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10 Environmental controls on the brGDGT and brGMGT distributions across the
11 Seine River basin (NW France): Implications for bacterial tetraethers as a proxy for
12 riverine runoff

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24
25

26 **Abstract**

27 Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are bacterial lipids that have been
28 largely used as environmental proxies in continental paleorecords. Another group of related lipids,
29 branched glycerol monoalkyl glycerol tetraethers (brGMGTs), has recently been proposed as a
30 potential paleotemperature proxy. Nevertheless, the sources and environmental dependencies of
31 both brGDGTs and brGMGTs along the river-sea continuum are still poorly understood,
32 complicating their application as paleoenvironmental proxies in aquatic settings. In this study, the
33 sources of brGDGTs and brGMGTs and the potential factors controlling their distributions are
34 explored across the Seine River basin (NW France), which encompasses the freshwater to seawater

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35 continuum. To this aim, brGDGTs and brGMGTs were analyzed in soils, Suspended Particulate
36 Matter (SPM) and sediments ($n=237$) collected all along this basin, from land to sea. Both types of
37 compounds are shown to be produced *in situ*, in freshwater as well as saltwater. Redundancy
38 analysis further shows that both salinity and nitrogen loadings dominantly control the brGDGT
39 distributions. Furthermore, the relative abundance of 6-methyl vs. 5-methyl brGDGTs (IR_{6Me} ratio),
40 Total Nitrogen (TN), $\delta^{15}N$ and chlorophyll *a* concentration co-vary in the upstream area, suggesting
41 that 6-methyl brGDGTs are preferentially produced under low-salinity and high-productivity
42 conditions. In contrast with brGDGTs, brGMGT distribution appear to be primarily regulated by
43 salinity, with a distinct influence on the individual homologues. Salinity is positively correlated
44 with homologues H1020a and H1020b, and negatively correlated with compounds H1020c,
45 H1034b, and H1034c. This suggests that bacteria thriving in freshwater preferentially produce
46 compounds H1020c, H1034b, and H1034c, whereas bacteria primarily growing in saltwater appear
47 to be predominantly responsible for the production of homologues H1020a and H1020b. Based on
48 the abundance ratio of the freshwater-derived compounds (H1020c, H1034b, and H1034c) vs.
49 saltwater-derived homologues (H1020a and H1020b), a novel proxy, Riverine Index (RIX) is
50 proposed to trace riverine organic matter inputs, with high values (>0.5) indicating higher riverine
51 contribution. As RIX relies on compounds that are specifically produced in certain settings
52 (freshwater or saltwater), this index has potential to serve as a powerful proxy for riverine runoff
53 in modern samples as well as in paleorecords.

54
55 *Keywords:* branched GDGTs; branched GMGTs; environmental proxies; land-ocean continuum;
56 riverine runoff

57

58 1. Introduction

59 Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are membrane lipids produced
60 by unknown bacteria, although some of them were attributed to the phylum *Acidobacteria*
61 (Sinninghe Damsté et al., 2011; Chen et al., 2022; Halamka et al., 2022). These compounds were
62 observed to occur ubiquitously in a wide range of terrestrial and aquatic environments (Schouten
63 et al., 2013; Raberg et al., 2022). The distribution of brGDGTs (number of cyclopentane moieties
64 and methyl groups; cf. structures in Fig. S1) was empirically linked with pH and Mean Annual Air
65 Temperature (MAAT) in soils (Weijers et al., 2007; De Jonge et al., 2014; Véquaud et al., 2022),
66 peats (Naafs et al., 2017; Véquaud et al., 2022) and lake sediments (Martínez-Sosa et al., 2021).
67 The brGDGT-based proxies (i.e. MBT'_{5ME} and CBT') have been largely applied to reconstruct
68 MAAT and pH from sedimentary archives (Coffinet et al., 2018; Harning et al., 2020; Wang et al.,
69 2020).

70 In aquatic settings, brGDGTs were initially suggested to be predominantly derived from
71 watershed soils and transported by erosion in the sediments (Hopmans et al., 2004). Based on this
72 assumption, the Branched and Isoprenoid Tetraethers (BIT) index was defined as the abundance
73 ratio of the major brGDGTs to crenarchaeol (isoprenoid GDGT mainly produced by marine
74 *Thaumarchaeota*). It is comprised between 0 and 1, with high BIT values (around 1) reflecting
75 higher contribution of terrestrial organic matter compared to marine organic matter (Hopmans et
76 al., 2004). Over the last years, the BIT index has been broadly used for quantifying the relative
77 contribution of terrestrial organic matter in aquatic systems (Xu et al., 2020; Yedema et al., 2023)
78 and evaluating the reliability of TEX₈₆ palaeothermometer (Cramwinckel et al., 2018). However,
79 several studies have shown that brGDGTs can also be produced *in situ* in aquatic settings (Peterse
80 et al., 2009; Tierney and Russell, 2009; Zell et al., 2014; De Jonge et al., 2015; Zhang et al., 2020),

81 adding complication for the identification of brGDGT sources in these ecosystems and for the
82 application of the brGDGTs as (paleo)environmental proxies, including the BIT index. The BIT
83 values have all the more to be carefully interpreted as they could also be influenced by the selective
84 degradation of branched *vs.* isoprenoid GDGTs (Smith et al., 2012). Thus, complementary
85 molecular proxies for quantifying the input of terrestrial organic matter to aquatic settings is still
86 needed.

87 The improvement of analytical methods allowed the separation and quantification of 5-, 6-
88 and 7-methyl brGDGTs (methyl groups at the fifth, sixth, and seventh positions; Fig. S1), that in
89 previous chromatographic protocols co-eluted (De Jonge et al., 2014, 2013; Ding et al., 2016).
90 Compounds eluting later than 7-methyl brGDGTs are tentatively designated 1050d and 1036d, as
91 their exact chemical structures are currently unknown (Wang et al., 2021). The fractional
92 abundance of the individual brGDGT isomers was shown to be influenced by distinct
93 environmental factors. For example, the relative abundance of 5-methyl brGDGTs was correlated
94 with temperature, whereas one of 6-methyl brGDGTs was correlated with pH (De Jonge et al.,
95 2014). In addition to temperature and pH, other environmental factors may influence brGDGT
96 distributions in terrestrial and aquatic settings and hence the application and interpretation of
97 brGDGT-derived proxies. For example, recent studies in lakes observed an influence of salinity on
98 the relative abundance of 6-methyl, 7-methyl brGDGTs and their late-eluting compounds (Wang
99 et al., 2021; Kou et al., 2022). This suggests that salinity could also control the distribution of these
100 compounds in other systems like river-sea continuums but this assumption has not yet been studied.

101 Compared with brGDGTs, the branched glycerol monoalkyl glycerol tetraethers
102 (brGMGTs) are a much less studied group of lipids. Recent studies have revealed their presence in
103 diverse environments, including peatlands (Naafs et al., 2018), marine settings (Liu et al., 2012),

104 rivers (Kirkels et al., 2022a) and lakes (Baxter et al., 2021, 2019). BrGMGTs are labelled as H1020,
105 H1034, and H1048 respectively (cf. Fig. S1), with isomers suggested by a suffix letter (a-c)
106 following the order in which they elute according to Baxter et al. (2019). These compounds are
107 structurally similar to brGDGTs, but possess an additional covalent carbon–carbon bond between
108 the alkyl chains, leading to “H-shaped” structure. The bridge of brGMGTs was considered to be a
109 primary adaptation to heat stress (Naafs et al., 2018; Baxter et al., 2019). Although a rigorous
110 chemical characterization of brGMGTs is lacking and the source organisms of brGMGTs are
111 unknown, correlations between the relative abundances of brGMGTs and MAAT were observed
112 in peat soils (Naafs et al., 2018) and lakes (Baxter et al., 2019), showing their potential as
113 temperature proxies. In addition to temperature, shifts in microbial community composition in
114 response to other unknown environmental factors seem to control the relative abundances of
115 brGMGTs in peats and lignites (Elling et al., 2023). Henceforth, in order to use the brGMGT as
116 environmental proxies in sedimentary records, it is still necessary to understand which factors
117 control their distributions in riverine and marine water columns and sediments, which remain to
118 date unclear (Bijl et al., 2021; Sluijs et al., 2020).

119 Based on previous studies of brGDGTs and brGMGTs in terrestrial and marine settings
120 (Dearing Crampton-Flood et al., 2019; Wang et al., 2021; Kirkels et al., 2022a, 2022b; Kou et al.,
121 2022), we hypothesize (1) that both brGDGTs and brGMGTs can be produced *in situ* in aquatic
122 systems and (2) that brGDGT and brGMGT distribution are influenced by surrounding
123 environmental factors and vary spatially along the land-sea continuum. These compounds have a
124 potential to be used as proxies of riverine organic matter inputs along estuaries. These hypotheses
125 were tested by examining and comparing the distribution of brGDGTs and brGMGTs in soils,
126 suspended particulate matter (SPM) and sediments ($n = 237$) collected all along the Seine River

127 basin (NW France), covering its riverine and estuarine parts. The aim of the present study was (1)
128 to investigate the sources of brGDGTs and brGMGTs along the Seine land-sea continuum, (2) to
129 determine the predominant environmental controls affecting the distribution of these molecules
130 and (3) to assess the potential of brGMGTs as a riverine runoff proxy.

131

132 **2. Material and methods**

133 *2.1. Study area*

134 The Seine River basin (Seine River and its estuary; Fig. 1a) is more than 760km long and
135 is characterized by high population density, draining through the greater Paris region (over 12
136 million inhabitants) to the English Channel (Flipo et al., 2021). The Seine Estuary is a macrotidal
137 estuary according to its high tidal range, small depth and morphology. The maximum flows are
138 generally observed in winter (over 700 m³/s; Fig. 1b), whereas the minimum flows are observed in
139 summer (below 250 m³/s; Fig. 1b). The tide influences the estuary up to the city of Poses (site 5,
140 KP 202 in Fig. 1a; KP represents kilometric point and is defined as the distance in kilometers from
141 the city of Paris), where a dam constitutes the boundary between the river and the estuary. The
142 estuary can be divided into two major parts: the upstream section mainly influenced by freshwater
143 (KP 202 to KP 298, from site 5 to site 12; Fig. 1a and Table 1) and the downstream section
144 predominantly influenced by saltwater intrusion (starting at KP 298, from site 12 to the coastal
145 area; Fig. 1a and Table 1).

146 *2.2. Sampling*

147 From June 2019 to March 2021, water samples ($n=102$) were collected across the Seine
148 River (Fig. 1a). Sub-surface water (ca. 1m depth) samples were collected in high-flow (over

149 250 m³/s) and low-flow (below 250 m³/s) seasons from the three zones (river, upstream estuary and
150 downstream estuary) of the Seine River basin (Table 1). At 5 sites (sites 4, 6, 10, 13, and 15, Fig.
151 1a and Table 1), both sub-surface and bottom water (2.2-16m depth) samples were retrieved using
152 a pump into precleaned 20L FLPE Nalgene carboys. Estuarine water samples (sites 6, 10, 13, and
153 15; Fig. 1a and Table 1) were collected at three tide periods (high tide, low tide and mid tide). For
154 these sites, 0.25-43L of water were immediately filtered using pre-combusted Whatman GF/F 0.7
155 µm glass fiber filters. After filtration, filters were freeze-dried, scratched and stored frozen at -20°C
156 prior to analysis.

157 Additional SPM samples ($n=16$; Table 1) used in this study for brGDGT and brGMGT
158 analysis were collected from the upstream and downstream estuary (site 5, 7, 13, 15, 17, 18, and
159 19; Fig. 1a and Table 1) in 2015 and 2016, as detailed by Thibault et al. (2019). Sediments ($n=68$)
160 from 8 cores (10cm depth) were collected at the same sites as these SPM samples in 2015 and 2016
161 using a UWITEC corer as described by Thibault et al. (2019) (Table 1). These sediments were
162 further sliced (1-cm thickness) and freeze-dried. Surficial soils ($n=9$) were collected in the lateral
163 area of the upstream section of the Seine river in 2021 (site A, B, and C, Fig. 1a and Table S1) and
164 freeze-dried. Additional wetland soils and mudflat sediments ($n=42$) were collected in the
165 downstream estuary in 2018, 2020, and 2021 (site D and E, Fig. 1a and Table S1), representing
166 allochthonous material transported into the estuary by tidal effect. These samples were collected at
167 low tide using a plexiglass® core (4.5 cm depth), and back to the laboratory, homogenized, freeze-
168 dried, and ground using a ball mill (model MM400, Retsch®)

169 *2.3. Elemental and isotopic analyses*

170 Elemental and isotopic analyses of the soils (surficial soils and mudflat sediments, $n=51$)
171 and SPM ($n=102$) collected from 2018 to 2021 were performed following the method described in

172 Thibault et al. (2019). Briefly, 40 mg of SPM and 1 g of soils/sediments samples were firstly
173 decarbonated by adding 10 mL of 3 M HCl for 2 h with magnetic stirring at room temperature.
174 Subsequently, these samples were rinsed using ultrapure water and centrifuged until reaching
175 neutral pH. The obtained decarbonated samples were stored at -20°C and freeze dried. Both
176 decarbonated and non-decarbonated samples (~ 6 mg for SPM and ~ 20 mg for soils) were enclosed
177 in a tin capsule. Total Organic Carbon content (TOC) and stable carbon isotopic composition ($\delta^{13}\text{C}$)
178 were measured in decarbonated samples using an elemental analyzer coupled with an isotope ratio
179 mass spectrometer (Thermo Fisher Scientific Delta V Advantage) at the ALYSES platform
180 (Sorbonne University / IRD, Bondy, France). Total Nitrogen (TN) and nitrogen isotope ($\delta^{15}\text{N}$) were
181 measured in non-decarbonated samples as acidification could impact the N contents (Ryba and
182 Burgess, 2002). The isotopic composition ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) was expressed as relative difference
183 between isotopic ratios in samples and in standards (Vienna Pee Dee Belemnite for carbon or
184 atmospheric N_2 for nitrogen). Additional elemental and isotopic analyses of SPM and sediments
185 collected in 2015 and 2016 ($n=84$) were carried out as described in Thibault et al. (2019).

186 2.4. *Lipid extraction and analyses*

187 The lipids from surficial soils and mudflat sediments (4-20g, $n=51$), and from SPM samples
188 (~ 150 mg, $n=102$) were extracted ultrasonically ($3\times$) with 20 to 40 mL of dichloromethane (DCM):
189 methanol (MeOH) (5/1, v/v) per extraction. Lipids from the SPM and sediments samples ($n=84$)
190 collected in 2015 and 2016 were previously extracted by Thibault (2018) following the same
191 method. The total lipid extracts were then separated into fractions of increasing polarity on an
192 activated silica gel column, using (i) 30 mL of heptane, (ii) 30 mL of heptane:DCM (1/4, v/v), and
193 (iii) 30 mL of DCM/MeOH (1/1, v/v) as eluents. An aliquot (30%) of the third (polar) fraction
194 containing GDGTs and GMGTs was dried, re-dissolved in heptane, and passed through a $0.2\mu\text{m}$

195 polytetrafluoroethylene (PTFE) filter (Ultrafree-MC; Merck). C₄₆ Glycerol Trialkyl Glycerol
196 Tetraether (GTGT) was used as an internal standard (Huguet et al., 2006). 5 µl of this standard
197 (0.01025 mg/mL) was typically added to 45 µl of sample.

198 GDGTs and GMGTs were analyzed using a Shimadzu LCMS 2020 high pressure liquid
199 chromatography coupled with mass spectrometry with an atmospheric pressure chemical ionization
200 source (HPLC-APCI-MS) in selected ion monitoring mode, modified from Hopmans et al. (2016)
201 and Huguet et al. (2019). Tetraether lipids were separated with two silica columns in tandem (BEH
202 HILIC columns, 2.1 × 150 mm, 1.7 µm; Waters) thermostated at 30°C. Injection volume was 30
203 µL. The flow rate was set at 0.2 mL/min. GDGTs and GMGTs were eluted isocratically for 25 min
204 with 82% A/18% B (A= hexane, B=hexane/isopropanol 9/1, v/v), followed by a linear gradient to
205 65% A/35% B in 25 min, then a linear gradient to 100% B in 30 min, and back to 82% A/18% B
206 in 4 min, maintained for 50 min. Semi-quantification of brGDGTs and brGMGTs was performed
207 by comparing the integrated signal of the respective compound with the signal of a C₄₆ synthesized
208 internal standard (Huguet et al., 2006) assuming their response factors to be identical.

209 2.5. Calculation of GDGT proxies

210 The IR_{6Me} index represents the proportion of 6-methyl brGDGTs vs. 5-methyl brGDGTs
211 and was calculated according to De Jonge et al. (2015; Eq. 1) with Roman numbers referring to the
212 structures in annex (Fig. S1):

$$213 \quad IR_{6Me} = \frac{II_{a_6} + II_{b_6} + II_{c_6} + III_{a_6} + III_{b_6} + III_{c_6}}{II_{a_5} + II_{b_5} + II_{c_5} + II_{a_6} + II_{b_6} + II_{c_6} + III_{a_5} + III_{b_5} + III_{c_5} + III_{a_6} + III_{b_6} + III_{c_6}} \quad (1)$$

214 The BIT index including the 6-methyl brGDGTs was calculated following De Jonge et al.
215 (2015; Eq. 2):

216
$$\text{BIT} = \frac{\text{I}_a + \text{II}_{a_5} + \text{II}_{a_6} + \text{III}_{a_5} + \text{III}_{a_6}}{\text{I}_a + \text{II}_{a_5} + \text{II}_{a_6} + \text{III}_{a_5} + \text{III}_{a_6} + \text{crenarchaeol}} \quad (2)$$

217 Based on duplicate injections, the average analytical error was 0.005 for IR_{6Me} and 0.06 for BIT.

218 *2.7. Water quality measurements*

219 Water turbidity was measured by a CTD Probe Sea-bird®. Water temperature, dissolved
 220 oxygen, salinity, and pH were measured using an automated YSI 6000 multi-parameter probe (YSI
 221 inc., Yellow springs, OH, USA). Chlorophyll *a* (Chl *a*) concentrations were measured on water
 222 samples after filtration on Whatman GF/F 0.7 μm glass fiber filters, which were stored frozen (-
 223 20° C) before analysis. Chl *a* was extracted from filters with incubation in 10 ml of 90% acetone
 224 for 12 hours in the dark at 4°C. After two centrifugations (1700 g, 5 min), Chl *a* concentrations
 225 were measured using a Turner Designs Fluorometer according to the method of Strickland and
 226 Parsons (1972) as described in the reference protocol of SNO SOMLIT (Service d'observation du
 227 Milieu Littoral).

228 *2.8. Statistical analyses*

229 All statistical analyses were performed using the R software (version 4.2.1). The non-
 230 parametric statistical tests were used due to the non-normal distribution of the dataset (tested by
 231 Shapiro–Wilk normality test; p-values < 0.05). Specifically, the Spearman's correlation was used
 232 to investigate potential correlations among different features (environmental parameters, fractional
 233 abundances of brGDGTs and brGMGTs, and proxies derived from these compounds), and the
 234 unpaired two-samples Wilcoxon test (also known as Mann-Whitney test or Wilcoxon rank sum
 235 test) was used for two independent group comparisons. Significance level is indicated by asterisks:
 236 *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001; ****p-value < 0.0001; NS (not significant),
 237 p-value > 0.05.

238 A Principal Component Analysis (PCA) was performed on the fractional abundances of
239 brGDGTs and brGMGTs, using the R packages factoextra and FactoMineR. The different groups
240 of samples were highlighted by adding 95% concentration ellipses. The proportion of variance in
241 brGDGT and brGMGT compositions that can be explained by different groups was evaluated by
242 permutational multivariate analysis of variance using distance matrices (adonis) in the adonis2
243 function of the R package Vegan, using the Bray-Curtis distances and 999 permutations.

244 A Redundancy analysis (RDA) was performed using the R package vegan to investigate
245 the relationship between environmental parameters and brGDGT or brGMGT distributions in SPM.
246 Angles between brGDGTs or brGMGTs and environmental factors were used to identify the
247 potential correlations. Right angles (90°) reflect a lack of linear correlations, whereas small or
248 straight angles (close to 0° or 180° , respectively) imply positive or negative linear correlations. The
249 compounds that are close to each other were assumed to be strongly linked, representing similar
250 distribution patterns and comparable responses to the environmental conditions. To evaluate the
251 relative importance of each explanatory variable (environmental parameters) on brGDGT or
252 brGMGT distributions, a hierarchical partitioning method implemented in the R package rdacca.hp
253 was used. This method calculated the individual importance (sum of the unique and total average
254 shared effects) from all subset models, generating an unordered assessment of variable importance
255 (Lai et al., 2022).

256 Spatial-temporal variations of environmental factors and proxies derived from brGDGTs
257 and brGMGTs were assessed after applying a locally estimated scatterplot smoothing (LOESS)
258 method. This method allows the identification of nonlinear data patterns and buffers the effect of
259 aberrant data and outliers. LOESS was implemented by the geom_smooth function of the R
260 package ggplot2.

261

262 **3. Results**

263 *3.1. Distribution of brGDGTs from land to sea*

264 The different brGDGTs were detected in all studied samples (Table S1). The brGDGT
265 chromatograms for downstream estuarine samples differed markedly from upstream samples (SPM
266 and sediments).

267 The brGDGT chromatograms from upstream samples (SPM and sediments) differed
268 markedly from downstream estuarine samples (SPM and sediments). For example, 6-methyl
269 brGDGTs were much more abundant than 5-methyl brGDGTs in the river (SPM) and upstream
270 estuary (SPM), whereas the strong predominance of 6-methyl vs. 5-methyl brGDGTs decreased in
271 the downstream samples (Fig. 2). Furthermore, the peaks of the recently described 7-methyl
272 brGDGTs and their late-eluting isomers (i.e. 1050d) were more pronounced in the downstream
273 estuary than in the rest of the Seine basin (Fig. 2).

274 The relative abundances of the brGDGTs were determined all along the Seine River basin
275 (Fig. 3 and Fig. S3). The 6-methyl brGDGTs (IIIa₆ and IIa₆) were significantly higher in river
276 (SPM) and upstream estuary (SPM and sediments) than in soils (surficial soils and mudflat
277 sediments) and downstream estuary (SPM and sediments). In addition, the relative abundances of
278 7-methyl brGDGTs (IIIa₇ and IIa₇) and their late-eluting compounds (1050d and 1036d) in
279 downstream estuary (SPM and sediments) were significantly higher than those in the upstream
280 estuary (SPM and sediments).

281 The concentration of total brGDGTs also showed differences along the land to sea
282 continuum (Fig. S2 and Table S1). The total brGDGTs concentration decreased from river (10.51

283 $\pm 5.91 \mu\text{g/g}$ organic carbon (C_{org} , based on SPM samples) to upstream estuary ($7.52 \pm 5.09 \mu\text{g/g}$
284 C_{org} , based on SPM and sediments) and downstream estuary ($4.95 \pm 4.09 \mu\text{g/g}$ C_{org} , based SPM
285 and sediments). In soils (surficial soils and mudflat sediments) from all the Seine basin, the
286 concentration in total brGDGTs was significantly lower than that in SPM and sediments (Fig. S2
287 and Table S1).

288 A Principal Component Analysis (PCA) was performed to statistically compare the
289 fractional abundances of brGDGTs from different location (river, upstream and downstream
290 estuary, based on SPM and sediments), which explained 40.9% of the variance in two dimensions,
291 with negative loadings for most of the 6-methyl brGDGTs and positive loadings for the remaining
292 brGDGTs (Fig. 4a). Samples from the downstream estuary clustered well apart from those from
293 the river and upstream parts. Specifically, the brGDGT distribution was dominated by 6-methyl
294 brGDGTs (IIIa₆, IIIb₆, IIIc₆, IIa₆, and IIb₆) in river and upstream estuarine samples, whereas in
295 downstream estuary, it was driven by 5-methyl brGDGTs (III₅, IIa₅, IIc₅, IIb₅, IIIb₅),
296 tetramethylated brGDGTs (Ia, Ib, and Ic), 7-methyl brGDGTs (IIIa₇, IIa₇, and IIb₇), and their late-
297 eluting compounds (1050d and 1036d).

298 A Redundancy analysis (RDA) was performed to investigate the influence of the
299 environmental factors (TOC, TN, temperature, and salinity) on the brGDGT distributions in SPM
300 samples (Fig. 5 and Table S2), which allowed to explain 38.9% of the variability through two
301 dimensions. The RDA triplot (Fig. 5a) showed how these factors correlate to the distributions of
302 individual brGDGTs. The first axis of the RDA explained 33.01% of the variability and was
303 primarily correlated with salinity and TN, whereas the second axis explained 5.89% of the
304 variability and was associated with temperature and TOC (Fig. 5a and Table S2). The first axis of
305 the RDA explained 33.01% of the variability and was primarily correlated with salinity (15.2%)

306 and TN (13.8%), whereas the second axis explained 5.89% of the variability and was associated
307 with temperature and TOC (< 6% of the variance; Fig. 5 and Table S2).

308 *3.2 Distribution of brGMGTs from land to sea*

309 The seven brGMGTs identified in previous studies were all detected in the samples
310 collected across the Seine River basin (Fig. 2; Table S1). The chromatograms revealed distinct
311 distributions in brGMGTs in the different parts of the basin (SPM and sediments), with e.g. a higher
312 intensity for the homologue H1020c in the river samples (SPM) than in those from the upstream
313 (SPM) and downstream estuary (SPM) (Fig. 2). These spatial variations were apparent when
314 calculating the fractional abundances of the individual brGMGTs (Fig. 6). From upstream to
315 downstream, the relative abundances in H1020a and H1020b increased, whereas those in 1020c,
316 H1034b, and H1034c decreased (Fig. 6). In SPM and sediments, the total brGMGT concentration
317 was observed to be slightly higher in the riverine part ($0.26 \pm 0.17 \mu\text{g/g C}_{\text{org}}$) than in downstream
318 ($0.20 \pm 0.14 \mu\text{g/g C}_{\text{org}}$) and upstream estuary samples ($0.18 \pm 0.15 \mu\text{g/g C}_{\text{org}}$; Fig. S2 and Table S1).
319 The total brGMGT concentrations were the lowest in soils all over the basin ($0.07 \pm 0.23 \mu\text{g/g C}_{\text{org}}$).

320 The PCA analysis based on the brGMGT relative abundances (Fig. 4b) explained 70.2% of
321 the variance, which allows to observe that samples from the different parts of the basin clustered
322 well apart from each other. The first axis explained 55.1% of the variance, separating downstream
323 samples from riverine and upstream samples, with negative loadings for two brGMGTs (H1020a
324 and H1020b), and positive loadings for the remaining brGMGTs (H1020c, H1034a, H1034b,
325 H1034c, and H1048). The second axis explained 15.1% of the variance and mainly separated the
326 riverine and upstream samples (Fig. 4b), with higher relative abundances of compounds H1020c
327 and H1034b in riverine samples.

328 The RDA was performed to investigate the factors that could explain the variability of
329 brGMGT distributions in SPM samples (Fig. 5 and Table S2), which allows to explain 25.39% of
330 the variance. The RDA triplot showed that the first axis, accounting for 21.59% of the variability,
331 was mainly associated with salinity and to a lesser extent TN, while the second axis (3.8%) was
332 mainly driven by temperature and TOC (Fig. 5a and Table S2). Based on hierarchical partitioning,
333 salinity had the highest variable importance and contributed to 13.22% of the brGMGT variations
334 (Fig. 5d and Table S2). The brGMGTs were slightly influenced by TN and temperature, as these
335 two factors contributed to 3.88% and 3.55% of brGMGT variations, respectively (Fig. 5d and Table
336 S2).

337

338 **4. Discussion**

339 *4.1. Sources of brGDGTs and environmental controls on their distribution*

340 *4.1.1 Sources of brGDGTs*

341 In order to determine the predominant origin of brGDGTs in the Seine River basin, the overall
342 brGDGT concentrations and distributions in SPM and sediments ($n=186$) were compared with
343 those in soils (surficial soils and mudflat sediments, $n=51$). The brGDGT concentrations
344 (normalized to C_{org}) and relative abundances of several brGDGTs (i.e. IIa₆ and IIIa₆) in the SPM
345 and sediments are significantly higher than those in soils ($p<0.05$, Wilcoxon test; Fig. S2a and Fig.
346 3). Such differences in brGDGT concentrations and relative abundances between soils and aquatic
347 settings (SPM and sediments) imply that at least part of the brGDGTs in the water column and
348 sediments of the Seine River basin is produced *in situ*. This is in agreement with previous findings

349 which suggested an *in situ* aquatic contribution to the brGDGT pool (Crampton-Flood et al., 2021;
350 De Jonge et al., 2015; Kirkels et al., 2022b; Peterse et al., 2009).

351 More specifically, the fractional abundances of the two major 6-methyl brGDGTs (IIa₆ and
352 IIIa₆) are significantly higher in the Seine River and upstream estuary than in soils (Fig. 3). This
353 confirms that these brGDGTs are mostly produced within the river, adding to the growing body of
354 evidence supporting riverine 6-methyl brGDGT production (De Jonge et al., 2015; Bertassoli et al.,
355 2022; Kirkels et al., 2022b). A subsequent shift in the brGDGT distributions in the downstream
356 compared to the upstream areas is observed in the Seine River basin. The PCA analysis shows a
357 separation of downstream estuarine samples (influenced by seawater intrusion) from riverine and
358 upstream estuary ones (without significant seawater intrusion) (Fig. 4a). This difference is
359 predominantly driven by the higher abundances of 6-methyl brGDGTs in riverine and upstream
360 estuarine samples *vs.* higher abundances of 5- and 7-methyl brGDGTs as well as compounds Ib,
361 Ic, and late eluting brGDGTs 1050d, 1036d in downstream estuarine samples (Figs. 3, 5a and A3).
362 This difference suggests that riverine 6-methyl brGDGTs may be more easily degraded than other
363 homologues and only partially transferred downstream. In addition to that, the riverine brGDGT
364 signal may be diluted by brGDGTs from other sources during downstream transport. This is in
365 agreement with a previous study, which showed a shift in brGDGT distribution from the Yenisei
366 River to the Kara Sea (De Jonge et al., 2015). They interpreted this to be a preferential degradation
367 of labile (riverine) 6-methyl brGDGTs and the enrichment in less labile (soil-derived) 5-methyl
368 brGDGTs during transport (De Jonge et al., 2015). This suggests that only limited amounts of
369 riverine 6-methyl brGDGTs are transferred to the ocean, as was also shown in other recent studies
370 (Cao et al., 2022; Kirkels et al., 2022b). In addition, a shift in brGDGT distribution during
371 downstream transport could be explained by mixing with autochthonous (i.e. estuarine-produced)

372 brGDGTs (Crampton-Flood et al., 2021). The relative abundance of several brGDGTs (i.e. Ib, Ic,
373 IIIa₇, IIa₇ and 1050d) in the downstream part of the Seine River basin is indeed significantly higher
374 than the one in the upstream part ($p < 0.05$, Wilcoxon test; Fig. 3), suggesting *in situ* brGDGT
375 production in saltwater. Such a saltwater contribution can be visualized by the PCA based on
376 brGDGT distribution, showing the positive score of the aforementioned compounds with the first
377 axis (Fig. 4a). This axis is dominated by downstream samples influenced by seawater intrusion in
378 the Seine Estuary (Fig. 4a). However, it should be noted that the relative abundance of compounds
379 Ib, Ic, IIIa₇, IIa₇ and 1050d is roughly similar in soils and in downstream estuary samples (Fig. 3).
380 Hence, it cannot be excluded that these brGDGTs detected in downstream samples are at least
381 partly derived from soils of the watershed.

382 4.1.2. Environmental controls on the brGDGT distribution

383 As several individual brGDGTs are suggested to be preferentially produced either in the
384 riverine or estuarine parts of the Seine basin, their distribution might be related to ambient
385 environmental factors. The RDA (performed on SPM samples) highlights the relationships
386 between the available environmental variables (salinity, TN, TOC, and temperature) and the
387 relative abundances of brGDGTs. Hierarchical partitioning indicates that salinity is the most
388 important factor influencing the brGDGT distribution (15.2%) in the Seine River basin (Fig. 5b
389 and Table S2). Salinity is related to the relative abundances of compounds Ib, Ic, 7-methyl
390 brGDGTs and the late-eluting homologs 1050d and 1036d that scored negatively on the first axis
391 of the RDA (Fig. 5a). This is in line with the positive significant correlations between salinity and
392 the relative abundances of these compounds (Fig. S4). This trends also support the assumption
393 made about the aquatic production of ring-containing tetramethylated brGDGTs (Ib and Ic) in
394 Svalbard fjords which was thought to be linked to a salinity change (Dearing Crampton-Flood et

395 al., 2019). The 7-methyl brGDGTs and their late-eluting isomers, were also shown to be much
396 more abundant in hypersaline lakes than those of lower salinity (Wang et al., 2021). Such a salinity-
397 dependent brGDGT composition has previously been interpreted by membrane adaptation to
398 salinity changes or by a shift in bacterial community composition (Dearing Crampton-Flood et al.,
399 2019; Wang et al., 2021). Hence, the significant positive correlations between salinity and these
400 compounds in the Seine River basin suggest that brGDGT-producing bacteria have similar
401 physiological mechanisms (i.e., membrane adaptation) to those reported in other aquatic settings
402 (lakes and fjords) and/or that the diversity of these bacteria changing along the river-sea continuum.

403 The relative abundances of several 6-methyl brGDGTs (i.e. IIa₆, IIIa₆, and IIb₆) in the Seine
404 River basin reveal significant negative correlations with salinity ($p < 0.05$, Wilcoxon test; Fig. S4),
405 which is in contrast with the positive relationships previously found in lakes (Wang et al., 2021).
406 The distinct behavior of 6-methyl brGDGTs between lakes and the Seine river-sea continuum
407 might be due to the lower salinity range in the Seine River basin (0-32 psu) vs. the lakes (0-376
408 psu) investigated by Wang et al. (2021). This suggests that the limited range of salinity variation
409 in the Seine River basin might be insufficient to trigger significant 6-methyl brGDGT production
410 as observed in hypersaline lakes.

411 Alternatively, the significant negative correlations between the salinity and the relative
412 abundance of 6-methyl brGDGTs in the Seine basin suggest that the bacteria producing 6-methyl
413 brGDGTs are preferentially present in the low salinity area of the estuary. To explore this
414 hypothesis, we investigate the spatio-temporal variations of the 6-methyl vs. 5-methyl brGDGTs
415 ratio: IR_{6Me} (Fig. 7). High IR_{6Me} values (0.69 ± 0.10) are associated with enhanced *in situ*
416 production of 6-methyl brGDGTs within the Yenisei river (De Jonge et al., 2015). In the Seine
417 River basin, seasonal variation in IR_{6me} is observed, especially in the upstream part with a low

418 salinity range (0-0.32 psu). Specifically, much higher IR_{6Me} values are observed in the freshwater
419 zone of the estuary (KP 243-297.6; site 7 to site 12) with a low salinity range (0-0.32 psu) during
420 low-flow season (Fig. 7), suggesting that 6-methyl brGDGTs are preferentially produced in this
421 zone when water discharge is low. Similarly, preferential production of 6-methyl brGDGT at low
422 discharges was previously observed in other river systems, including the Amazon River basin
423 (Kirkels et al., 2020; Crampton-Flood et al., 2021; Bertassoli et al., 2022) as well as Black and
424 White Rivers (Dai et al., 2019). It was suggested that the enhanced 6-methyl brGDGT production
425 at low flows was due to slow flow velocity and reduced soil mobilization. Although these
426 hypotheses could account for the temporal variation in IR_{6Me} in the Seine River basin, they are
427 unlikely to explain the substantially high IR_{6Me} values in this specific zone. Other environmental
428 variables such as dissolved oxygen contents (Wu et al., 2021) and pH (De Jonge et al., 2014, 2015)
429 were previously suggested to have a potential influence on 6-methyl brGDGT distributions.
430 Nevertheless, these two environmental factors do not co-vary with IR_{6Me} in the present study and
431 can be ruled out as causes of variation in 6-methyl brGDGT distribution along the Seine river-sea
432 continuum (Fig. 7). Hence, the production of 6-methyl brGDGTs in the upstream zone of the Seine
433 Estuary has to be triggered by other factors, such as the nutrient concentration.

434 High nutrient levels were shown to favor the production of 6-methyl versus 5-methyl
435 brGDGTs in the water column of mesocosm experiments (Martínez-Sosa and Tierney, 2019). As
436 the nutrient concentration is higher in the upstream part of the Seine estuary (Wei et al., 2022), the
437 substantial 6-methyl brGDGT production observed in the aforementioned zone (KP 243-297.6, Fig.
438 7) at low flows could be due to the high amount of nutrients, especially nitrogen. This is supported
439 by the RDA triplot showing strong correlation of TN with the brGDGT distribution in the Seine
440 basin (Fig. 5b), with the major 6-methyl brGDGTs (i.e. IIa₆ and IIIa₆) plotting close to TN in the

441 RDA triplot (Fig. 5a). In addition, TN and $\delta^{15}\text{N}$ are observed to co-vary with $\text{IR}_{6\text{Me}}$ and to peak in
442 the same zone (KP 243-297.6; Fig. 7) during the low-flow season. Nitrate from sewage effluents
443 and manure are generally enriched in ^{15}N compared to other sources, leading to much elevated $\delta^{15}\text{N}$
444 values (10–25‰) (Andrisoa et al., 2019; Leavitt et al., 2006). Nutrients, in the form of nitrogen,
445 can be concentrated at low discharges, thus triggering phytoplankton blooms (Romero et al., 2019).
446 Hence, the elevated TN and $\delta^{15}\text{N}$ signals in SPM of the upstream estuary could be attributed to the
447 increase of nitrogen loadings and ^{15}N -enriched nitrate uptake by phytoplankton developing
448 intensively during the low-flow season. The much higher chlorophyll *a* concentrations in the
449 upstream estuary under low discharge conditions support the hypothesis of phytoplankton blooms
450 (Fig. 7). This high phytoplankton biomass might consequently create an environment that
451 accelerates the growth and production of heterotrophic bacteria, which can in turn transform
452 phytoplankton-derived organic matter (Buchan et al., 2014). As the brGDGT-producers were
453 suggested to have a heterotrophic lifestyle (Weijers et al., 2010; Huguet et al., 2017; Blewett et al.,
454 2022), they may transform phytoplankton-derived organic matter and thus participate in N-cycling
455 during blooms. Hence, the co-variations of all the parameters ($\text{IR}_{6\text{Me}}$, TN, $\delta^{15}\text{N}$, and Chl *a*
456 concentration) peaking in the upstream area during low-flow season suggest that low salinity range
457 and high phytoplankton productivity represent favorable conditions for 6-methyl brGDGT
458 production.

459 4.2. Sources of brGMGTs and environmental controls on their distribution

460 4.2.1 Sources of brGMGTs

461 Similarly to the brGDGTs, the brGMGTs can also be produced *in situ* within the aquatic
462 settings (Baxter et al., 2021; Kirkels et al., 2022a). In previous studies, brGMGTs were detected
463 only in part of the soils surrounding the Godavari River basin (India; Kirkels et al., 2022a) and

464 Lake Chala (East Africa; Baxter et al., 2021), suggesting a limited brGMGT production in soils in
465 comparison to aquatic settings. Consistently, in the Seine River basin, concentrations of brGMGTs
466 in SPM and sediment samples are significantly higher than those in soils ($p < 0.05$, Wilcoxon test;
467 Fig. S2b), pointing out their predominant aquatic source.

468 A notable compositional shift in brGMGT distribution is observed along the Seine River
469 basin, as revealed by the separation of riverine, upstream and downstream estuarine samples in the
470 PCA (Fig. 4b). The relative abundance of 3 brGMGTs (H1020c, H1034b, and H1034c) gradually
471 decreases across the basin (Fig. 6) and is significantly correlated with those of 6-methyl brGDGTs
472 (Fig. S5a). As 6-methyl brGDGTs are mainly produced in freshwaters in the Seine basin, this
473 suggests that brGMGTs H1020c, H1034b and H1034c and 6-methyl brGDGTs have a common
474 freshwater origin and that the mixture of fresh and marine waters along the estuary leads to the
475 dilution of these compounds during downstream transport. H1020c is the dominant brGMGT
476 homologue in SPM from the riverine zone of the Seine and one of the most abundant brGMGT in
477 the upstream part of the estuary (Fig. 6). Such a trend was also observed in SPM and riverbed
478 sediments from the upper part of the Godavari River basin, which was attributed to *in situ* riverine
479 brGMGT production of this compound (Kirkels et al., 2022a).

480 The fractional abundance of H1020a and H1020b homologues gradually increases along
481 the Seine River basin. This is consistent with the higher abundances of H1020a and H1020b
482 previously reported in marine sediments from the Bay of Bengal (Kirkels et al., 2022a). The
483 predominance of these compounds in such samples was attributed to their *in situ* production in the
484 marine realm. In line with this hypothesis, the relative abundances of brGMGTs H1020a and
485 H1020b significantly correlate with brGDGTs Ib, Ic, IIIa₇, IIa₇ and 1050d (Fig. S5a) in the Seine
486 Estuary, suggesting a similar marine origin.

487 4.2.2. Environmental controls on the distribution of brGMGTs

488 The current knowledge on the parameters controlling the brGMGT distributions in the
489 terrestrial and marine realm is still limited. The correlations between the brGDGT and brGMGT
490 relative abundances in the Seine River basin (Fig. S5a) suggest that both types of compounds might
491 be derived from overlapping source microorganisms, with common environmental factors
492 controlling their membrane lipid composition. In the Seine River basin, salinity is shown to be the
493 main environmental parameter influencing the brGMGT distribution, as also observed for
494 brGDGTs (Fig. 5). This is reflected in the significant ($p < 0.05$) increase in the relative abundances
495 of homologues H1020a and H1020b with salinity and a concomitant significant negative
496 correlation between this parameter and the relative abundances of homologues H1020c, H1034b,
497 and H1034c ($p < 0.05$, Wilcoxon test; Fig. 8a). Nevertheless, the individual effect of TN on
498 brGMGT relative abundances is observed to be much lower compared to that observed for
499 brGDGTs (Fig. 5 and Table S2). This implies that, while having common controlling factors such
500 as the salinity, they are also influenced by distinct parameters (i.e. TN), likely indicating distinct
501 sources. This is consistent with a recent study showing that brGDGTs and brGMGTs likely
502 originate from overlapping, but not identical origins (Elling et al., 2023).

503 The shift in brGMGT distribution observed across the Seine River basin (Figs. 5b and 8a)
504 could be due to a change in the diversity of brGMGT-producing bacteria and/or to an adaptation
505 of these microorganisms to environmental changes occurring from upstream to downstream. The
506 latter hypothesis seems unlikely, as a physiological adaptation of a given bacterial community
507 would make it difficult to explain why the relative abundance of three isomers of compound H1020,
508 which share a similar structure, varies differently in response to salinity changes. Hence, a shift in
509 brGMGT-producing bacterial communities across the basin is more likely. Compounds H1020c,

510 H1034b, and H1034c could predominantly be produced by bacteria preferentially growing in
511 freshwater, and homologues H1020a and H1020b by bacteria preferentially living in brackish or
512 saltwater.

513 *4.3. Potential implications for brGMGTs as a proxy for riverine runoff*

514 The distinct brGMGT distributions in freshwater and saltwater could be used to trace the
515 Organic Matter (OM) produced upstream all along the Seine basin. To trace such a riverine runoff
516 signal, we propose a new proxy, the Riverine IndeX (RIX), based on the fractional abundances of
517 brGMGTs H1020c, H1034c, and H1034b versus H1020a and H1020b (Eq. 3):

$$518 \quad \text{RIX} = \frac{H1020c+H1034c+H1034b}{H1020c+H1034c+H1034b+H1020a+H1020b} \quad (3)$$

519 The RIX is calculated for the SPM and sediment samples from the Seine River basin,
520 showing an obvious decreasing trend from upstream to downstream (Fig. 8b). The RIX in river
521 (0.54±0.13, SPM) and upstream estuarine (0.44±0.12, SPM and sediments) samples is significantly
522 higher than for downstream estuarine (0.27±0.12, SPM and sediments) samples. RIX values around
523 0.50 could therefore be considered reflecting the riverine endmember, while those below 0.30 could
524 represent the saltwater endmember.

525 As it cannot be completely ruled out that part of the brGMGT signal in the water masses of
526 the Seine may be partially derived from surrounding soils, this index is also calculated for the soil
527 samples. The RIX values of the soil samples are 0.26±0.17, close to those of the downstream
528 estuarine samples. However, the average concentrations of brGMGTs are an order of magnitude
529 lower in the soils than in the sediments and SPM samples of the Seine basin. Therefore, it can be
530 assumed that the impact of soil-derived brGMGTs on the observed RIX signal in the water column
531 of the Seine basin is low.

532 In order to test the general applicability of the RIX, it was then applied to riverine and
533 marine samples (SPM and sediments) collected in the Godavari River basin and Bay of Bengal
534 (Kirkels et al., 2022a). This site represents the only other river-sea continuum besides the Seine
535 basin for which brGMGT data are presently available. Significant differences in RIX between the
536 SPM and sediment samples from the Godavari River basin are observed ($p < 0.05$, Wilcoxon test;
537 Fig. 8b). In addition, 96% of the RIX values in riverine SPM and riverbed sediments from the
538 Godavari basin exceed 0.5, whereas all of the RIX values observed in marine sediments from the
539 Bay of Bengal are below 0.3. This suggests that the RIX cutoff values defined using the samples
540 from the Seine basin may be broadly applicable and valid across other river-sea continuums. This
541 deserves further studies.

542 Further confirmation of the RIX potential as a tracer of riverine OM comes from the
543 significant correlations observed between this index and other commonly used proxies for tracing
544 OM sources, i.e. the BIT and $\delta^{13}\text{C}_{\text{org}}$ ($p < 0.05$, Wilcoxon test; Fig. S5b). These proxies show roughly
545 similar spatial and temporal variations in the Seine River basin. In the low-flow season, RIX and
546 BIT gradually decrease while $\delta^{13}\text{C}_{\text{org}}$ increase across the basin (Fig. 9). Such trends during the low
547 discharge periods likely reflects the continuous dilution process of riverine OM caused by the
548 mixing of fresh and marine water masses (Thibault et al., 2019). The gradual dilution of the riverine
549 OM signal along the Seine River basin could be due to the increase of seawater intrusion, and thus
550 marine-derived OM, at low discharges (Kolb et al., 2022; Ralston and Geyer, 2019). In contrast,
551 during the high-flow season, no such gradual dilution trend is observed. Instead, at high discharges,
552 the RIX, BIT and $\delta^{13}\text{C}_{\text{org}}$ remain roughly stable from KP 202 to 310.5, before, steeply decreasing
553 for BIT and RIX, and increasing for $\delta^{13}\text{C}_{\text{org}}$. This trend can be explained by the fact that at high
554 flow rates, the limit of saltwater intrusion in the estuary shifts seawards rather than landwards,

555 allowing the riverine OM to be flushed further downstream than under low discharge conditions.
556 After KP 310.5, the riverine OM is diluted because of the mixing with marine water masses, as
557 observed during the low-flow season. The trends observed in the Seine Estuary are consistent with
558 previous studies in other regions, showing that terrestrial OM was only effectively transported
559 downstream at high flow rates (Kirkels et al., 2022b, 2020).

560 Although the BIT is successfully used in the Seine River basin as well as in previous studies
561 to trace riverine (terrestrial) OM inputs (Hopmans et al., 2004; Xu et al., 2020), this index can be
562 biased by *in situ* production of brGDGTs in aquatic settings (Dearing Crampton-Flood et al., 2019;
563 Sinninghe Damsté, 2016) and selective degradation of crenarchaeol vs. brGDGTs (Smith et al.,
564 2012). Hence, high BIT values do not necessarily indicate higher contribution of terrestrial OM in
565 some settings (Smith et al., 2012). Unlike the BIT index, based on two different families of
566 compounds (isoGDGTs and brGDGTs), the RIX is based on 5 compounds from the same family
567 (brGMGTs) that likely have similar degradation rates and therefore not influenced by selective
568 degradation. Furthermore, the RIX is based on the relative abundances of brGMGTs which are all
569 predominantly produced in aquatic settings, three of them (H1020c, H1034b, and H1034c) being
570 mainly produced in freshwater and two of them (H1020a and H1020b) mainly in saltwater.
571 Therefore, the RIX is based on compounds which are more specifically produced in the two
572 endmembers (freshwater or saltwater), which could avoid the biases encountered with the BIT.
573 Overall, our work shows that, in addition to the BIT and $\delta^{13}\text{C}_{\text{org}}$, the RIX successfully captures the
574 spatio-temporal dynamics of riverine OM in the Seine River basin, making this proxy a promising
575 and complementary one tracing riverine runoff in modern samples as well as paleorecords.

576

577

578 **5. Conclusions**

579 In this study, the brGDGT and brGMGT concentrations and distributions in soils, SPM,
580 and sediments ($n=237$) across the Seine River basin were investigated. Higher concentrations and
581 distinct distributions of brGDGTs and brGMGTs in SPM and sediments compared with soils imply
582 that both types of compounds can be produced *in situ* in aquatic settings. The distribution of both
583 brGDGTs and brGMGTs are largely related to salinity, but only brGDGT distributions are
584 significantly influenced by nitrogen nutrient loadings. In addition, covariations of IR_{6Me}, TN, $\delta^{15}\text{N}$,
585 and Chl *a* concentration within the low salinity region suggest that riverine (6-methyl) brGDGT
586 production is favored by low-salinity and high-productivity conditions.

587 In the Seine River basin, salinity correlate positively with H1020a and H1020b, and
588 negatively with H1020c, H1034b, and H1034c. This indicates that compounds H1020c, H1034b,
589 and H1034c could be produced by bacteria that preferentially grow in freshwater, while
590 homologues H1020a and H1020b could be produced by bacteria that mainly live in saltwater.
591 Based on this, a novel proxy, the Riverine IndeX (RIX) is proposed to trace riverine OM input. The
592 average value of RIX for the riverine samples is 0.54, which is much higher than that in soils (0.26
593 on average) and downstream estuarine (0.27 on average) samples. We thus recommend that RIX
594 values over 0.5 imply considerable riverine contributions, whereas RIX values below 0.3 indicate
595 higher marine contributions. This cutoff value defined in the Seine River basin also works in the
596 Godavari River basin (India), which implies that this novel proxy based on brGMGTs may be
597 broadly applicable and warrants further exploration.

598

599

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610

611 **Appendix A. Supplementary material**

612 The brGDGT and brGMGT data are made available in the Supplementary material and will be
613 archived in PANGAEA by the time of publication.

614

615 **References**

- 616 Andrisoa, A., Stieglitz, T.C., Rodellas, V., Raimbault, P., 2019. Primary production in coastal
617 lagoons supported by groundwater discharge and porewater fluxes inferred from nitrogen
618 and carbon isotope signatures. *Mar. Chem.* 210, 48–60.
- 619 Baxter, A.J., Hopmans, E.C., Russell, J.M., Sinninghe Damsté, J.S., 2019. Bacterial GMGTs in
620 East African lake sediments: Their potential as palaeotemperature indicators. *Geochim.*
621 *Cosmochim. Acta* 259, 155–169.
- 622 Baxter, A.J., Peterse, F., Verschuren, D., Sinninghe Damsté, J.S., 2021. Anoxic in situ production
623 of bacterial GMGTs in the water column and surficial bottom sediments of a meromictic
624 tropical crater lake: Implications for lake paleothermometry. *Geochim. Cosmochim. Acta*
625 306, 171–188.

- 626 Bertassoli, D.J., Häggi, C., Chiessi, C.M., Schefuß, E., Hefter, J., Akabane, T.K., Sawakuchi, A.O.,
627 2022. Controls on the distributions of GDGTs and n-alkane isotopic compositions in
628 sediments of the Amazon River Basin. *Chem. Geol.* 594, 120777.
- 629 Bijl, P.K., Frieling, J., Cramwinckel, M.J., Boschman, C., Sluijs, A., Peterse, F., 2021.
630 Maastrichtian–Rupelian paleoclimates in the southwest Pacific – a critical re-evaluation of
631 biomarker paleothermometry and dinoflagellate cyst paleoecology at Ocean Drilling
632 Program Site 1172. *Clim. Past* 17, 2393–2425.
- 633 Blewett, J., Elling, F.J., Naafs, B.D.A., Kattein, L., Evans, T.W., Lauretano, V., Gallego-Sala, A.V.,
634 Pancost, R.D., Pearson, A., 2022. Metabolic and ecological controls on the stable carbon
635 isotopic composition of archaeal (isoGDGT and BDGT) and bacterial (brGDGT) lipids in
636 wetlands and lignites. *Geochim. Cosmochim. Acta* 320, 1–25.
- 637 Buchan, A., LeClerc, G.R., Gulvik, C.A., González, J.M., 2014. Master recyclers: features and
638 functions of bacteria associated with phytoplankton blooms. *Nat. Rev. Microbiol.* 12, 686–
639 698.
- 640 Cao, J., Lian, E., Yang, S., Ge, H., Jin, X., He, J., Jia, G., 2022. The distribution of intact polar
641 lipid-derived branched tetraethers along a freshwater-seawater pH gradient in coastal East
642 China Sea. *Chem. Geol.* 596, 120808.
- 643 Chen, Y., Zheng, F., Yang, H., Yang, W., Wu, R., Liu, X., Liang, H., Chen, H., Pei, H., Zhang, C.,
644 Pancost, R.D., Zeng, Z., 2022. The production of diverse brGDGTs by an *Acidobacterium*
645 providing a physiological basis for paleoclimate proxies. *Geochim. Cosmochim. Acta*.
- 646 Coffinet, S., Huguet, A., Bergonzini, L., Pedentchouk, N., Williamson, D., Anquetil, C., Gałka, M.,
647 Kołaczek, P., Karpińska-Kołaczek, M., Majule, A., Laggoun-Défarge, F., Wagner, T.,
648 Derenne, S., 2018. Impact of climate change on the ecology of the Kyambangunguru crater
649 marsh in southwestern Tanzania during the Late Holocene. *Quat. Sci. Rev.* 196, 100–117.
- 650 Crampton-Flood, E.D., van der Weijst, C.M., van der Molen, G., Bouquet, M., Yedema, Y.,
651 Donders, T.H., Sangiorgi, F., Sluijs, A., Damsté, J.S.S., Peterse, F., 2021. Identifying
652 marine and freshwater overprints on soil-derived branched GDGT temperature signals in
653 Pliocene Mississippi and Amazon River fan sediments. *Org. Geochem.* 154, 104200.
- 654 Cramwinckel, M.J., Huber, M., Kocken, I.J., Agnini, C., Bijl, P.K., Bohaty, S.M., Frieling, J.,
655 Goldner, A., Hilgen, F.J., Kip, E.L., Peterse, F., van der Ploeg, R., Röhl, U., Schouten, S.,
656 Sluijs, A., 2018. Synchronous tropical and polar temperature evolution in the Eocene.
657 *Nature* 559, 382–386.
- 658 Dai, G., Zhu, E., Liu, Z., Wang, Y., Zhu, S., Wang, S., Ma, T., Jia, J., Wang, X., Hou, S., Fu, P.,
659 Peterse, F., Feng, X., 2019. Compositional Characteristics of Fluvial Particulate Organic
660 Matter Exported From the World’s Largest Alpine Wetland. *J. Geophys. Res.*
661 *Biogeosciences* 124, 2709–2727.
- 662 De Jonge, C., Hopmans, E.C., Stadnitskaia, A., Rijpstra, W.I.C., Hofland, R., Tegelaar, E.,
663 Sinninghe Damsté, J.S., 2013. Identification of novel penta- and hexamethylated branched
664 glycerol dialkyl glycerol tetraethers in peat using HPLC–MS2, GC–MS and GC–SMB-MS.
665 *Org. Geochem.* 54, 78–82.
- 666 De Jonge, C., Hopmans, E.C., Zell, C.I., Kim, J.-H., Schouten, S., Sinninghe Damsté, J.S., 2014.
667 Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in
668 soils: Implications for palaeoclimate reconstruction. *Geochim. Cosmochim. Acta* 141, 97–
669 112.
- 670 De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A., Streletskaia, I.D.,
671 Vasiliev, A.A., Sinninghe Damsté, J.S., 2015. Drastic changes in the distribution of
672 branched tetraether lipids in suspended matter and sediments from the Yenisei River and

673 Kara Sea (Siberia): Implications for the use of brGDGT-based proxies in coastal marine
674 sediments. *Geochim. Cosmochim. Acta* 165, 200–225.

675 Dearing Crampton-Flood, E., Peterse, F., Sinninghe Damsté, J.S., 2019. Production of branched
676 tetraethers in the marine realm: Svalbard fjord sediments revisited. *Org. Geochem.* 138,
677 103907.

678 Ding, S., Schwab, V.F., Ueberschaar, N., Roth, V.-N., Lange, M., Xu, Y., Gleixner, G., Pohnert,
679 G., 2016. Identification of novel 7-methyl and cyclopentanyl branched glycerol dialkyl
680 glycerol tetraethers in lake sediments. *Org. Geochem.* 102, 52–58.

681 Elling, F.J., Kattein, L., David A. Naafs, B., Lauretano, V., Pearson, A., 2023. Heterotrophic origin
682 and diverse sources of branched glycerol monoalkyl glycerol tetraethers (brGMGTs) in
683 peats and lignites. *Org. Geochem.* 104558.

684 Flipo, N., Lestel, L., Labadie, P., Meybeck, M., Garnier, J., 2021. Trajectories of the Seine River
685 Basin, in: Flipo, N., Labadie, P., Lestel, L. (Eds.), *The Seine River Basin, The Handbook
686 of Environmental Chemistry*. Springer International Publishing, Cham, pp. 1–28.

687 Halamka, T.A., Raberg, J.H., McFarlin, J.M., Younkin, A.D., Mulligan, C., Liu, X.-L., Kopf, S.H.,
688 2022. Production of diverse brGDGTs by *Acidobacterium Solibacter usitatus* in response
689 to temperature, pH, and O₂ provides a culturing perspective on brGDGT proxies and
690 biosynthesis. *Geobiology*.

691 Harning, D.J., Curtin, L., Geirsdóttir, Á., D’Andrea, W.J., Miller, G.H., Sepúlveda, J., 2020. Lipid
692 Biomarkers Quantify Holocene Summer Temperature and Ice Cap Sensitivity in Icelandic
693 Lakes. *Geophys. Res. Lett.* 47, e2019GL085728.

694 Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2016. The effect of improved
695 chromatography on GDGT-based palaeoproxies. *Org. Geochem.* 93, 1–6.

696 Hopmans, E.C., Weijers, J.W.H., Schefuß, E., Herfort, L., Sinninghe Damsté, J.S., Schouten, S.,
697 2004. A novel proxy for terrestrial organic matter in sediments based on branched and
698 isoprenoid tetraether lipids. *Earth Planet. Sci. Lett.* 224, 107–116.

699 Huguet, A., Coffinet, S., Roussel, A., Gayraud, F., Anquetil, C., Bergonzini, L., Bonanomi, G.,
700 Williamson, D., Majule, A., Derenne, S., 2019. Evaluation of 3-hydroxy fatty acids as a pH
701 and temperature proxy in soils from temperate and tropical altitudinal gradients. *Org.
702 Geochem.* 129, 1–13.

703 Huguet, A., Meador, T.B., Laggoun-Défarage, F., Könneke, M., Wu, W., Derenne, S., Hinrichs, K.-
704 U., 2017. Production rates of bacterial tetraether lipids and fatty acids in peatland under
705 varying oxygen concentrations. *Geochim. Cosmochim. Acta* 203, 103–116.

706 Huguet, C., Hopmans, E.C., Febo-Ayala, W., Thompson, D.H., Sinninghe Damsté, J.S., Schouten,
707 S., 2006. An improved method to determine the absolute abundance of glycerol
708 dibiphytanyl glycerol tetraether lipids. *Org. Geochem.* 37, 1036–1041.

709 Kirkels, F.M.S.A., Ponton, C., Galy, V., West, A.J., Feakins, S.J., Peterse, F., 2020. From Andes
710 to Amazon: Assessing Branched Tetraether Lipids as Tracers for Soil Organic Carbon in
711 the Madre de Dios River System. *J. Geophys. Res. Biogeosciences* 125, e2019JG005270.

712 Kirkels, F.M.S.A., Usman, M.O., Peterse, F., 2022a. Distinct sources of bacterial branched GMGTs
713 in the Godavari River basin (India) and Bay of Bengal sediments. *Org. Geochem.* 167,
714 104405.

715 Kirkels, F.M.S.A., Zwart, H.M., Usman, M.O., Hou, S., Ponton, C., Giosan, L., Eglinton, T.I.,
716 Peterse, F., 2022b. From soil to sea: sources and transport of organic carbon traced by
717 tetraether lipids in the monsoonal Godavari River, India. *Biogeosciences* 19, 3979–4010.

- 718 Kolb, P., Zorndt, A., Burchard, H., Gräwe, U., Kösters, F., 2022. Modelling the impact of
719 anthropogenic measures on saltwater intrusion in the Weser estuary. *Ocean Sci.* 18, 1725–
720 1739.
- 721 Kou, Q., Zhu, L., Ju, J., Wang, J., Xu, T., Li, C., Ma, Q., 2022. Influence of salinity on glycerol
722 dialkyl glycerol tetraether-based indicators in Tibetan Plateau lakes: Implications for
723 paleotemperature and paleosalinity reconstructions. *Palaeogeogr. Palaeoclimatol.*
724 *Palaeoecol.* 601, 111127.
- 725 Lai, J., Zou, Y., Zhang, J., Peres-Neto, P.R., 2022. Generalizing hierarchical and variation
726 partitioning in multiple regression and canonical analyses using the *rdacca.hp* R package.
727 *Methods Ecol. Evol.* 13, 782–788.
- 728 Leavitt, P.R., Brock, C.S., Ebel, C., Patoine, A., 2006. Landscape-scale effects of urban nitrogen
729 on a chain of freshwater lakes in central North America. *Limnol. Oceanogr.* 51, 2262–2277.
- 730 Liu, X.-L., Summons, R.E., Hinrichs, K.-U., 2012. Extending the known range of glycerol ether
731 lipids in the environment: structural assignments based on tandem mass spectral
732 fragmentation patterns. *Rapid Commun. Mass Spectrom.* 26, 2295–2302.
- 733 Martínez-Sosa, P., Tierney, J.E., 2019. Lacustrine brGDGT response to microcosm and mesocosm
734 incubations. *Org. Geochem.* 127, 12–22.
- 735 Martínez-Sosa, P., Tierney, J.E., Stefanescu, I.C., Dearing Crampton-Flood, E., Shuman, B.N.,
736 Routson, C., 2021. A global Bayesian temperature calibration for lacustrine brGDGTs.
737 *Geochim. Cosmochim. Acta* 305, 87–105.
- 738 Naafs, B.D.A., Inglis, G.N., Zheng, Y., Amesbury, M.J., Biester, H., Bindler, R., Blewett, J.,
739 Burrows, M.A., del Castillo Torres, D., Chambers, F.M., Cohen, A.D., Evershed, R.P.,
740 Feakins, S.J., Gałka, M., Gallego-Sala, A., Gandois, L., Gray, D.M., Hatcher, P.G., Honorio
741 Coronado, E.N., Hughes, P.D.M., Huguet, A., Könönen, M., Laggoun-Défarge, F.,
742 Lähteenoja, O., Lamentowicz, M., Marchant, R., McClymont, E., Pontevedra-Pombal, X.,
743 Ponton, C., Pourmand, A., Rizzuti, A.M., Rochefort, L., Schellekens, J., De Vleeschouwer,
744 F., Pancost, R.D., 2017. Introducing global peat-specific temperature and pH calibrations
745 based on brGDGT bacterial lipids. *Geochim. Cosmochim. Acta* 208, 285–301.
- 746 Naafs, B.D.A., McCormick, D., Inglis, G.N., Pancost, R.D., 2018. Archaeal and bacterial H-
747 GDGTs are abundant in peat and their relative abundance is positively correlated with
748 temperature. *Geochim. Cosmochim. Acta* 227, 156–170.
- 749 Peterse, F., Kim, J.-H., Schouten, S., Kristensen, D.K., Koç, N., Sinninghe Damsté, J.S., 2009.
750 Constraints on the application of the MBT/CBT palaeothermometer at high latitude
751 environments (Svalbard, Norway). *Org. Geochem.* 40, 692–699.
- 752 Raberg, J.H., Miller, G.H., Geirsdóttir, Á., Sepúlveda, J., 2022. Near-universal trends in brGDGT
753 lipid distributions in nature. *Sci. Adv.* 8, eabm7625.
- 754 Ralston, D.K., Geyer, W.R., 2019. Response to Channel Deepening of the Salinity Intrusion,
755 Estuarine Circulation, and Stratification in an Urbanized Estuary. *J. Geophys. Res. Oceans*
756 124, 4784–4802.
- 757 Romero, E., Garnier, J., Billen, G., Ramarson, A., Riou, P., Le Gendre, R., 2019. Modeling the
758 biogeochemical functioning of the Seine estuary and its coastal zone: Export, retention, and
759 transformations. *Limnol. Oceanogr.* 64, 895–912.
- 760 Ryba, S.A., Burgess, R.M., 2002. Effects of sample preparation on the measurement of organic
761 carbon, hydrogen, nitrogen, sulfur, and oxygen concentrations in marine sediments.
762 *Chemosphere* 48, 139–147.
- 763 Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol
764 dialkyl glycerol tetraether lipids: A review. *Org. Geochem.* 54, 19–61.

765 Sinninghe Damsté, J.S., 2016. Spatial heterogeneity of sources of branched tetraethers in shelf
766 systems: The geochemistry of tetraethers in the Berau River delta (Kalimantan, Indonesia).
767 *Geochim. Cosmochim. Acta* 186, 13–31.

768 Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Weijers, J.W.H., Foesel, B.U.,
769 Overmann, J., Dedysh, S.N., 2011. 13,16-Dimethyl octacosanedioic acid (iso-diabolic acid),
770 a common membrane-spanning lipid of Acidobacteria subdivisions 1 and 3. *Appl. Environ.*
771 *Microbiol.* 77, 4147–4154.

772 Sluijs, A., Frieling, J., Inglis, G.N., Nierop, K.G.J., Peterse, F., Sangiorgi, F., Schouten, S., 2020.
773 Late Paleocene–early Eocene Arctic Ocean sea surface temperatures: reassessing
774 biomarker paleothermometry at Lomonosov Ridge. *Clim. Past* 16, 2381–2400.

775 Smith, R.W., Bianchi, T.S., Li, X., 2012. A re-evaluation of the use of branched GDGTs as
776 terrestrial biomarkers: Implications for the BIT Index. *Geochim. Cosmochim. Acta* 80, 14–
777 29.

778 Strickland, J.D.H., Parsons, T.R., 1972. *A Practical Handbook of Seawater Analysis*, 2nd edition.

779 Thibault, A., 2018. *Dynamique de la matière organique dans la Seine : approche globale et*
780 *moléculaire (phdthesis)*. Sorbonne Université.

781 Thibault, A., Derenne, S., Parlanti, E., Anquetil, C., Sourzac, M., Budzinski, H., Fuster, L.,
782 Laverman, A., Roose-Amsaleg, C., Viollier, E., Huguet, A., 2019. Dynamics of organic
783 matter in the Seine Estuary (France): Bulk and structural approaches. *Mar. Chem.* 212, 108–
784 119.

785 Tierney, J.E., Russell, J.M., 2009. Distributions of branched GDGTs in a tropical lake system:
786 Implications for lacustrine application of the MBT/CBT paleoproxy. *Org. Geochem.* 40,
787 1032–1036.

788 Véquaud, P., Thibault, A., Derenne, S., Anquetil, C., Collin, S., Contreras, S., Nottingham, A.T.,
789 Sabatier, P., Werne, J.P., Huguet, A., 2022. FROG: A global machine-learning temperature
790 calibration for branched GDGTs in soils and peats. *Geochim. Cosmochim. Acta* 318, 468–
791 494.

792 Wang, H., An, Z., Lu, H., Zhao, Z., Liu, W., 2020. Calibrating bacterial tetraether distributions
793 towards in situ soil temperature and application to a loess-paleosol sequence. *Quat. Sci.*
794 *Rev.* 231, 106172.

795 Wang, H., Liu, W., He, Y., Zhou, A., Zhao, H., Liu, H., Cao, Y., Hu, J., Meng, B., Jiang, J.,
796 Kolpakova, M., Krivonogov, S., Liu, Z., 2021. Salinity-controlled isomerization of
797 lacustrine brGDGTs impacts the associated MBT5ME' terrestrial temperature index.
798 *Geochim. Cosmochim. Acta* 305, 33–48.

799 Wei, X., Garnier, J., Thieu, V., Passy, P., Le Gendre, R., Billen, G., Akopian, M., Laruelle, G.G.,
800 2022. Nutrient transport and transformation in macrotidal estuaries of the French Atlantic
801 coast: a modeling approach using the Carbon-Generic Estuarine Model. *Biogeosciences* 19,
802 931–955.

803 Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007.
804 Environmental controls on bacterial tetraether membrane lipid distribution in soils.
805 *Geochim. Cosmochim. Acta* 71, 703–713.

806 Weijers, J.W.H., Wiesenberg, G.L.B., Bol, R., Hopmans, E.C., Pancost, R.D., 2010. Carbon
807 isotopic composition of branched tetraether membrane lipids in soils suggest a rapid
808 turnover and a heterotrophic life style of their source organism(s). *Biogeosciences* 7, 2959–
809 2973.

810 Wu, J., Yang, H., Pancost, R.D., Naafs, B.D.A., Qian, S., Dang, X., Sun, H., Pei, H., Wang, R.,
811 Zhao, S., Xie, S., 2021. Variations in dissolved O₂ in a Chinese lake drive changes in

812 microbial communities and impact sedimentary GDGT distributions. *Chem. Geol.* 579,
813 120348.

814 Xu, S., Zhang, Z., Jia, G., Yu, K., Lei, F., Zhu, X., 2020. Controlling factors and environmental
815 significance of BIT and $\delta^{13}\text{C}$ of sedimentary GDGTs from the Pearl River Estuary, China
816 over recent decades. *Estuar. Coast. Shelf Sci.* 233, 106534.

817 Yedema, Y.W., Sangiorgi, F., Sluijs, A., Sinninghe Damsté, J.S., Peterse, F., 2023. The dispersal
818 of fluviially discharged and marine, shelf-produced particulate organic matter in the
819 northern Gulf of Mexico. *Biogeosciences* 20, 663–686.

820 Zell, C., Kim, J.-H., Balsinha, M., Dorhout, D., Fernandes, C., Baas, M., Sinninghe Damsté, J.S.,
821 2014. Transport of branched tetraether lipids from the Tagus River basin to the coastal
822 ocean of the Portuguese margin: consequences for the interpretation of the MBT'/CBT
823 paleothermometer. *Biogeosciences* 11, 5637–5655.

824 Zhang, Z.-X., Li, J., Chen, Z., Sun, Z., Yang, H., Fu, M., Peng, X., 2020. The effect of methane
825 seeps on the bacterial tetraether lipid distributions at the Okinawa Trough. *Mar. Chem.* 225,
826 103845.

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Captions to figures and tables

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837 **Table 1.** Location of the sampling sites along the Seine Basin, with the type of samples collected.

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839 **Fig. 1.** (a) Geographical locations of sampling sites in the Seine River Basin (KP: kilometric point,
840 the distance in kilometers from the city of Paris (KP 0)). (b) Mean monthly water discharge for the
841 Seine River at the Paris Austerlitz station from 2015 to 2021 (data from
842 <https://www.hydro.eaufrance.fr/>). Bullets represent the sampling period in high-flow ($>250 \text{ m}^3/\text{s}$ -
843 blue) and low-flow ($<250 \text{ m}^3/\text{s}$ - red) seasons.

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845 **Fig. 2.** Extracted chromatograms of brGDGTs and brGMGTs for the SPM samples collected in (a)
846 site 3 (Triel sur Seine, November 2020), (b) site 10 (Val-des-Leux, July 2019) and (c) site 18
847 (Honfleur, April 2015). The nomenclature for the penta- and hexamethylated brGDGTs: 5-methyl
848 brGDGTs (IIIa₅, IIIb₅, IIIc₅, IIa₅, IIb₅, and IIc₅); 6-methyl brGDGTs (IIIa₆, IIIb₆, IIIc₆, IIa₆, IIb₆,
849 and IIc₆); 7-methyl brGDGTs (IIIa₇, IIIb₇, and IIa₇).

850 **Fig. 3.** Relative abundances of selected individual brGDGTs from soils (surficial soils and mudflat
851 soils/sediments, $n=51$), river ($n=9$), upstream estuary ($n=56$), and downstream estuary ($n=121$)
852 samples across the Seine River basin: cyclopentane-containing tetramethylated brGDGTs (Ib and
853 Ic), 6-methyl brGDGTs (IIa₆ and IIIa₆), 7-methyl brGDGTs (IIa₇ and IIIa₇) and brGDGTs 1050d
854 and 1036d. Box plots of upstream and downstream estuary samples are based on SPM and
855 sediments, whereas those of river samples are based only on SPM. Boxes are color-coded based
856 on the sample type (soil in brown, river in red, upstream estuary in yellow, and downstream estuary
857 in blue). Statistical testing was performed by a Wilcoxon test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;
858 **** $P < 0.0001$; ns, not significant, $P > 0.05$).

859 **Fig. 4.** PCA analysis of fractional abundances of (a) brGDGTs and (b) brGMGTs. The dataset used
860 for PCA analysis is composed of SPM and sediments. Adonis analysis was used to evaluate how
861 variation can be explained by the variables (999 permutations).

862 **Fig. 5.** RDA analysis showing relationships between environmental factors (TN, TOC, salinity,
863 temperature, purple arrows) and fractional abundances of (a) brGDGTs and (c) brGMGTs. The
864 individual importance of the environmental factors (TN, TOC, salinity, temperature) explaining
865 the variation in (b) brGDGT and (d) brGMGT distributions was determined by hierarchical
866 partitioning analysis. The dataset used for RDA analysis is composed of SPM from river ($n=6$; red),
867 upstream estuary ($n=42$; yellow), and downstream estuary ($n=59$; blue). Significance level is
868 indicated by asterisks: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

869 **Fig. 6.** Relative abundance of distinct brGMGTs from soils (surficial soils and mudflat
870 soils/sediments, $n=51$), river ($n=9$), upstream estuary ($n=56$) and downstream estuary ($n=121$)
871 across the Seine River basin. Box plots of upstream and downstream estuary are composed of SPM
872 and sediments, whereas those of river are composed of SPM. Boxes are color-coded based on the
873 sample type (soil in brown, river in red, upstream estuary in yellow, and downstream estuary in
874 blue). Statistical testing was performed by a Wilcoxon test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;
875 **** $P < 0.0001$; ns, not significant, $P > 0.05$).

876 **Fig. 7.** Spatio-temporal variations of IR_{6Me} and several environmental factors, including TN (%),
877 $\delta^{15}\text{N}$ (‰), Chla ($\mu\text{g/L}$), TOC (%), turbidity (NTU), pH, and dissolved oxygen saturation (DO, %).
878 The trends showing variations were based on locally estimated scatterplot smoothing (LOESS)
879 method with 95% confidence intervals. KP (kilometric point) represents the distance in kilometers
880 from the city of Paris (KP 0). Dataset is composed of SPM. The shaded area highlights a zone (KP
881 243-297.6) where IR_{6Me} and several environmental parameters co-vary.

882 **Fig. 8.** (a) Salinity plotted versus relative abundance of brGMGTs. Shaded area represent 95%
883 confidence intervals. Vertical error bars indicate mean \pm s.d for samples with the same salinity.
884 Dataset is composed of SPM. (b) Distribution of RIX across the Seine River basin. Boxes are color-
885 coded based on the sample type (river in red, upstream estuary in yellow, and downstream estuary
886 in blue). Dataset is composed of SPM and sediments. (c) RIX in the Godavari River basin (India)
887 and Bay of Bengal sediments (data from Kirkels et al. (2022a)). Statistical testing was performed
888 by a Wilcoxon test.

889 **Fig. 9.** Spatio-temporal variations of RIX and several other terrestrial proxies, including BIT and
890 $\delta^{13}\text{C}$ (‰). The trends showing spatio-temporal variations were based on locally estimated
891 scatterplot smoothing (LOESS) method with 95% confidence intervals. KP (kilometric point)
892 represents the distance in kilometers from the city of Paris (KP 0). Dataset is composed of SPM.

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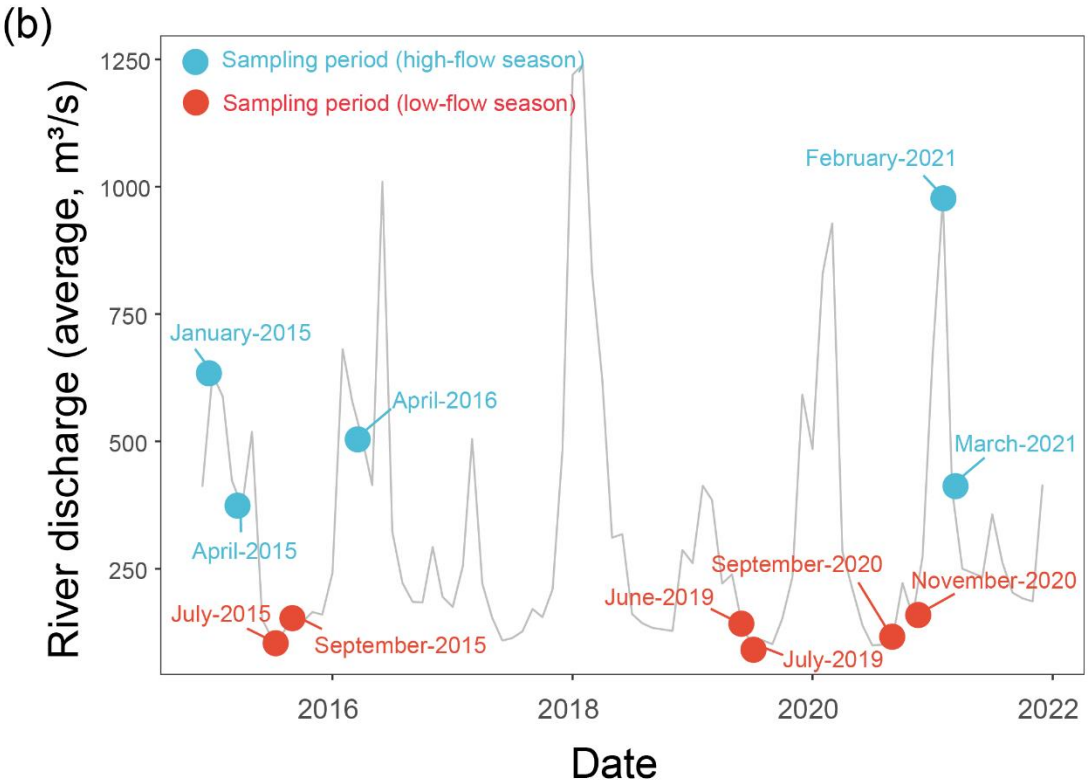
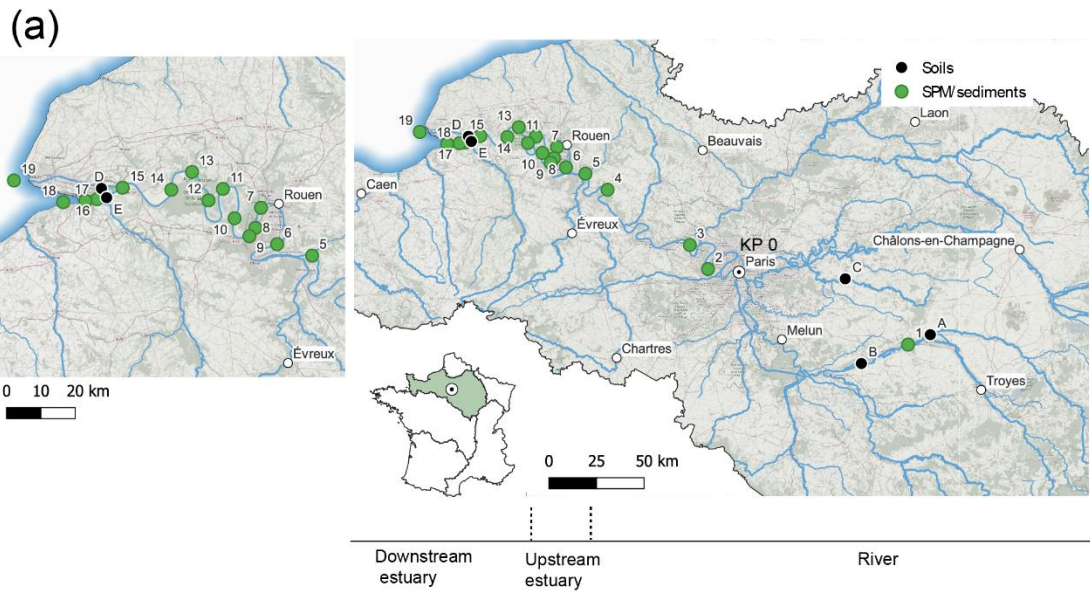
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909 **Table 1.** Location of the sampling sites along the Seine Basin, with the type of samples collected

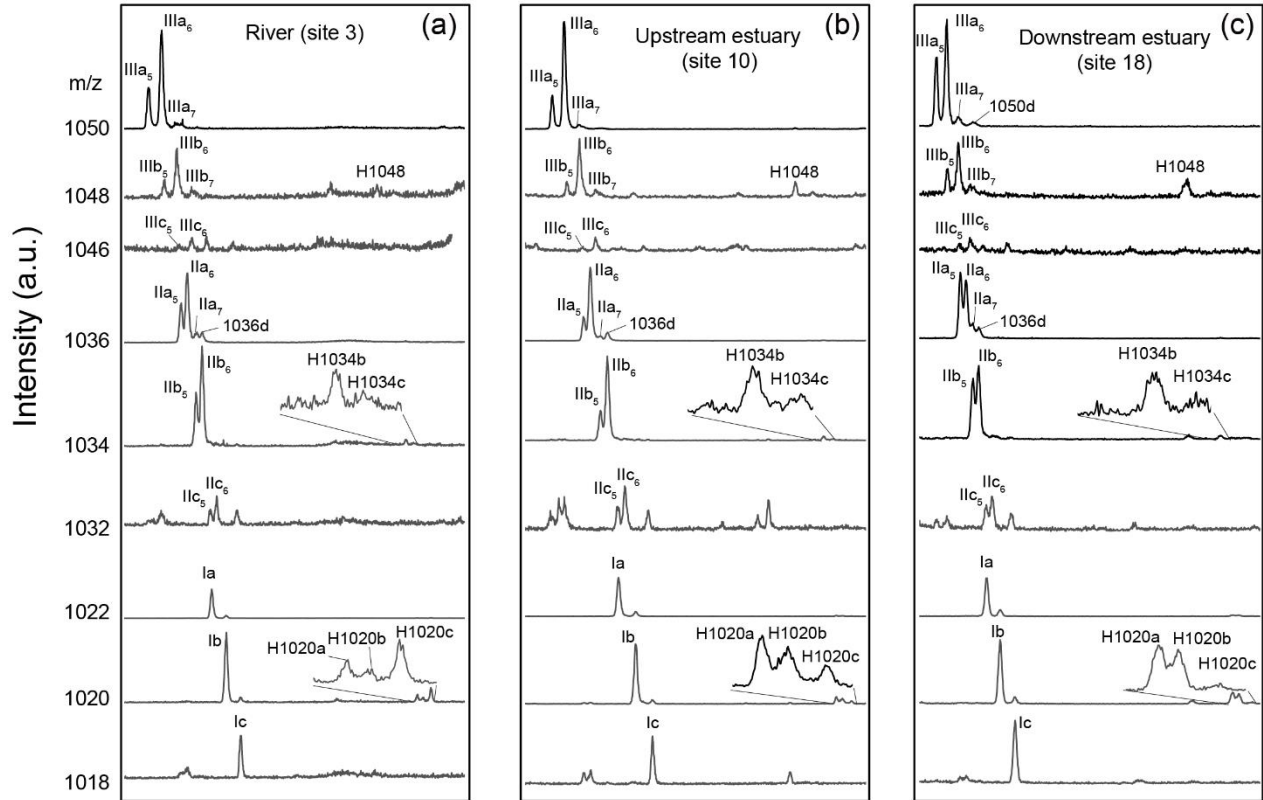
Site	Name	Longitude (°)	Latitude (°)	KP	Zone	Date	Type
1	Marnay sur Seine	3.56	48.51	-200	River	2020-11	SPM
2	Bougival	2.13	48.87	40	River	2020-11	SPM
3	Triel sur Seine	2.00	48.98	80	River	2020-11	SPM
4	Les Andelys	1.40	49.24	175	River	2019-6; 2019-7; 2020-9	SPM
5	Poses	1.24	49.31	202	Upstream estuary	2016-4; 2020-11	SPM
6	Oissel	1.10	49.34	229.4	Upstream estuary	2019-6; 2019-7; 2020-9	SPM
7	Rouen	1.03	49.43	243	Upstream estuary	2016-4	SPM; Sediments
8	Petit Couronne	1.01	49.38	251.3	Upstream estuary	2020-9; 2021-2; 2021-3	SPM
9	Grand-Couronne	0.98	49.36	255.6	Upstream estuary	2019-6	SPM
10	Val des Leux	0.92	49.40	265.55	Upstream estuary	2019-6; 2019-7; 2020-9	SPM
11	Duclair	0.87	49.48	278	Upstream estuary	2020-9; 2021-2; 2021-3	SPM
12	Heurtauville	0.82	49.45	297.65	Downstream estuary	2019-6	SPM
13	Caudebec	0.75	49.52	310.5	Downstream estuary	2015-4; 2015-9; 2016-4; 2019-6; 2019-7; 2020-9; 2021-2; 2021-3	SPM; Sediments
14	Vatteville-La-Rue	0.67	49.47	318	Downstream estuary	2019-6	SPM
15	Tancarville	0.47	49.47	337	Downstream estuary	2015-1; 2015-4; 2015-9; 2019-6; 2019-7; 2020-9; 2021-2; 2021-3	SPM; Sediments
16	Berville-Sur-Mer	0.37	49.44	346	Downstream estuary	2019-6	SPM
17	Fatouville	0.32	49.44	350	Downstream estuary	2015-4; 2015-7; 2015-9; 2016-4	SPM; Sediments

18	Honfleur	0.23	49.43	355.8	Downstream estuary	2015-4; 2015-9; 2019-6; 2020-9; 2021-2; 2021-3	SPM
19	La Carosse	0.03	49.48	370	Downstream estuary	2015-7; 2016-4; 2016-4	SPM; Sediments

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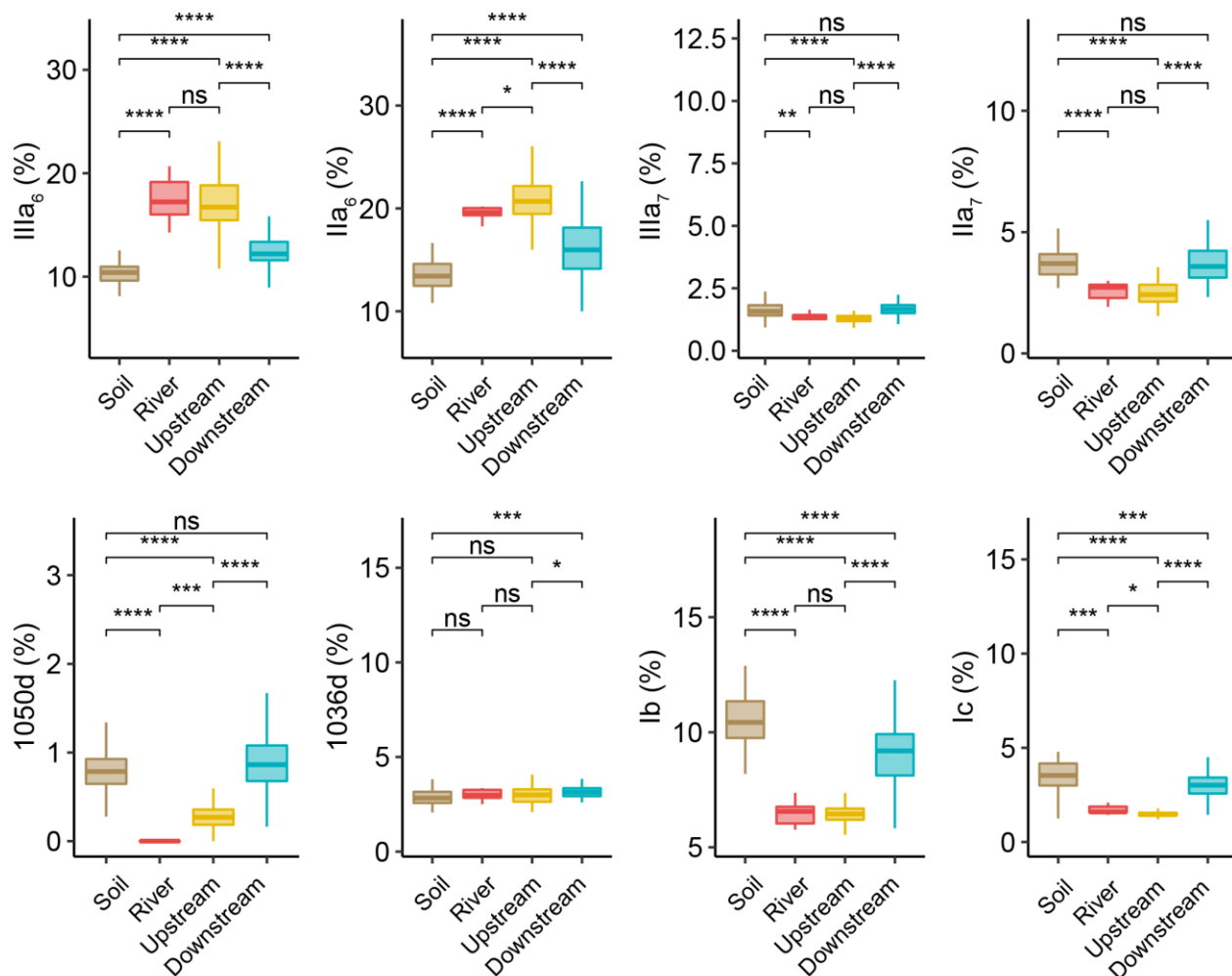


911
 912 **Fig. 1.** (a) Geographical locations of sampling sites in the Seine River Basin (KP: kilometric point,
 913 the distance in kilometers from the city of Paris (KP 0)). (b) Mean monthly water discharge for the
 914 Seine River at the Paris Austerlitz station from 2015 to 2021 (data from
 915 <https://www.hydro.eaufrance.fr/>). Bullets represent the sampling period in high-flow (>250 m³/s -
 916 blue) and low-flow (<250 m³/s - red) seasons.



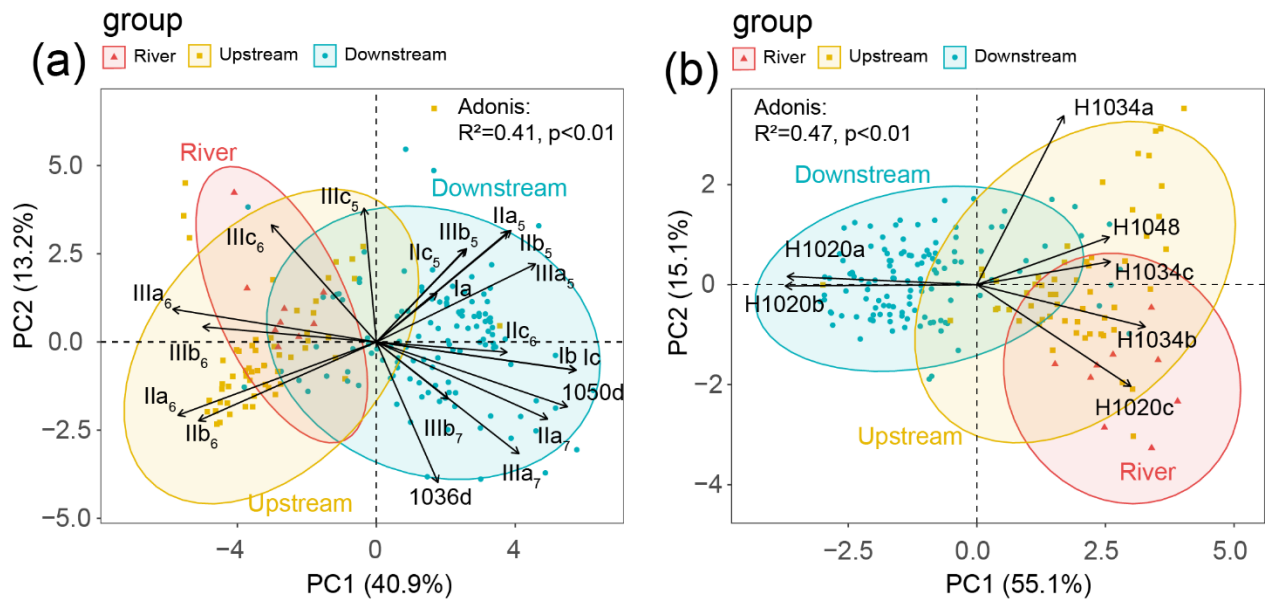
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919 **Fig. 2.** Extracted chromatograms of brGDGTs and brGMGTs for the SPM samples collected in (a)
 920 site 3 (Triel sur Seine, November 2020), (b) site 10 (Val-des-Leux, July 2019) and (c)
 921 site 18 (Honfleur, April 2015). The nomenclature for the penta- and hexamethylated brGDGTs: 5-methyl
 922 brGDGTs (IIIa₅, IIIb₅, IIIc₅, IIa₅, IIb₅, and IIc₅); 6-methyl brGDGTs (IIIa₆, IIIb₆, IIIc₆, IIa₆, IIb₆,
 923 and IIc₆); 7-methyl brGDGTs (IIIa₇, IIIb₇, and IIa₇).



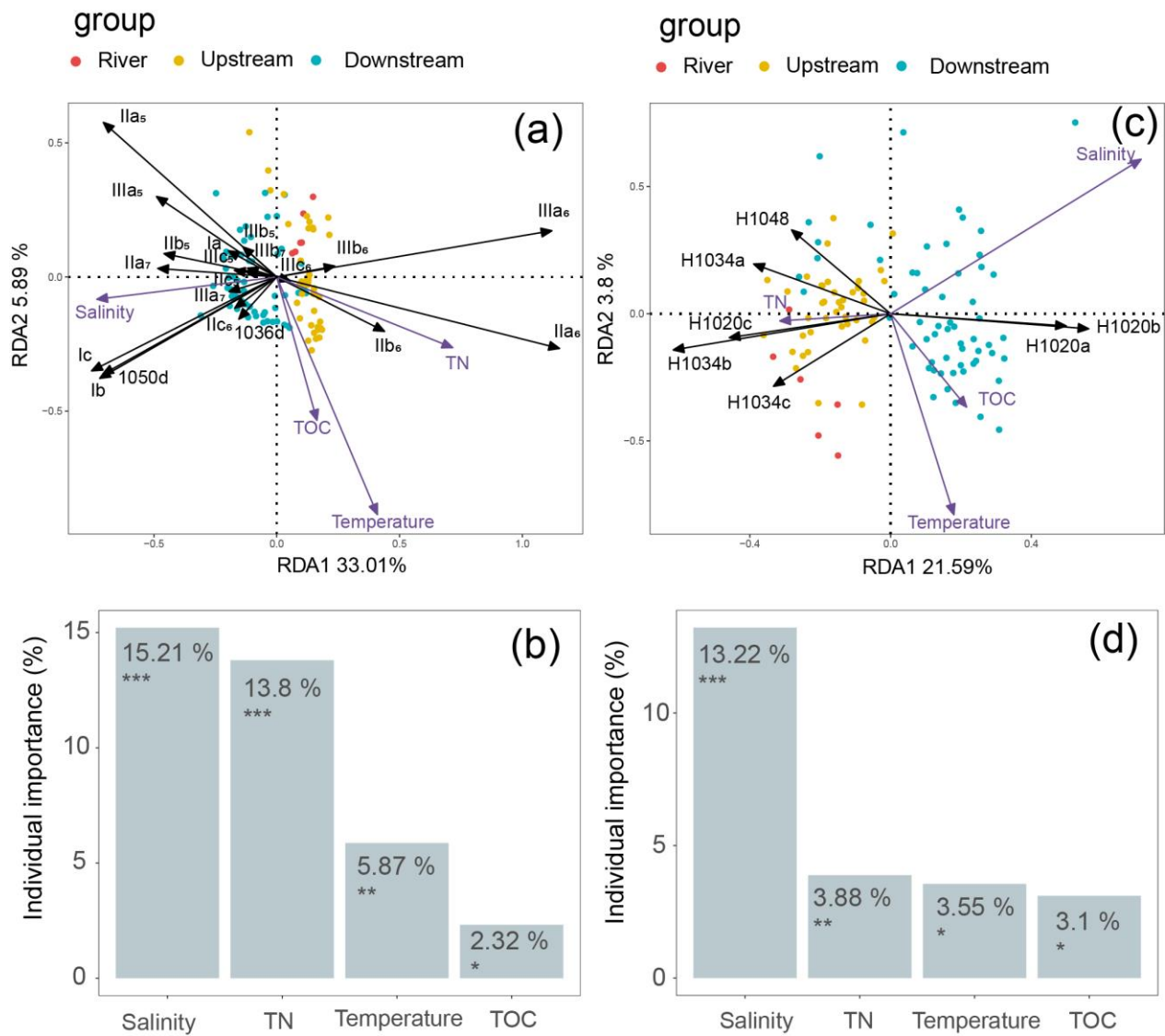
924

925 **Fig. 3.** Relative abundances of selected individual brGDGTs from soils (surficial soils and mudflat
 926 soils/sediments, $n=51$), river ($n=9$), upstream estuary ($n=56$), and downstream estuary ($n=121$)
 927 samples across the Seine River basin: cyclopentane-containing tetramethylated brGDGTs (Ib and
 928 Ic), 6-methyl brGDGTs (IIa₆ and IIIa₆), 7-methyl brGDGTs (IIa₇ and IIIa₇) and brGDGTs 1050d
 929 and 1036d. Box plots of upstream and downstream estuary samples are based on SPM and
 930 sediments, whereas those of river samples are based only on SPM. Boxes are color-coded based
 931 on the sample type (soil in brown, river in red, upstream estuary in yellow, and downstream estuary
 932 in blue). Statistical testing was performed by a Wilcoxon test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;
 933 **** $P < 0.0001$; ns, not significant, $P > 0.05$).



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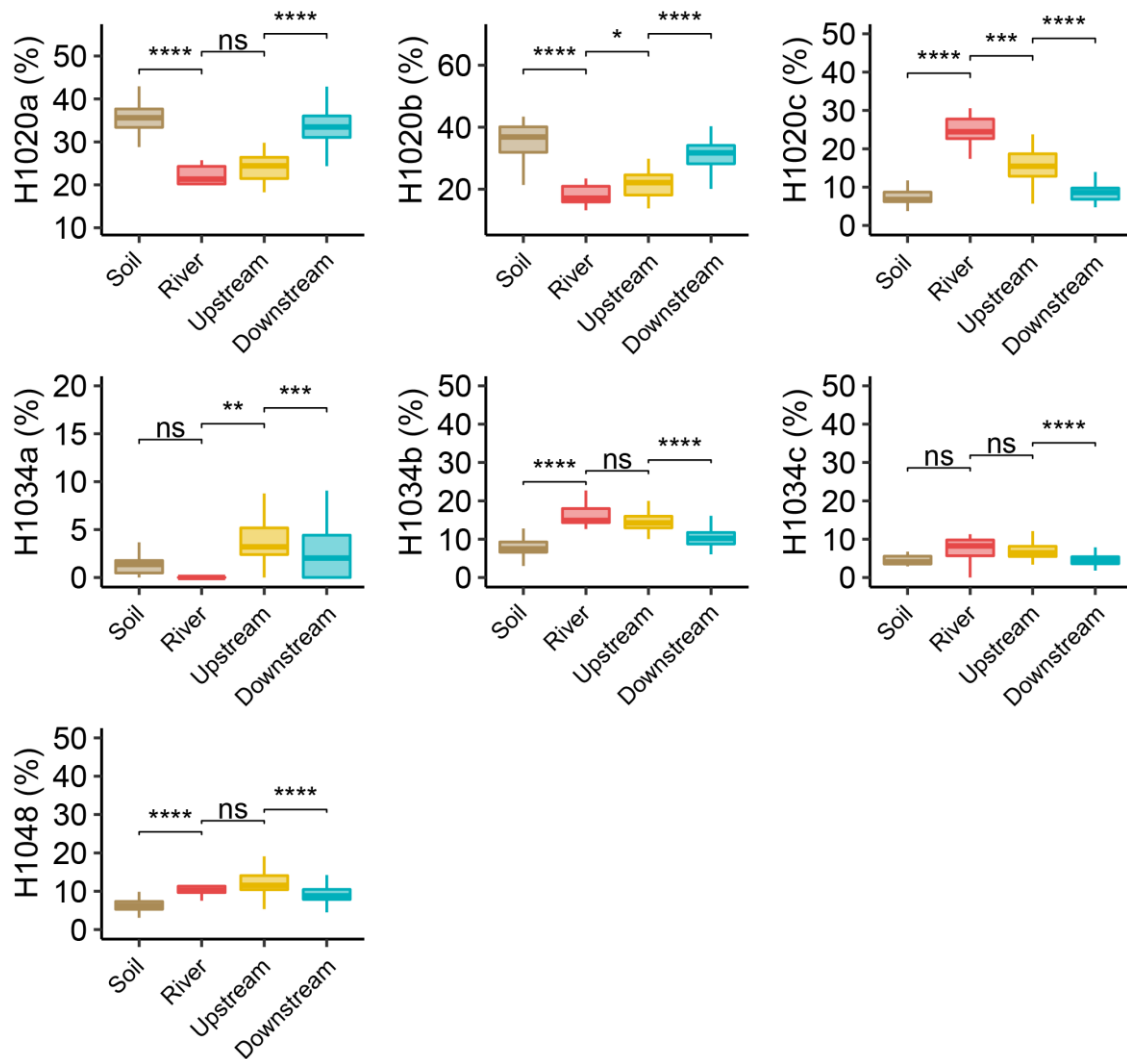
935 **Fig. 4.** PCA analysis of fractional abundances of (a) brGDGTs and (b) brGMGTs. The dataset used
 936 for PCA analysis is composed of SPM and sediments. Adonis analysis was used to evaluate how
 937 variation can be explained by the variables (999 permutations).



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939 **Fig. 5.** RDA analysis showing relationships between environmental factors (TN, TOC, salinity, temperature, purple arrows) and fractional abundances of (a) brGDGTs and (c) brGMGTs. The
 940 individual importance of the environmental factors (TN, TOC, salinity, temperature) explaining
 941 the variation in (b) brGDGT and (d) brGMGT distributions was determined by hierarchical
 942 partitioning analysis. The dataset used for RDA analysis is composed of SPM from river ($n=6$; red),
 943 upstream estuary ($n=42$; yellow), and downstream estuary ($n=59$; blue). Significance level is
 944 indicated by asterisks: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

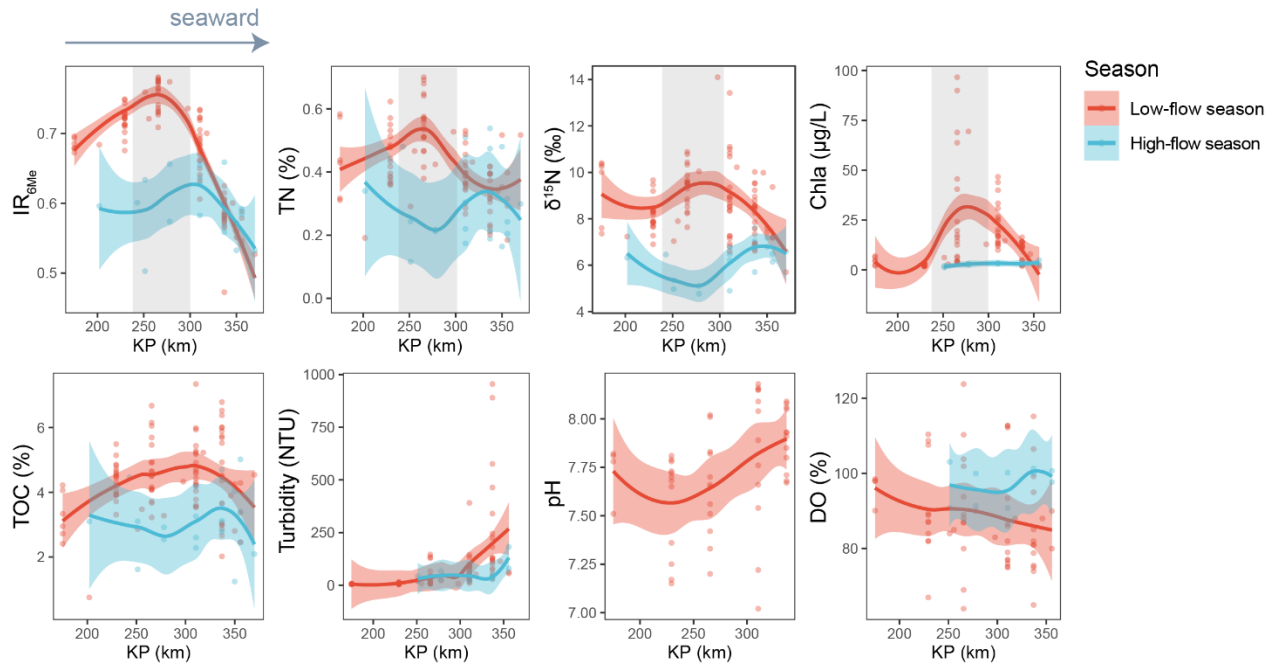
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 948 **Fig. 6.** Relative abundance of distinct brGMGTs from soils (surficial soils and mudflat
 949 soils/sediments, $n=51$), river ($n=9$), upstream estuary ($n=56$) and downstream estuary ($n=121$)
 950 across the Seine River basin. Box plots of upstream and downstream estuary are composed of SPM
 951 and sediments, whereas those of river are composed of SPM. Boxes are color-coded based on the
 952 sample type (soil in brown, river in red, upstream estuary in yellow, and downstream estuary in
 953 blue). Statistical testing was performed by a Wilcoxon test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;
 954 **** $P < 0.0001$; ns, not significant, $P > 0.05$).

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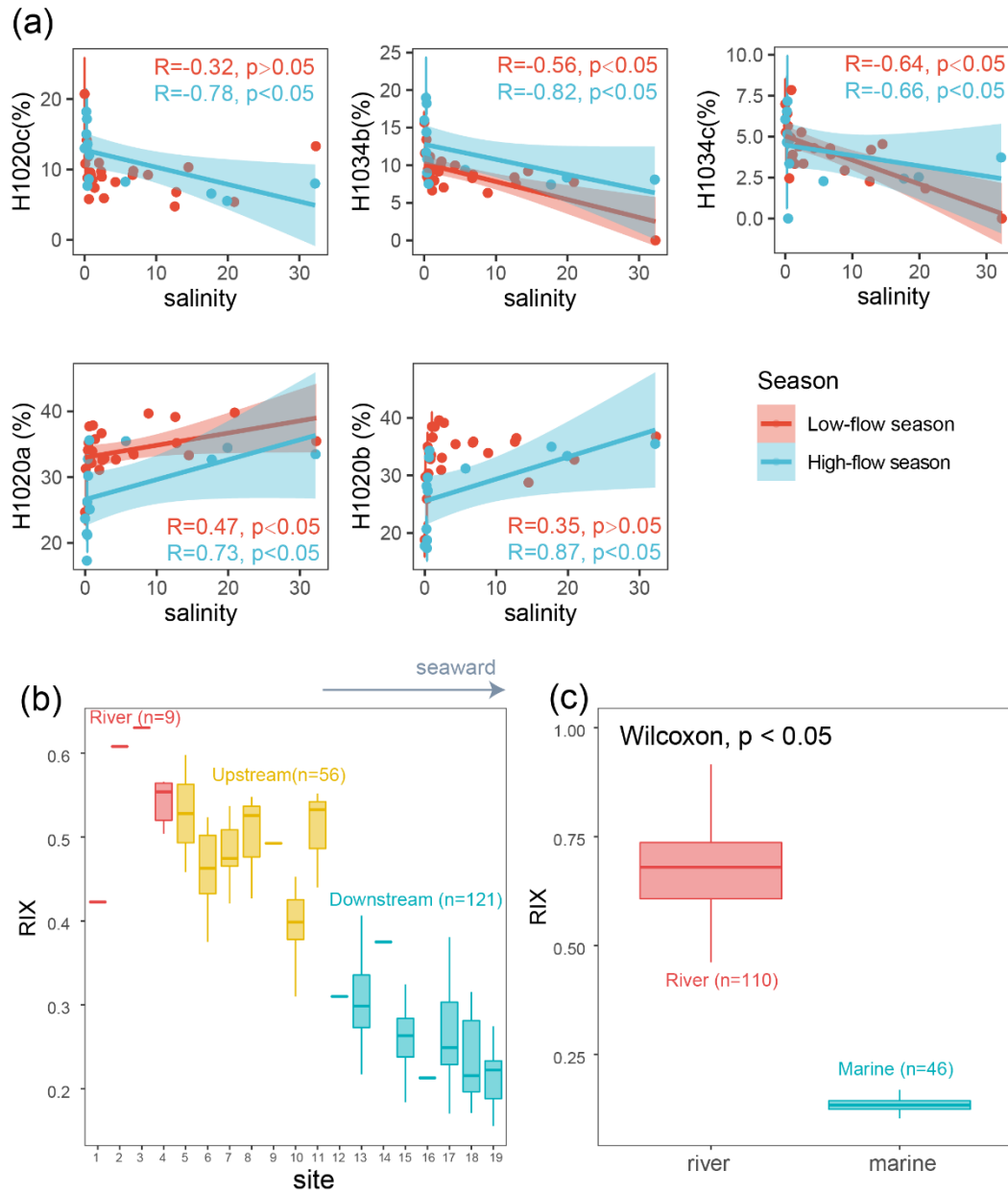


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958 **Fig. 7.** Spatio-temporal variations of IR_{6Me} and several environmental factors, including TN (%),
 959 $\delta^{15}N$ (‰), Chla ($\mu\text{g/L}$), TOC (%), turbidity (NTU), pH, and dissolved oxygen saturation (DO, %).
 960 The trends showing variations were based on locally estimated scatterplot smoothing (LOESS)
 961 method with 95% confidence intervals. KP (kilometric point) represents the distance in kilometers
 962 from the city of Paris (KP 0). Dataset is composed of SPM. The shaded area highlights a zone (KP
 963 243-297.6) where IR_{6Me} and several environmental parameters co-vary.

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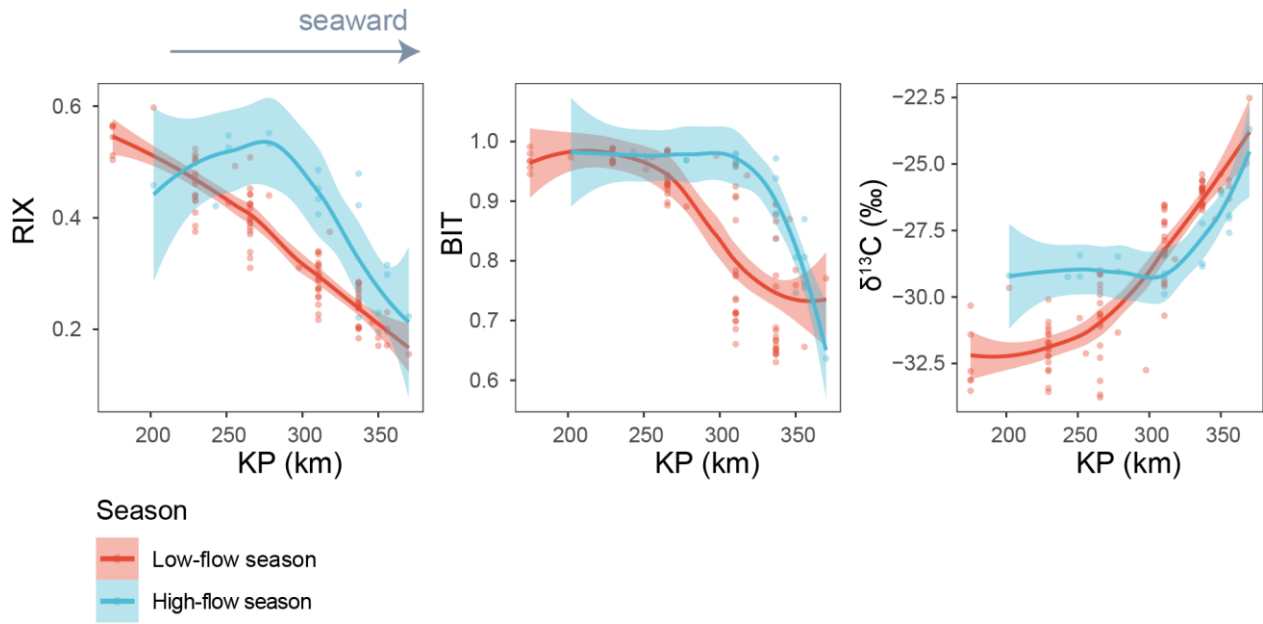
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967 **Fig. 8.** (a) Salinity plotted versus relative abundance of brGMGTs. Shaded area represent 95%
 968 confidence intervals. Vertical error bars indicate mean \pm s.d. for samples with the same salinity.
 969 Dataset is composed of SPM. (b) Distribution of RIX across the Seine River basin. Boxes are color-
 970 coded based on the sample type (river in red, upstream estuary in yellow, and downstream estuary
 971 in blue). Dataset is composed of SPM and sediments. (c) RIX in the Godavari River basin (India)
 972 and Bay of Bengal sediments (data from Kirkels et al. (2022a)). Statistical testing was performed
 973 by a Wilcoxon test.

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975

976 **Fig. 9.** Spatio-temporal variations of RIX and several other terrestrial proxies, including BIT and
 977 $\delta^{13}\text{C}$ (‰). The trends showing spatio-temporal variations were based on locally estimated
 978 scatterplot smoothing (LOESS) method with 95% confidence intervals. KP (kilometric point)
 979 represents the distance in kilometers from the city of Paris (KP 0). Dataset is composed of SPM.

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