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9

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'<sub>5ME</sub> 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 TEX<sub>86</sub> 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 m<sup>3</sup>/s; Fig. 1b), whereas the minimum flows are observed in  
139 summer (below 250 m<sup>3</sup>/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 m<sup>3</sup>/s) and low-flow (below 250 m<sup>3</sup>/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  $-20^{\circ}\text{C}$  and freeze dried. Both  
176 decarbonated and non-decarbonated samples ( $\sim 6$  mg for SPM and  $\sim 20$  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  $\text{N}_2$  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 ( $\sim 150$ 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). C<sub>46</sub> 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 C<sub>46</sub> 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<sub>6Me</sub> 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<sub>6Me</sub> 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 ( $90^\circ$ ) reflect a lack of linear correlations, whereas small or  
248 straight angles (close to  $0^\circ$  or  $180^\circ$ , 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 (IIIa<sub>6</sub> and IIa<sub>6</sub>) 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 (IIIa<sub>7</sub> and IIa<sub>7</sub>) 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 ( $C_{\text{org}}$ , based on SPM samples) to upstream estuary ( $7.52 \pm 5.09 \mu\text{g/g}$   
284  $C_{\text{org}}$ , based on SPM and sediments) and downstream estuary ( $4.95 \pm 4.09 \mu\text{g/g}$   $C_{\text{org}}$ , 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 (IIIa<sub>6</sub>, IIIb<sub>6</sub>, IIIc<sub>6</sub>, IIa<sub>6</sub>, and IIb<sub>6</sub>) in river and upstream estuarine samples, whereas in  
295 downstream estuary, it was driven by 5-methyl brGDGTs (III<sub>5</sub>, IIa<sub>5</sub>, IIc<sub>5</sub>, IIb<sub>5</sub>, IIIb<sub>5</sub>),  
296 tetramethylated brGDGTs (Ia, Ib, and Ic), 7-methyl brGDGTs (IIIa<sub>7</sub>, IIa<sub>7</sub>, and IIb<sub>7</sub>), 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. IIa<sub>6</sub> and IIIa<sub>6</sub>) 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 (IIa<sub>6</sub> and  
352 IIIa<sub>6</sub>) 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 IIIa<sub>7</sub>, IIa<sub>7</sub> 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, IIIa<sub>7</sub>, IIa<sub>7</sub> 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. IIa<sub>6</sub>, IIIa<sub>6</sub>, and IIb<sub>6</sub>) 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<sub>6Me</sub> (Fig. 7). High IR<sub>6Me</sub> values ( $0.69 \pm 0.10$ ) 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<sub>6me</sub> is observed, especially in the upstream part with a low

418 salinity range (0-0.32 psu). Specifically, much higher IR<sub>6Me</sub> 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<sub>6Me</sub> in the Seine River basin, they are  
427 unlikely to explain the substantially high IR<sub>6Me</sub> 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<sub>6Me</sub> 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. IIa<sub>6</sub> and IIIa<sub>6</sub>) 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  $^{15}\text{N}$  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  $^{15}\text{N}$ -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, IIIa<sub>7</sub>, IIa<sub>7</sub> 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<sub>6Me</sub>, 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

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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.

838

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.

844

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 (IIIa<sub>5</sub>, IIIb<sub>5</sub>, IIIc<sub>5</sub>, IIa<sub>5</sub>, IIb<sub>5</sub>, and IIc<sub>5</sub>); 6-methyl brGDGTs (IIIa<sub>6</sub>, IIIb<sub>6</sub>, IIIc<sub>6</sub>, IIa<sub>6</sub>, IIb<sub>6</sub>,  
849 and IIc<sub>6</sub>); 7-methyl brGDGTs (IIIa<sub>7</sub>, IIIb<sub>7</sub>, and IIa<sub>7</sub>).

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 (IIa<sub>6</sub> and IIIa<sub>6</sub>), 7-methyl brGDGTs (IIa<sub>7</sub> and IIIa<sub>7</sub>) 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<sub>6Me</sub> 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<sub>6Me</sub> 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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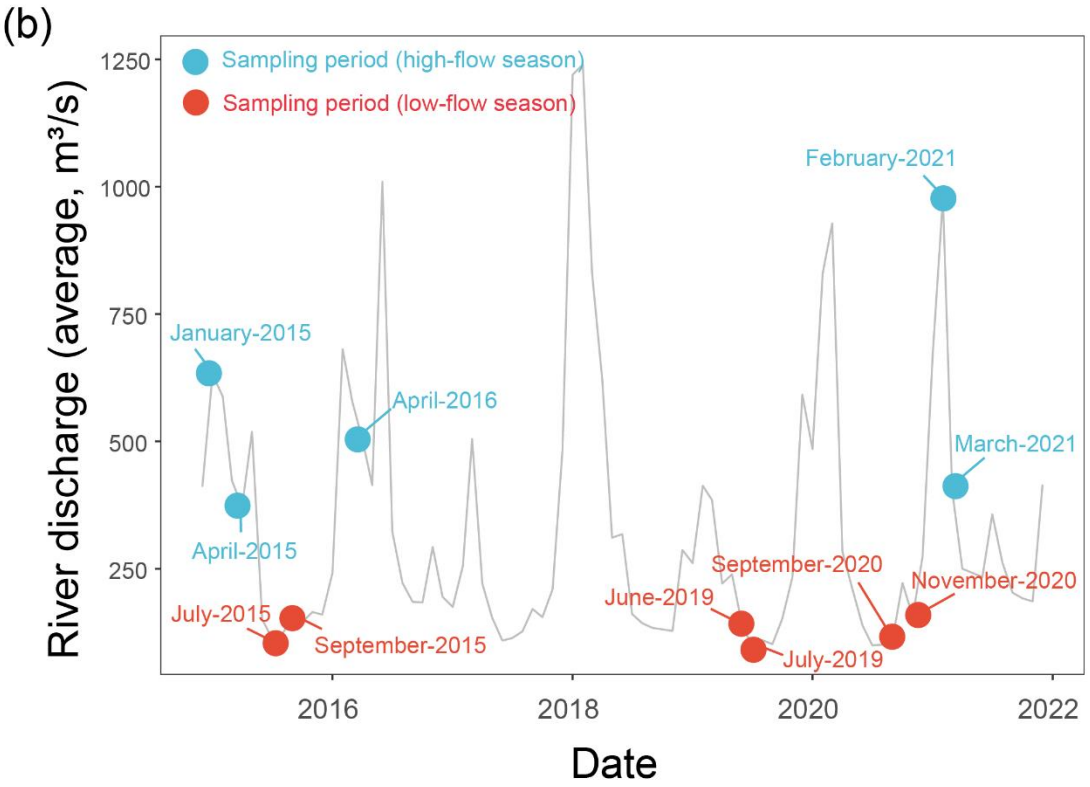
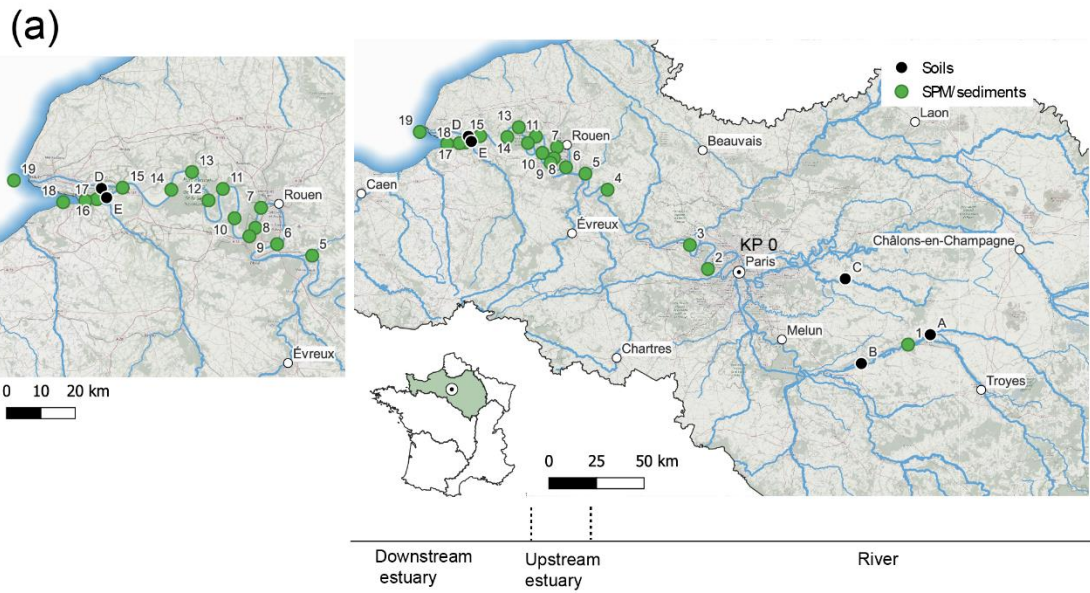
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909 **Table 1.** Location of the sampling sites along the Seine Basin, with the type of samples collected

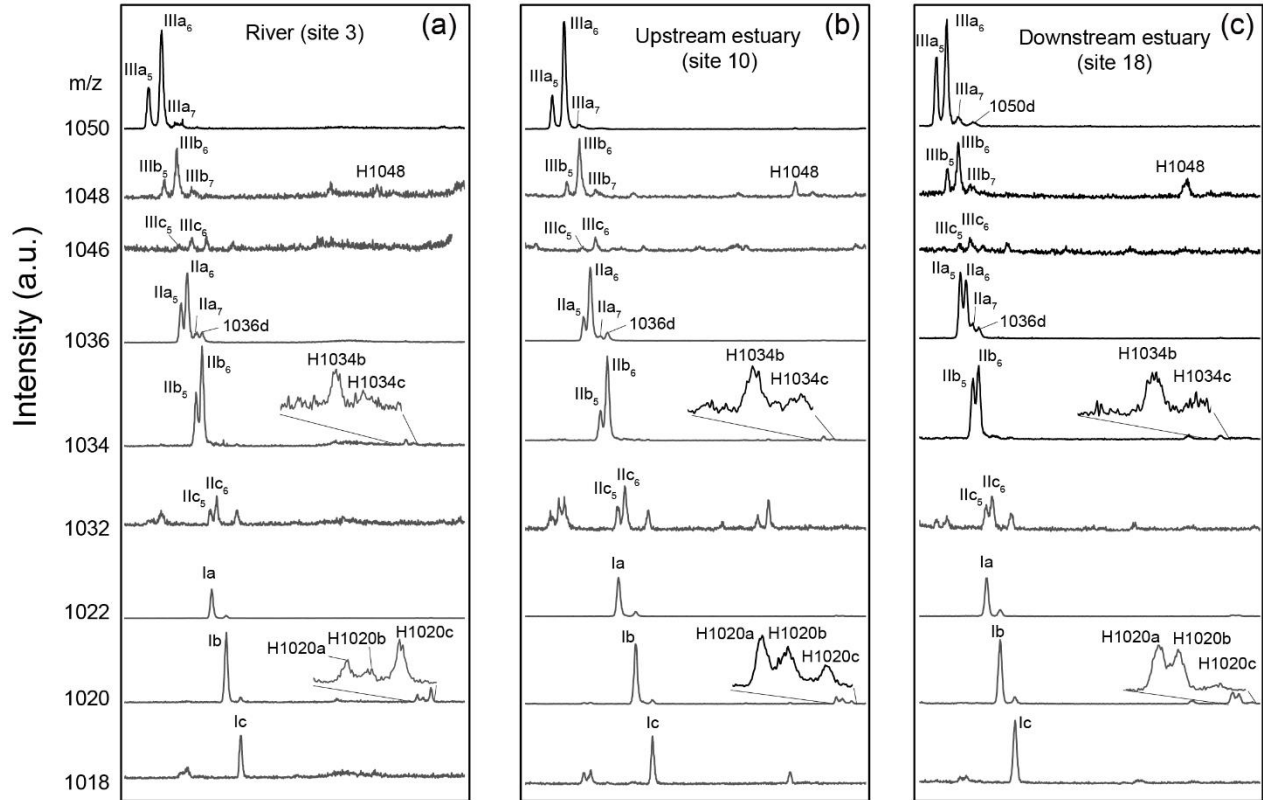
Site	Name	Longitude (°)	Latitude (°)	KP	Zone	Date	Type
1	Marnay sur Seine	3.56	48.51	-200	River	2020-11	SPM
2	Bougival	2.13	48.87	40	River	2020-11	SPM
3	Triel sur Seine	2.00	48.98	80	River	2020-11	SPM
4	Les Andelys	1.40	49.24	175	River	2019-6; 2019-7; 2020-9	SPM
5	Poses	1.24	49.31	202	Upstream estuary	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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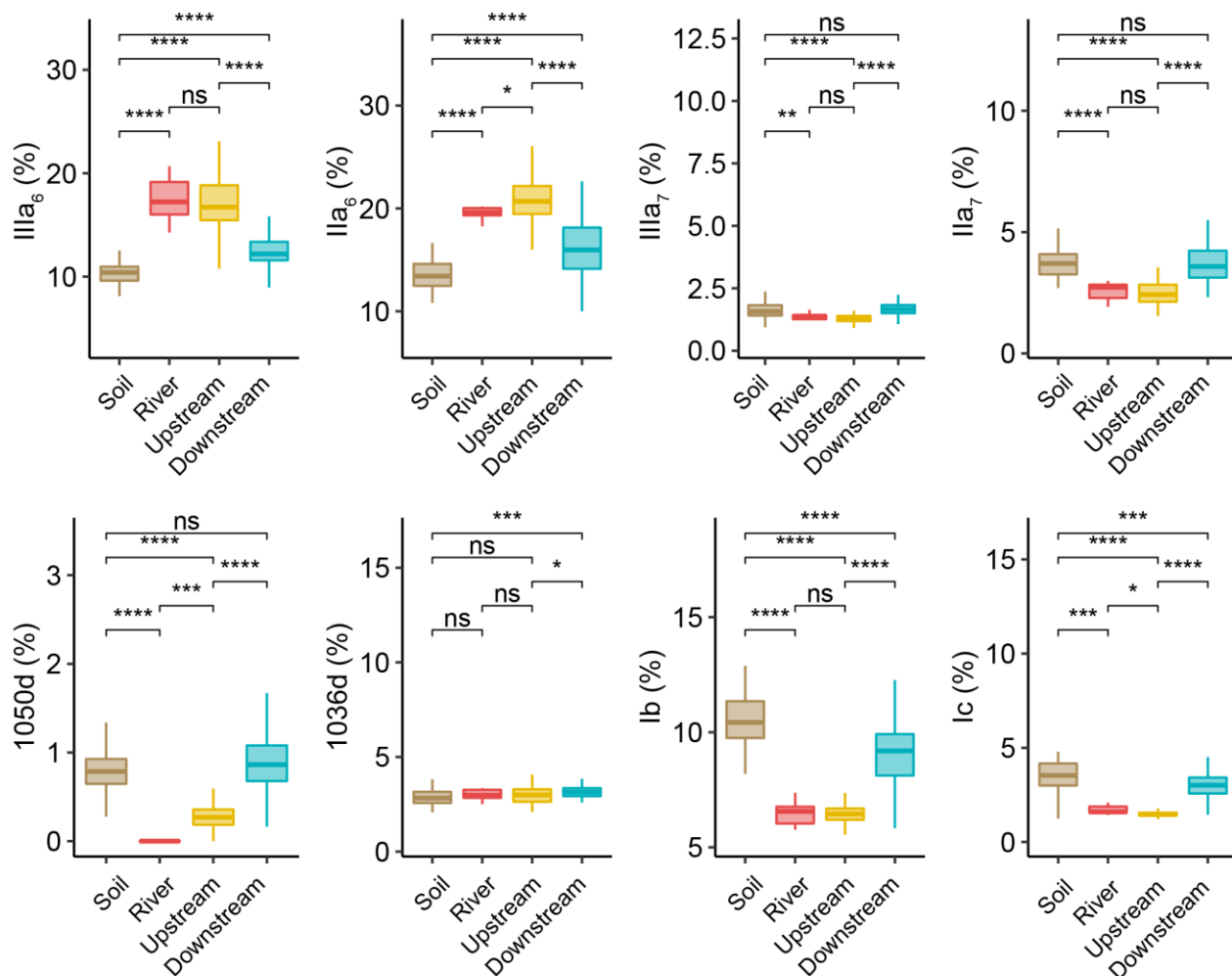


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 912 **Fig. 1.** (a) Geographical locations of sampling sites in the Seine River Basin (KP: kilometric point,  
 913 the distance in kilometers from the city of Paris (KP 0)). (b) Mean monthly water discharge for the  
 914 Seine River at the Paris Austerlitz station from 2015 to 2021 (data from  
 915 <https://www.hydro.eaufrance.fr/>). Bullets represent the sampling period in high-flow (>250 m<sup>3</sup>/s -  
 916 blue) and low-flow (<250 m<sup>3</sup>/s - red) seasons.



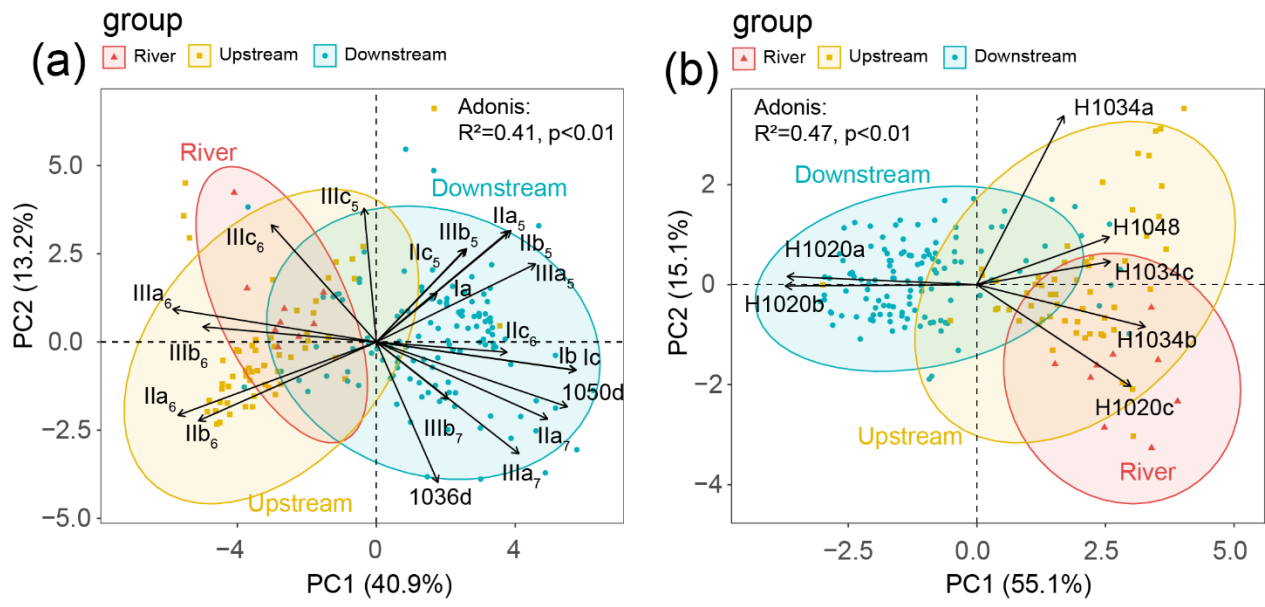
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919 **Fig. 2.** Extracted chromatograms of brGDGTs and brGMGTs for the SPM samples collected in (a)  
 920 site 3 (Triel sur Seine, November 2020), (b) site 10 (Val-des-Leux, July 2019) and (c)  
 921 site 18 (Honfleur, April 2015). The nomenclature for the penta- and hexamethylated brGDGTs: 5-methyl  
 922 brGDGTs (IIIa<sub>5</sub>, IIIb<sub>5</sub>, IIIc<sub>5</sub>, IIa<sub>5</sub>, IIb<sub>5</sub>, and IIc<sub>5</sub>); 6-methyl brGDGTs (IIIa<sub>6</sub>, IIIb<sub>6</sub>, IIIc<sub>6</sub>, IIa<sub>6</sub>, IIb<sub>6</sub>,  
 923 and IIc<sub>6</sub>); 7-methyl brGDGTs (IIIa<sub>7</sub>, IIIb<sub>7</sub>, and IIa<sub>7</sub>).



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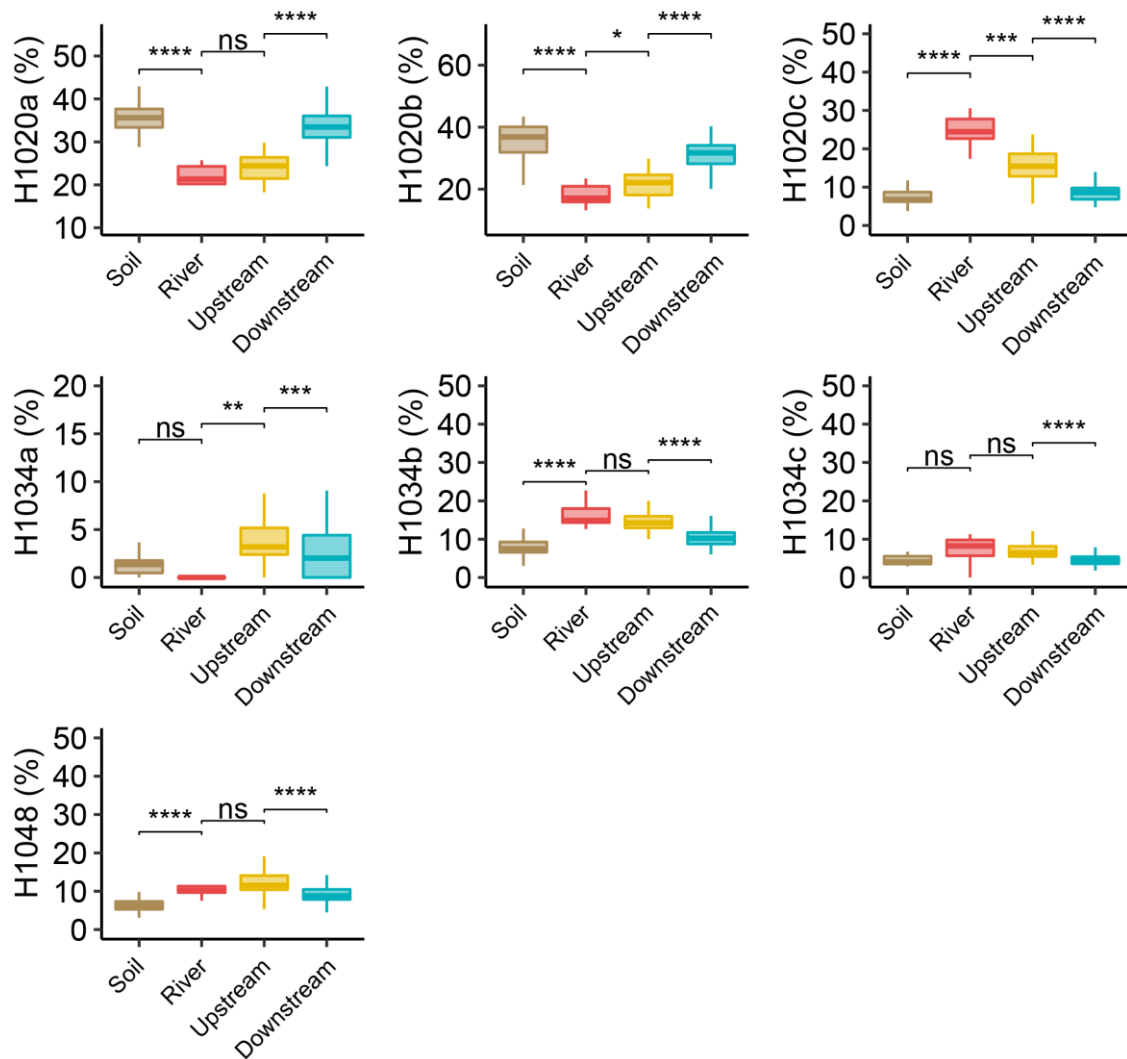
925 **Fig. 3.** Relative abundances of selected individual brGDGTs from soils (surficial soils and mudflat  
 926 soils/sediments,  $n=51$ ), river ( $n=9$ ), upstream estuary ( $n=56$ ), and downstream estuary ( $n=121$ )  
 927 samples across the Seine River basin: cyclopentane-containing tetramethylated brGDGTs (Ib and  
 928 Ic), 6-methyl brGDGTs (IIa<sub>6</sub> and IIIa<sub>6</sub>), 7-methyl brGDGTs (IIa<sub>7</sub> and IIIa<sub>7</sub>) 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).

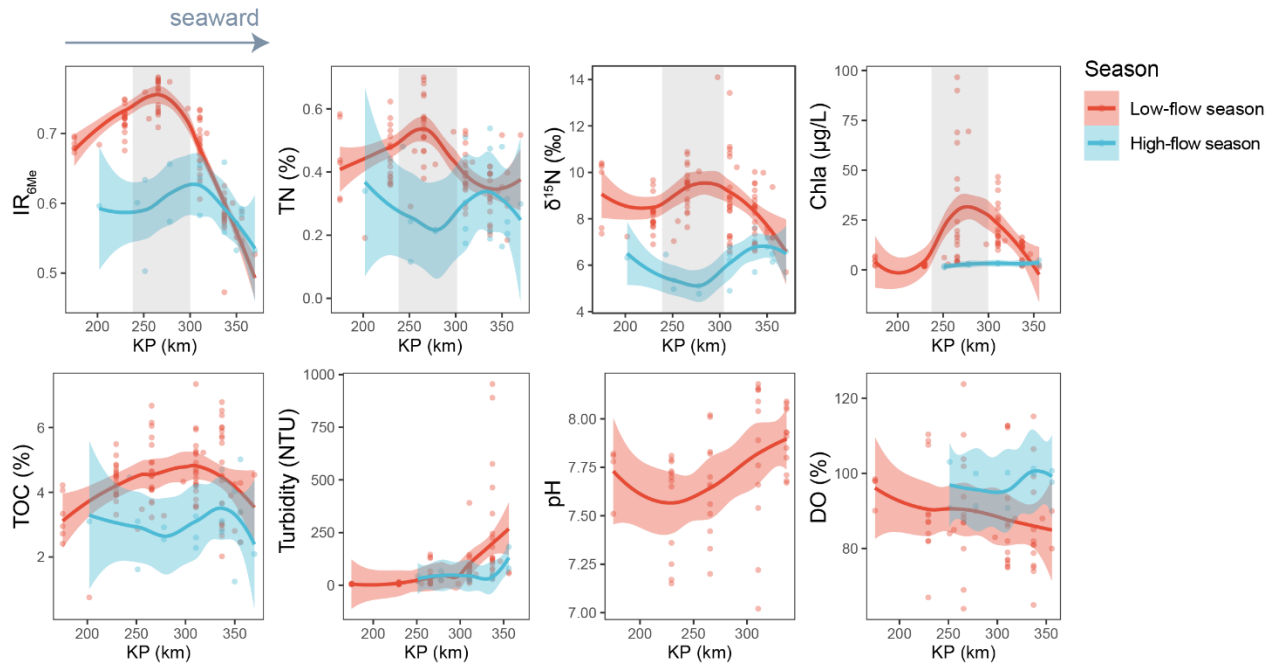




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 948 **Fig. 6.** Relative abundance of distinct brGMGTs from soils (surficial soils and mudflat  
 949 soils/sediments,  $n=51$ ), river ( $n=9$ ), upstream estuary ( $n=56$ ) and downstream estuary ( $n=121$ )  
 950 across the Seine River basin. 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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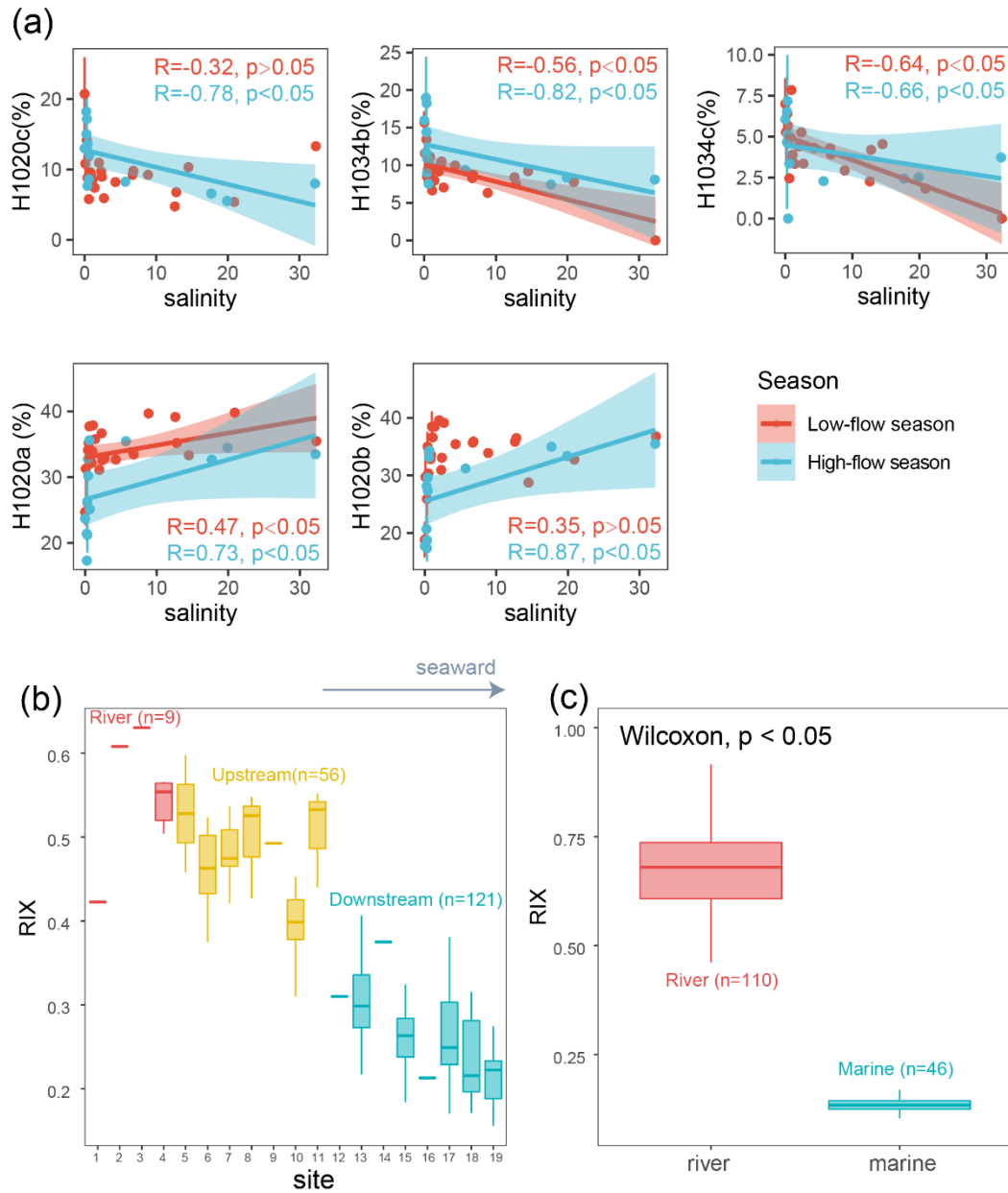


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958 **Fig. 7.** Spatio-temporal variations of  $IR_{6Me}$  and several environmental factors, including TN (%),  
 959  $\delta^{15}N$  (‰), Chla ( $\mu\text{g/L}$ ), TOC (%), turbidity (NTU), pH, and dissolved oxygen saturation (DO, %).  
 960 The 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  
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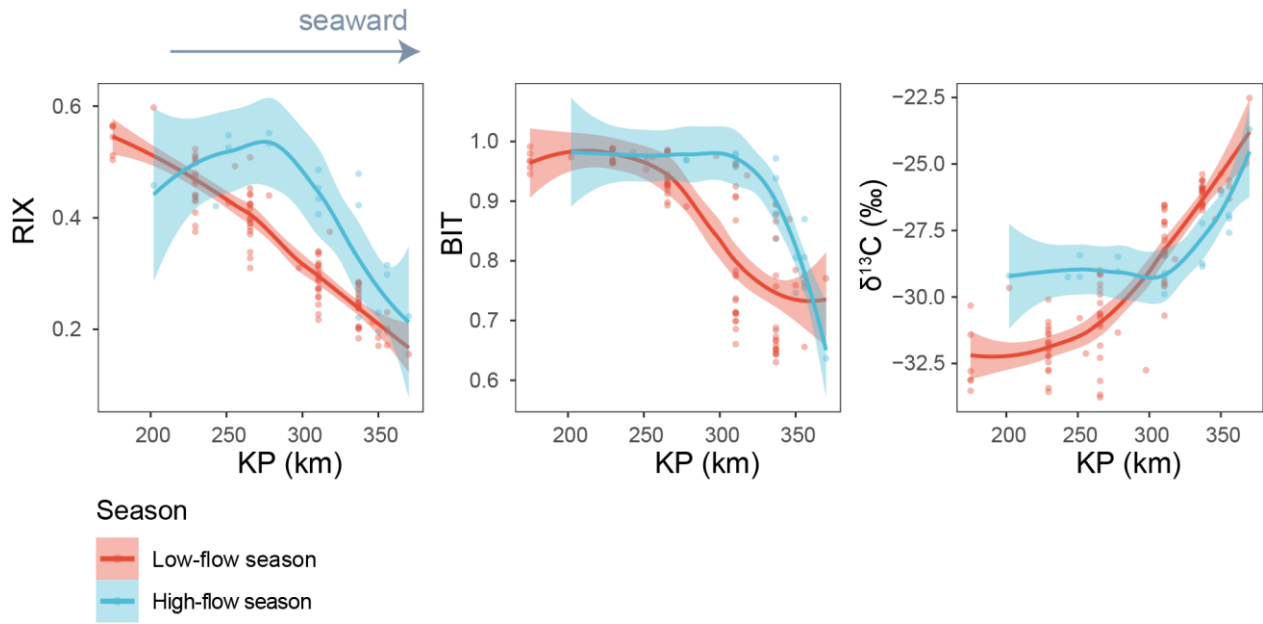
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967 **Fig. 8.** (a) Salinity plotted versus relative abundance of brGMGTs. Shaded area represent 95%  
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 969 Dataset is composed of SPM. (b) Distribution of RIX across the Seine River basin. Boxes are color-  
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 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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 979 represents the distance in kilometers from the city of Paris (KP 0). Dataset is composed of SPM.

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