has also been reported in deep (2m depth)
594 peat horizons (Kluber et al., 2020). As *Acidobacteria* are producers of both hopanes (Welander et al.,
595 2010) and brGDGTs (Sinninghe Damsté et al., 2014, 2011; Chen et al., 2022; Halamka et al., 2023), the
596 potential influence of the presence of this phylum in the lower peat layer on pH reconstructions should
597 be considered. Although brGDGTs have mostly been associated with aerobic phyla so far (e.g.,
598 Sinninghe Damsté et al., 2018), brGDGT production may require low oxygen availability (Halamka et
599 al., 2021) and the occurrence of anaerobic producers cannot be excluded. Lipid production in the lower
600 peat layers may cause an offset between the molecular signal and the age of the bulk peat sediment.
601 The latter may be older than the compounds considered for pH reconstruction.
602 To test this hypothesis, we artificially added a positive or negative offset in one of the two pH
603 reconstructions and recalculated the correlation between the two pH reconstructions. Specifically,
604 $\text{pH}_{\text{C}_{31} \text{ hopane}}$ at time t_n was compared to the $\text{pH}_{\text{brGDGT}}$ at time t_{n+1} with n the sample number increasing
605 with depth, and vice versa. Adding an offset did not improve the correlation, suggesting that there is
606 no offset between the two pH reconstructions (see supplementary material, Fig. S.8 A and B).
607 Compound-specific ^{14}C dating, as proposed by Pancost et al. (2000), could confirm the limited impact
608 of microbial activity in deeper layers, and provides a better understanding of microbial activity in deep
609 peat layers. Another possibility to identify an offset is to compare the pH reconstructions with other
610 non-microbial proxies. Major climate transitions affect both vegetation and microbial communities. In
611 the NGaoundaba peat record, the end of the African Humid Period (AHP) is well marked in bulk organic
612 parameters (HI, $\text{TOC}_{\text{residual}}$ and $\delta^{13}\text{C}_{\text{bulk}}$; Schaaff et al. (2023)) around 5.8 and 5.7 ka cal BP (Fig. 4 B, C
613 and D, Schaaff et al., (2023)). A transition from low pH values during the AHP to higher pH values during
614 the middle and late Holocene as well as changes in hopane and brGDGT concentrations are recorded
615 between 6.0 and 5.8 ka cal BP (Fig. 4 E and F). A limited impact of the difference in response time to
616 climate change between the microbial community and vegetation is expected (e.g., Webb, 1986;
617 Hughen et al., 2004; Waldrop and Firestone, 2006). Thus, an offset of less than 0.3 ka cal BP may occur
618 at the NGaoundaba peat deposit between the age model and the variations recorded in vegetation-
619 based proxies and the variations recorded in the microbially-based proxies. The pH signal may also be
620 an integrated signal of compounds produced in a centimetric or even decametric depth range. Weijers
621 et al. (2011) showed an absence of correspondence between CBT-based pH values and *in situ*
622 measured pore water pH, further suggesting that the brGDGT signal is more likely to represent a
623 preserved 'fossil' biosignature than an *in situ* production in deep peat.
624 Caution is needed, however, when interpreting results from the upper part of the peat deposits.
625 Analyses of short recent peat sequences can lead to misinterpretations, corresponding to recent
626 changes in microbial community structure rather than to a paleoclimatic signal.

627 628 **4.3.2. A seasonal bias for pH reconstruction?** 629

630
631 Seasonality is a significant bias in brGDGT temperature reconstructions in various settings, most likely
632 due to bacterial membrane adaptation to environmental changes or changes in the microbial
633 community structure (e.g., Sun et al., 2011; Loomis et al., 2014; Cao et al., 2018; Miller et al., 2018).
634 Except for seasonally frozen sites, temperature seasonality has been shown to have little impact on
635 brGDGT temperature calibration in peats (Dearing Crampton-Flood et al., 2020; Naafs et al., 2017).
636 While temperature seasonality is a major concern in high-latitude and temperate records, tropical
637 regions have narrower temperature variations throughout the year, making tropical peat records less
638 susceptible to affect temperature seasonality bias (Loomis et al., 2012; Pérez-Angel et al., 2020).
639 However, as aforementioned, precipitation can have an impact on pH values. Because of the strong
640 seasonal variations in precipitation that occurs in the tropics, this could affect the microbial community
641 structure and activity, and, in turn, severely bias pH reconstructions. Marwanto et al. (2018), for
642 example, show that drought and rewetting events affected the pH values of two tropical peatlands in
643 Indonesia. During a drought event, the pH at 50 cm-depth decreases due to increased oxidative
644 conditions but remains low long after the next rewetting event begins (Marwanto et al., 2018). It is
645 beyond the scope of this study to determine how seasonal climate and environmental changes will
646 affect pH reconstructions in peat, but additional research is undoubtedly required. Differences in
647 sensitivity to seasonal sensitivity between brGDGT producers and hopane producers may explain, in
648 part, the observed difference in the range of reconstructed pH values (Fig. 4 E and F and Fig. 6).

649 **4.4. A multi-causal interpretation of pH reconstructions**

651
652 Changes in reconstructed pH have been proposed to reflect past changes in precipitation, with
653 increased precipitation leading to a dilution effect and an increase in pH (Pancost et al., 2003; Inglis et
654 al., 2018). The minimum reconstructed pH values for the NGaoundaba peat deposit, between 8.4 and
655 6.2 ka cal BP, coincide with a period of increased precipitation in agreement with the timing of the
656 African Humid Period (Fig. 4 B, C and D, Schaaff et al., (2023)) and are therefore not consistent with a
657 potential dilution effect. Consistently, higher brGDGT-reconstructed pH values were recorded in a “dry”
658 site compared to a “wet” site from the Frasné peatland (Jura Mountains, France), suggesting an
659 influence of peat moisture level or oxygen availability on brGDGT distribution (Huguet et al., 2010). In
660 paleorecords, similar relationship between peat moisture and/or precipitation and CBT-based pH (soil
661 calibration) have been suggested (Zheng et al., 2015; Wang et al., 2017). The low pH recorded at the
662 NGaoundaba peat deposit is also consistent with ombrotrophic conditions (wetland hydrology mostly
663 controlled by precipitation) as opposed to minerotrophic conditions (wetland hydrology modulated by
664 precipitation and groundwater input) which usually present higher pH (e.g., Swanson and Grigal, 1991).
665 Two other parameters seem to strongly influence pH variations and can be responsible of pH variations
666 in paleorecords. First, catchment lithology may have a strong impact on long term variations of peat
667 pH. More specifically, the presence of carbonate in the catchment area and its leaching can induce
668 alkaline input into the peatland. Among the paleopH records explored here (Zheng et al., 2015; Wang
669 et al., 2017; Zhang et al., 2020, this study), the Dajiuhu peat deposit is the only site situated in a
670 carbonate-dominated catchment (Zhang et al., 2022). Interestingly, this site records a decrease in
671 hopane-based pH during the dry deglaciation period (Zhang et al., 2020) while other sites record an
672 increase in reconstructed pH during drier periods. This suggests that the decrease in precipitation
673 during the deglaciation at the Dajiuhu peatland decreased the runoff from the catchment and the input
674 of alkali resulting from carbonate alteration, reducing the buffering effect of carbonate at this site.
675 Second, changes in the peat-forming vegetation influence peat pH since some plants, such as
676 *Sphagnum* mosses, directly contribute to peat acidification through cation exchange (Clymo, 1963).
677 Major transitions previously observed in CBT-based pH reconstructions were attributed to major
678 changes in vegetation, with the transition from sedge-dominated peat to *Sphagnum*-dominated peat
679 associated with lower pH values during *Sphagnum*-dominated interval (Nichols et al., 2014; Weijers et
680 al., 2011). Weijers et al. (2011) associated these changes with changes in the trophic status of the peat
681 from a fen system to an ombrotrophic bog. As the catchment area of the NGaoundaba peat deposit is

682 small and located in a maar crater with a slightly higher elevation than the surrounding area, this
683 deposit has probably remained an ombrotrophic peat over the last 10 ka, fed primarily by rainfall water
684 without a permanent water inlet. Despite the absence of *Sphagnum* mosses at NGaoundaba, pH
685 variations based on brGDGTs and hopanes may coincide with major changes in local vegetation, as
686 suggested by significant variations in $\delta^{13}\text{C}_{\text{bulk}}$ (Fig. 4 D, E and F). Such vegetation changes observed at
687 the NGaoundaba peat deposit may coincide with changes in precipitation and associated changes such
688 as water table depth and oxygenation level. Therefore, in the absence of *Sphagnum* mosses,
689 simultaneous changes in vegetation and pH may only reflect a correlation between these two
690 parameters without any causal effect.

691 The interpretation of past pH variations based on organic proxies remains complex, and pH variations
692 cannot be attributed to a single cause. Prior to any interpretation in terms of change in precipitation
693 and precipitation/evaporation balance, a good understanding of catchment characteristics (size,
694 lithology, vegetation, and trophic status of the peat) and their evolution through time is fundamental.
695 At the NGaoundaba peat deposit, a decrease of reconstructed pH is recorded from 9.7 to 5.8 ka cal BP
696 which coincides with the African Humid Period. It is likely that paleo-pH reconstructions based on
697 microbial compounds produced in-situ are also affected by changes in peat moisture level, reflecting
698 the balance between precipitation and evapotranspiration. The progressive decrease of pH between
699 8.9 and 8.0 ka cal BP coincides with a period of low $\text{TOC}_{\text{residual}}$ and HI values interpreted as a potential
700 drier intermission during the AHP (Schaaff et al., 2023). During this period, a change in
701 evapotranspiration or in the seasonality of precipitation rather than in the amount of precipitation
702 could explain the discrepancy between low reconstructed pH suggesting more precipitation, and low
703 TOC and HI values suggesting a drier period (Fig. 4). Between 8.0 and 5.8 ka cal BP, reconstructed pH
704 values present their lowest values consistent with increase precipitation interpreted based on bulk
705 organic data (Fig 4.). Low pH values during the African Humid Period are consistent with good
706 preservation of organic matter (Moore, 1989) and coincide with high $\text{TOC}_{\text{residual}}$ and HI values that may
707 be associated with better preservation of peat organic matter (Schaaff et al., 2023). The increase in pH
708 recorded in the mid- and late Holocene probably led to an increase in methanotrophy and possibly in
709 methanogenesis (Dunfield et al., 1993) which is consistent with previous studies suggesting more
710 methanogenesis at neutral pH values (Inglis et al., 2019).

711 712 **Conclusions and perspectives**

713 Through the joint investigation of bacterial hopanes and brGDGTs in a sequence of African peat, our
714 study validates the use of these families of molecules for the reconstruction of pH variations but also
715 highlights their limitations and perspectives for improvement. The comparison of C_{30} and C_{31}
716 $\beta\beta/(\alpha\beta+\beta\beta)$ hopane ratios as well as the carbon isotopic composition of C_{31} $\beta\beta$ and $\alpha\beta$ isomers
717 demonstrate that the direct acid-catalyzed isomerization of $\beta\beta$ into $\alpha\beta$ isomers is unlikely without
718 biological mediation. The influence of deep peat hopane and brGDGT production appears to be limited
719 at the NGaoundaba peat deposit since no downcore trend in hopane and brGDGT concentrations and
720 abundances is observed and limited offset with proxies independent of microbial production has been
721 found. Since C_{30} hopanes do not follow the same trends as other hopanes, it is not advisable to use
722 this class of molecules to reconstruct paleo-pH.

723 Multiple causes explain the variations in paleo-pH records. Firstly, a comparison of existing paleo-pH
724 records shows that catchment lithology, particularly the presence of carbonate, likely strongly
725 influences pH variations. Secondly, changes in vegetation can explain some of the variations in paleo-
726 pH records. Catchment lithology and vegetation changes can be constrained by literature data and
727 additional analyses such as pollen. Variations in pH values can then be interpreted in terms of the
728 balance between precipitation and evapotranspiration.

729 Future culturing and laboratory experiments should help confirming the potential occurrence of $\alpha\beta$
730 hopanes in bacteria or other types of living (micro)organisms, or their potential formation during
731 microbial adaptation to changing environment and/or biodegradation of organic matter. Additionally,
732 new sets of data (both paleo-records and modern data) should improve the pH calibrations based on
733 hopane and brGDGT distributions and their potential as paleoenvironmental and paleoclimatic proxies.

734

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745

746 **Data availability**

747 Data are available on Pangaea (<https://doi.org/10.1594/PANGAEA.963887>).

748

749 **Appendix A. Supplementary Material**

750 Supplementary material includes compound structures (Fig. S.1), complements on hopane analyses
751 (uncertainty measurements) and an annotated HPLC-APCI-MS chromatogram (Fig. S.2), additional
752 tables and figures on relative abundances and concentrations of brGDGTs and hopanes (Table S.1 and
753 figure S.3) and $C_{31} 22S/(22S + 22R)$ (Fig. S.4). It also includes additional correlations that were tested
754 for this study and comparison with global peat database (Fig. S.5 to S.8) as well as a figure presenting
755 the isotopic composition difference between $C_{31} \beta\beta$ and $\alpha\beta$ hopanes (Fig. S.9).

756

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