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## Multidisciplinary study to monitor consequences of pollution on intertidal benthic ecosystems (Hauts de France, English Channel, France): Comparison with natural areas

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1 **Multidisciplinary study to monitor consequences of pollution on intertidal benthic**  
2 **ecosystems (Hauts de France, English Channel, France): comparison with natural areas**

3  
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21

22 **Abstract**

23 The intertidal areas of the Hauts-de-France (English Channel – France) stand out for the  
24 occurrence of fragile ecosystems that are exposed to natural and human-induced stress. Over the  
25 last two centuries, the northern part of this region has experienced a strong human pressure, with  
26 the settlement of numerous activities (i.e., metallurgic factories, harbors, embankments). On the  
27 contrary, the southern part includes mostly natural areas. The whole region is influenced by a  
28 macrotidal regime. A multidisciplinary approach based on sedimentological (grain-size),  
29 geochemical (trace metals, biomarkers) and biological (foraminifera) proxies was used to unravel  
30 the contrasting environmental conditions in the Hauts-de-France. Three foraminiferal-types  
31 communities, which reflect different ecological characteristics at regional scale, were identified:  
32 1) estuarine macrotidal assemblages (*Haynesina germanica* associated to Elphidiidae) in low  
33 impacted estuaries; 2) industrial-perturbed assemblages (*H. germanica* and *Criboelphidium*  
34 *excavatum*) in harbor areas; and 3) infaunal-dominant assemblages (*Bolivina variabilis* and *B.*  
35 *pseudoplicata*) in embankment areas. The outcomes of this study show that a multiproxy  
36 procedure needs to be adopted for properly characterizing intertidal ecosystems, where human  
37 impacts and natural stresses overlap and are hard to disentangle.

38

39 **Key words**

40 Benthic foraminifera; benthic ecology; estuaries; intertidal; Hauts-de-France; multiproxy  
41 approach; biomarkers

42

## 43 **1 Introduction**

44 Intertidal areas are fragile environments that are naturally stressed by high spatial and temporal  
45 variability of physico-chemical parameters (Elliott and Quintino, 2007). In conjunction with it,  
46 anthropogenic activities (e.g., sewage, dredging, urbanization, aquaculture) strongly contribute to  
47 the degradation of these critical ecotones. The distribution of benthic organisms (e.g.,  
48 foraminifera, nematodes, macro-invertebrates, macro-algae) in intertidal areas is the result of a  
49 complex interplay among these natural and human-induced features (Armynot du Châtelet et al.,  
50 2016; Müller-Navarra et al., 2019; Rolet et al., 2015; Semprucci et al., 2016). Hence,  
51 disentangling human impacts from natural stresses can be difficult and remains controversial in  
52 these areas (Dauvin, 2007; Elliott and Quintino, 2007). Agricultural and urban run-off are among  
53 the most important sources of contaminants to intertidal areas. Run-off includes organic  
54 compounds like pesticides, pharmaceutical products and fossil fuel-by-products (aromatic  
55 polycyclic hydrocarbons and fecal materials), and inorganic ones such as nutrients and trace  
56 elements including toxic ones (i.e., Pb, Hg, Cd). Some of these contaminants can be accumulated  
57 in coastal soft sediments through long-time periods, affecting benthic and epibenthic fauna and  
58 inducing major ecological damages (Frontalini and Coccioni, 2011; Levin et al., 2001). Hence,  
59 the use of multidisciplinary approaches combining sedimentological, geochemical and biological  
60 proxies is recommended for unravelling environmental features in intertidal systems. Such an  
61 approach will allow for a better interpretation of the modern environments (Francescangeli et al.,  
62 2018; Grauel et al., 2013; Leorri et al., 2008; Martins et al., 2016).

63 The use of biological indicators to monitor the environmental pollution has been enforced  
64 worldwide by marine legislations and, hence, has kindled the interest of numerous researchers  
65 suggesting different biological quality elements to assess the health of marine systems such as

66 macroalgae, macrofauna and meiofauna (e.g. Ar Gall et al., 2016; Borja et al., 2000; Bouchet et  
67 al., 2012; Rosenberg et al., 2004; Semprucci et al., 2016). The benthic macrofauna is currently  
68 the most widely used biological quality element in monitoring the ecological quality status of  
69 marine environments (Birk et al., 2012; Blanchet et al., 2008; Bouchet and Sauriau, 2008;  
70 Dauvin, 2018; Pinto et al., 2009). Within benthic meiofauna, foraminifera (protozoans) represent  
71 a promising tool for detecting the environmental quality of water bodies and for assessing its  
72 deviation from natural reference conditions (Alve et al., 2019; Dimiza et al., 2016; El Kateb et  
73 al., 2020; Hess et al., 2020; Jorissen et al., 2018). Their sensibility and rapid response to  
74 environmental stress, as well as specific ecological requirements of benthic foraminifera, make  
75 them an efficient environmental proxy (Schönfeld et al., 2012) in intertidal areas (e.g. Alve,  
76 1995; Bouchet et al., 2007, 2018; Debenay, 2001; Martins et al., 2015). These studies confirmed  
77 the reliability of benthic foraminifera in biomonitoring programs. However, there is still a lack of  
78 knowledge on foraminiferal ecology and a need of further quantitative studies to improve the  
79 application of foraminifera in environmental biomonitoring, particularly in coastal and intertidal  
80 areas.

81 The northern part of the intertidal areas of the Hauts-de-France (northern France) has  
82 experienced strong human-induced modifications over the last two centuries. Since the second  
83 industrial revolution, numerous polluting activities (i.e., metallurgic, textile and chemical  
84 factories, harbors of Boulogne-sur-Mer, Calais and Dunkirk, nuclear power plant of Gravelines)  
85 have developed, leading to a large degradation of environmental quality (Amara et al., 2007;  
86 Francescangeli et al., 2016; Rolet et al., 2015). Conversely, the coast of Picardy, in the southern  
87 part of the Hauts-de-France, includes mostly natural areas that are protected by natural parks and  
88 less impacted by pollution (Henry et al., 2004). A general littoral drift that drives the coastal

89 pollutants northward from the English Channel to the North Sea enhances this North/South  
90 distinction (Anthony, 2000). The differential degree of disturbance makes the coastlines of the  
91 region an excellent laboratory for observing the response of benthic foraminifera to  
92 environmental variations both in the natural and human-affected zones. So far, three studies have  
93 focused on the response of living benthic foraminifera to environmental conditions in the Hauts-  
94 de-France (Armynot du Châtelet et al 2009, 2011, Francescangeli et al. 2017). These studies  
95 were, however, very local (Boulogne-sur-Mer Harbor and Canche Estuary), and were not  
96 sufficient to give a comprehensive overview on the distribution of benthic foraminifera and their  
97 response to environmental conditions. Two other studies provided a wider view on the  
98 foraminiferal communities in the intertidal areas of the northern France and the English Channel  
99 (Armynot du Châtelet et al., 2018a; Armynot du Châtelet et al., 2018b).

100 The objective of the present work is to document the foraminiferal assemblages in the intertidal  
101 areas of the region of Hauts-de-France. This will allow us to assess how the different parts of the  
102 coastline are affected by pollution by using a multiproxy approach based on the ecological  
103 response of benthic foraminifera, and sedimentological and geochemical parameters. To further  
104 assess the quality of the organic matter, different biomarkers are used: i) the aliphatic fractions,  
105 to understand the origin of the organic matter (i.e. Peters and Moldowan, 1993; Volkman et al.,  
106 1994), and ii) the aromatic fractions and steroid compounds, to highlight anthropogenic  
107 contaminations (i.e. Nacci et al., 2002; Standley et al., 2000; Tuvikene, 1995). This study also  
108 aims at contributing on the research of benthic foraminifera as bioindicators of environmental  
109 quality in intertidal and coastal areas.

## 110 **2 Materials and methods**

### 111 **2.1 Study area**

112 The coastal areas of Hauts-de-France extend for about 240 km along the eastern part of the  
113 English Channel (from the Belgium border to the Bay of Somme down to the Calais-Dover  
114 Strait) (Figure 1a). The continental shelf corresponding to the current English Channel  
115 underwent important erosion leading to the accumulation of fluvial deposits. In these relatively  
116 sediment-starved conditions, recent sedimentation occurs offshore as tidal sand banks and, when  
117 wind deposits develop, as coastal dunes facing macrotidal beaches (Battiau-Queney et al., 2001).  
118 The net transport drives sediment from the English Channel to the North Sea, being influenced  
119 by a strong northward littoral drift (Anthony, 2000). Along this overall movement, estuarine  
120 areas act as traps for coarse and fine sediments and represent places of maximum sedimentation.  
121 The region is characterized by a semidiurnal (Chabert d'Hieres and Le Provost, 1978) macro-to  
122 hyper-tidal regime (following the classification of McLusky and Elliott, 2004). To the south of  
123 the study area, the tidal range exceeds 10 m during the highest astronomical tides. The tidal  
124 range decreases northward to the Belgian border, where it reaches about 4 m. The combination  
125 of dominant macrotidal conditions with the relatively limited action of wave dynamics leads us  
126 to define this region as being a tide-dominated system (for more details on regional sedimentary  
127 processes look at Margotta, 2014). Elongated northward drifting sand- or gravel-spit tend to  
128 close the estuaries, leading to the formation of extended tidal flats in their inner part (Dalrymple  
129 et al., 1992).

130 The area is drained by five main rivers (Figure 1A). The northernmost one is the Aa River that  
131 opens into the English Channel in Grand-Fort-Philippe – an embankment area where the natural

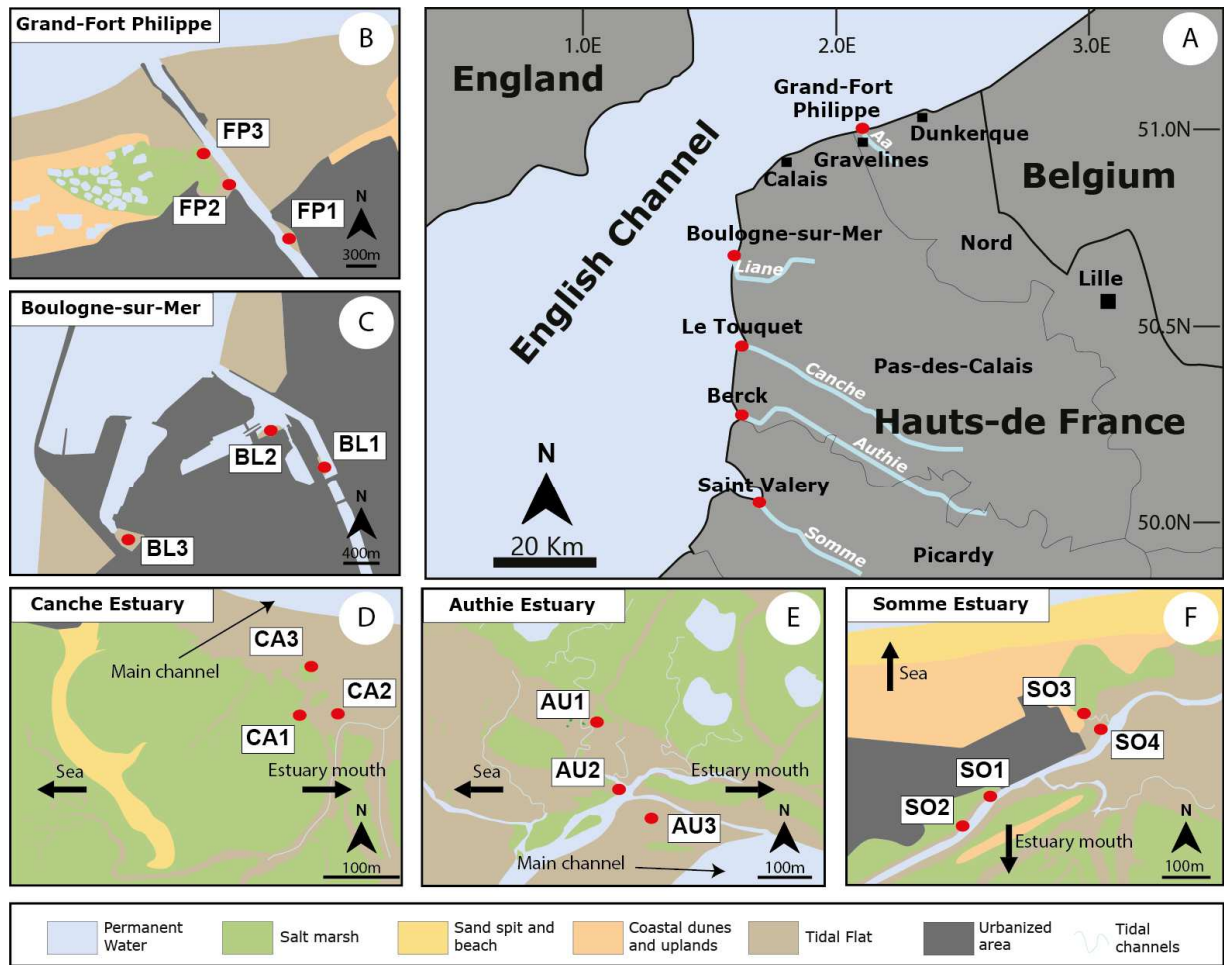
132 flowing of the river channel was modified by human action during the 17<sup>th</sup> centuries. The estuary  
133 presently consists of a four-kilometre-long artificial channel. The Liane River has been heavily  
134 modified since the 18<sup>th</sup> century with the building of Boulogne-sur-Mer harbor, the biggest of the  
135 area (Armynot du Châtelet et al., 2017). The Canche, Authie and Somme rivers open onto the  
136 English Channel at Le Touquet-Paris-Plage, Berck-sur-Mer and Saint-Valéry and Le Crotoy,  
137 respectively. These estuaries were weakly modified compared to the two former ones, with only  
138 littoral defences.

## 139 2.2 Sampling strategy

140 Sixteen sampling stations were selected from five estuaries along the intertidal zones of the  
141 Hauts-de-France region. These stations are from north to south (Figure 1B-F): the Aa Estuary, in  
142 the embanked area of Grand-Fort-Philippe (FP1, FP2, FP3), the Liane Estuary in the harbor of  
143 Boulogne-sur-Mer (BL1, BL2, BL3) then the Canche (CA1, CA2, CA3), Authie (AU1, AU2,  
144 AU3) and Somme Estuaries (SO1, SO2, SO3, SO4). Information on regional pollution levels  
145 were retrieved from Billon (2001), Berthet et al. (2003), Henry et al. (2004), Amara et al. (2007)  
146 and Kerambrun et al. (2012). Overall, analyses of the sediment indicate that Bay of Somme is the  
147 least contaminated in the region, while the harbour of Boulogne-sur-Mer is the most impacted in  
148 terms of trace metals. At each station, four replicated sediment samples were collected (10 dm<sup>3</sup>,  
149 the uppermost 1 cm over a surface of 1 m<sup>2</sup>). One replicate was used to measure sediment  
150 properties (grain-size, calcium carbonate, organic matter, major and trace elements). The other  
151 three replicates were used for the benthic foraminiferal analyses. The 48 foraminiferal replicated  
152 samples were stored in transparent graduated containers and stained with buffered Rose Bengal  
153 dye (2 g of Rose Bengal in 1000 ml of ethyl alcohol) to distinguish living (stained) from dead  
154 (unstained) tests at time of collection (Lutze and Altenbach, 1991; Walton, 1952). Six additional



155 samples were collected for biomarkers analysis in glass vials to prevent any contamination (FP1,  
156 BL3, CA3, AU3, SO1 and SO3). Sampling station positions and elevations were measured using  
157 a Trimble GeoXT dGPS allowing a precision higher than 10-15 cm (in elevation) (Table 1). We  
158 used the WGS 84 coordinate system to position the sampling stations. Elevations were  
159 referenced to the mean sea level (MSL) calculated after elevation reference system IGN69.



160

161 Figure 1. A) Hauts-de-France region with the position of the 16 sampling stations within the 5  
 162 selected sampling areas: B) Grand-Fort-Philippe - Aa river (FP), C) Boulogne-sur-Mer harbor -  
 163 Liane river (BL), D) Le Touquet - Canche Estuary (CA), E) Berck - Authie Estuary (AU), F)  
 164 Saint Valery-sur-Somme - Somme Estuary (SO).

Chart Datum IGN69	MLWST	MLWNT	MHWNT	MHWST	Stations	Elevation	East-X	North-Y	Descriptions
<b>Grand-Fort Philippe</b>	-2.10	-3.1	2.5	3.6	FP1	-0.51	2.110	51.003	Tidal flat
					FP2	-1.1	2.099	51.008	Tidal flat
					Dunkirk	FP3	1.74	2.103	51.006
<b>Boulogne-sur-mer</b>	-3.3	-1.8	2.8	4.5	BL1	-0.51	1.599	50.724	Harbor
					BL2	1.15	1.592	50.727	Harbor
					Boulogne-sur-Mer	BL3	-1.15	1.574	50.718
<b>Canche Estuary</b>	-3.2	-1.5	3.4	5.5	CA1	3.09	1.599	50.534	Upper tidal flat
					CA2	2.73	1.599	50.534	Upper tidal flat
					Boulogne-sur-Mer	CA3	1.87	1.599	50.535
<b>Authie Estuary</b>	-3.2	-1.5	3.4	5.2	AU1	3.49	1.595	50.373	Upper tidal flat
					AU2	2.85	1.596	50.372	Tidal flat
					Boulogne-sur-Mer	AU3	3.17	1.597	50.372
<b>Somme Estuary</b>	-3.3	-1.8	3.8	5.8	SO1	1.96	1.564	50.214	Tidal flat
					SO2	1.39	1.563	50.213	Tidal flat
					SO3	3.25	1.567	50.216	Upper tidal flat
					Boulogne-sur-Mer	SO4	1.88	1.567	50.215

165

166 Table 1. Tidal elevation (m) for Grand-Fort-Philippe (FP), Boulogne-sur-Mer (BL), Canche  
167 Estuary (CA), Authie Estuary (AU) and Somme Estuary (SO) (associated reference harbor tidal  
168 gauge: Dunkirk harbor for FP and Boulogne-sur-Mer for BL, CA, AU and SO), relative to  
169 French geodetic vertical datum IGN69. Mean low water spring tide (MLWST), mean low water  
170 neap tide (MLWNT), mean high water neap tide (MHWNT), and mean high water spring tide  
171 (MHWST) are provided. Elevation (m) on mean sea level, geographic coordinates (WGS 84),  
172 description of the 16 sampling stations are also provided.

### 173 2.3 Sedimentological analyses

174 Sediment grain-size analyses were performed using the principle of diffraction and diffusion of a  
175 monochromatic laser beam on suspended particles (Malvern Mastersizer 2000, red He-Ne laser).  
176 The method is based on near-forward scattering of a laser beam by particles in suspension  
177 (Trentesaux et al., 2001). Measurements can range from 0.02 to 2,000  $\mu\text{m}$ , with an obscuration  
178 ranging between 10 and 20%. Four grain-size fractions were considered: clay ( $<4 \mu\text{m}$ ), fine silt  
179 ( $4\text{-}10 \mu\text{m}$ ), sortable silt ( $10\text{-}63 \mu\text{m}$ ), and sand ( $63\text{-}2000 \mu\text{m}$ ). Silt fraction and motility are  
180 discussed in McCave et al. (1995).

181 The C, H, N and S for Total Organic Carbon (TOC) estimation was determined using a Flash  
182 EA 1112 Elemental Analyzer (Thermo) equipped with an auto-sampler. The analysis was  
183 performed on 1.5 to 2 mg of dried and crushed sediment, added to approximately 5 mg of  
184 vanadium pentoxide, used as a combustion catalyst. 2,5-Bis (5-tert-butyl-benzoxazol-2-yl)  
185 thiophene (BBOT) was used as standard. The TOC was determined by subtracting carbonate  
186 carbon from total carbon concentration. Calcium carbonate content was determined using a  
187 Bernard calcimeter. Triplicated measurements were carried out for each sample using 0.5 g of  
188 finely crushed dry sediment (Armynot du Châtelet et al., 2009). The C/N ratios were used to  
189 distinguish the origin of sedimentary organic matter (Meyers, 1994). To evaluate the organic  
190 load, the Organic Sediment Index (OSI) (Ballinger and McKee, 1971) was calculated as follows  
191 (Eq. 1):

$$192 \quad OSI = [TOC \text{ wt}\%] \times [ON \text{ wt}\%] \quad (\text{Eq. 1})$$

193 where [OC] is the total organic carbon and [ON] is the total organic nitrogen multiplied by 0.95.  
194 This index was developed for the classification of sedimentary deposits affected or not by  
195 organic pollution in rivers, lakes and estuaries (Rostan et al., 1987).

## 196 2.4 Trace metal analysis

### 197 2.4.1 Total concentration

198 Total concentrations of Al, Co, Cr, Cu, Fe, Mn, V and Zn were analyzed at each sampling  
199 station. After drying the sediment particles at room temperature, the raw samples were gently  
200 crushed to limit the destruction of soft particles. Then 200 mg of the fine fraction recovered after  
201 grinding at 63  $\mu\text{m}$  were attacked first with a mixture of 5 mL of Suprapur<sup>®</sup> nitric acid (65%) and  
202 10 mL of a concentrated HF boiling solution over 48 h. After the acids' evaporation, 10 mL of a

203 freshly prepared HNO<sub>3</sub>/HCl mixture (1/2 v:v) was added in order to eliminate the remaining  
204 solid grains. The recovered solutions were subsequently diluted in a known volume of ultrapure  
205 water and analyzed using ICP-AES (inductively coupled plasma – atomic emission  
206 spectroscopy; Varian Vista-PRO, axial view) and ICP-MS (inductively coupled plasma – mass  
207 spectroscopy; Thermo Elemental X Series). This attack procedure was validated and the  
208 accuracy of the analytical procedure was checked by means of the following sediment standard  
209 reference materials (Canadian International Standards): HISS-1, MESS-3 and PACS-2.

#### 210 2.4.2 *Enrichment factor (EF) and pollution load index (PLI)*

211 Commonly, normalization of the metals concentration to a conservative element such as Al is  
212 used for calculating, as an index, the enrichment factor (EF, Eq. 2). This index allow a more  
213 accurate evaluation of anthropogenic influences on the sediments (Francescangeli et al., 2016;  
214 Hasan et al., 2013; Woitke et al., 2003). The EF was calculated for each element, as follows (Eq.  
215 2):

$$216 \quad EF = ([X_i]/[Y_i])/([X_0]/[Y_0]) \quad (\text{Eq. 2})$$

217 Where [X<sub>i</sub>] and [Y<sub>i</sub>] are the concentrations of the target element and a conservation element,  
218 respectively in the sample *i*; [X<sub>0</sub>] and [Y<sub>0</sub>] are the local geochemical background concentrations  
219 the target element and a conservation element, respectively. The EF is normalized against  
220 aluminum (conservation element) following for instance Delgado et al. (2010).

221 Fine particles (silts and clays), can partly overcome the bias bound to the nature of the sediment.  
222 The following EF categories, estimated by previous studies (Birth, 2003), are used to evaluate  
223 the degrees of pollution: EF<3: no relevant contamination or minor contamination; 3<EF<5:

224 moderate contamination;  $5 < EF < 10$  severe contamination;  $10 < EF < 25$  high contamination;  $EF > 25$   
225 extremely high contamination.

226 The Tomlinson's Pollution Load Index (PLI) (Tomlinson et al., 1980) was calculated to highlight  
227 anthropogenic contributions of metals in the study area (Eq. 3). The PLI is obtained as a  
228 concentration factor (Eq. 4) of each metal, whose higher concentrations may be toxic (Co, Cr,  
229 Cu, Mn and Zn), with respect to the background values. The fingerprint of the global enrichment  
230 of chemical elements has been calculated as follows:

$$231 \quad PLI = \sqrt[n]{(CF_X \times \dots \times CF_n)} \quad (\text{Eq. 3})$$

$$232 \quad CF_X = [X_i] / [X_0] \quad (\text{Eq. 4})$$

233 Where  $[X_i]$  and  $[X_0]$  are the concentrations of the element  $X$  and relative geochemical  
234 background, respectively. As local background values were not available for all the sampling  
235 stations, the calculation of EFs and PLI were based on reference regional values from  
236 Sterckeman et al. (2002). These represent the lowest average composition of soils in marine  
237 flood plains from the Hauts-de-France region. The results were then compared with local  
238 references (Billon et al., 2002; Francescangeli et al., 2016; Francescangeli et al., 2018) and  
239 reported in Supplementary materials.

## 240 **2.5 Biomarkers**

### 241 *2.5.1 Samples treatment*

242 Between 5 and 10 g of dry sediments were extracted using an azeotrope mixture of  
243 dichloromethane (DCM) and methanol (MeOH) 2:1 (v:v) by means of a Soxhlet extractor for  
244 48h. Subsequent separation of the total lipids extracts (TLE's) into apolar, oxygenated and polar  
245 fractions was achieved via silica column chromatography using solvent mixtures of 9:1 (v:v)

246 hexane/DCM, 8:2 (v:v) DCM/ethyl acetate, and 1:1 (v:v) DCM/MeOH, respectively. All the  
247 resulting fractions were dried using a rotary evaporator. The oxygenated and polar fraction  
248 containing sterols and acids, respectively, were derivatized using BSTFA-TMCS 99:1, Supelco  
249 (N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane) to improve the  
250 chromatographic resolution. Briefly, 200  $\mu$ L of BSTFA-TMCS 99:1 were added to the dry  
251 extracts and ultrasonicated for 10 min. The remaining solution was dried under N<sub>2</sub> atmosphere.  
252 All alcohols and acids were later identified and semi quantified as trimethylsilyl-derivatives.

### 253 *2.5.2 Gas chromatography - mass spectrometry analysis*

254 One mL of extract was injected into a gas chromatograph (Perkin Elmer 680) coupled with a  
255 mass spectrometer (Perkin Elmer 600C). Chromatographic conditions were as follow: inlet  
256 heated at 250°C; DB5-MS-UI column initially at 40°C for 1 min, then heated to 320°C and  
257 maintained 10 min at 320°C; helium column flow of 1 mL min<sup>-1</sup>, split ratio of 10. Mass  
258 spectrometer conditions were as follow: scan time 0.2 s, inter-delay scan 0.1 s, and ionization  
259 energy 70 eV. Identification of compounds was based on a comparison with the NIST mass  
260 spectra database and/or on the comparison of retention times of standards. For the purpose of  
261 product semi-quantification, standard solutions of androstanol, anthracene D<sub>10</sub> and nonadecanone  
262 C<sub>29</sub> were used.

263 In this study, different fractions were used to detect: a) origin of the organic matter (aliphatic  
264 fractions) and b) anthropogenic contaminations (aromatic fractions and steroid compounds).  
265 Details about biomarker approach were reported as Supplementary materials (Biomarkers).

### 266 **2.6 Foraminiferal analysis**

267 In the laboratory, wet samples were gently washed with tap water, through 315 and 63  $\mu\text{m}$  mesh  
268 sieves, and dried at 40°C. Foraminiferal tests of the intermediate fraction were concentrated by  
269 flotation on trichloroethylene (Horton and Murray, 2006; Semensatto and Dias-Brito, 2007).  
270 Although heavy liquid separation should be avoided, it may be used in environments with low  
271 foraminiferal density, due to a high sedimentation rate as in estuarine areas resulting in a strong  
272 dilution of foraminifera within mineral grains. Only specimens containing dense, brightly red-  
273 stained protoplasm were considered as living (Alve and Murray, 1999; de Stigter et al., 1999).  
274 All living specimens were counted and identified following the generic classification Loeblich  
275 (1987), and the specific ones of Debenay (2012) and Debenay et al. (2001). The observations  
276 were carried out under a binocular microscope, model Olympus SZX16. The foraminiferal  
277 density (FD; absolute abundance; specimens/cm<sup>3</sup>) and the relative abundances of living taxa  
278 were calculated for each station. For diversity, the  $\exp(H'_{bc})$  (Chao and Shen, 2003) index was  
279 considered in the present study (for details Bouchet et al., 2012).

## 280 2.7 Data analysis

281 After testing the normality, a Spearman's Rho correlation was used to identify significant  
282 relationships between the environmental parameters. Spearman's correlation coefficient is a  
283 nonparametric method to measure correlation using rank values. It measures the monotonic  
284 relationship of the variables rather than the linear association of the Pearson matrix (Daniel,  
285 1990). For non-linear data, Spearman's correlation coefficient is more reliable compared to  
286 Pearson's  $r$ . A Canonical Correspondent Analysis (CCA) was used to disentangle the  
287 relationships between environmental parameters and benthic foraminifera. Four synthetic  
288 environmental parameters were considered to model and constrain the variance: substrate (Sand),  
289 Elevation, metal contaminations (PLI), and organic load (OSI) in the CCA. Only species that



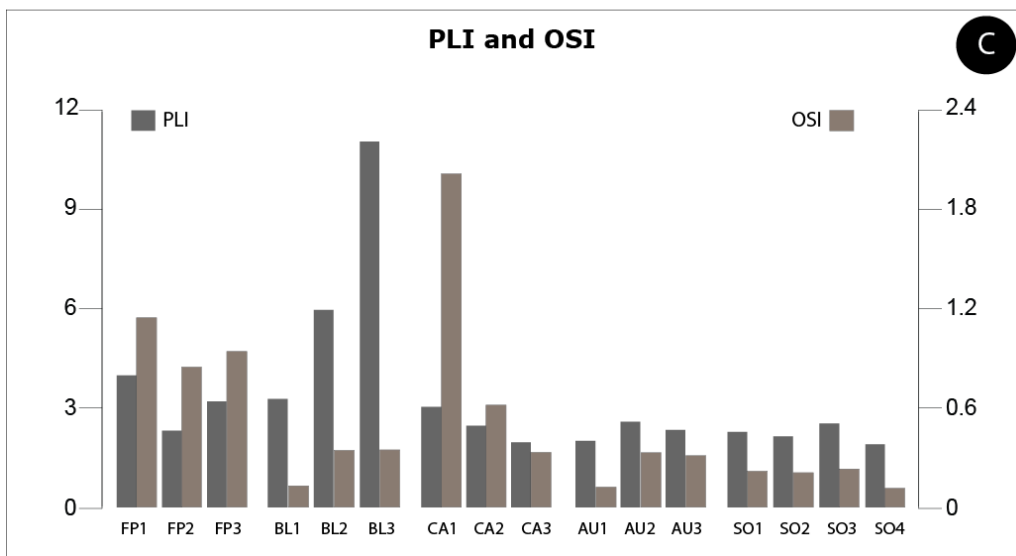
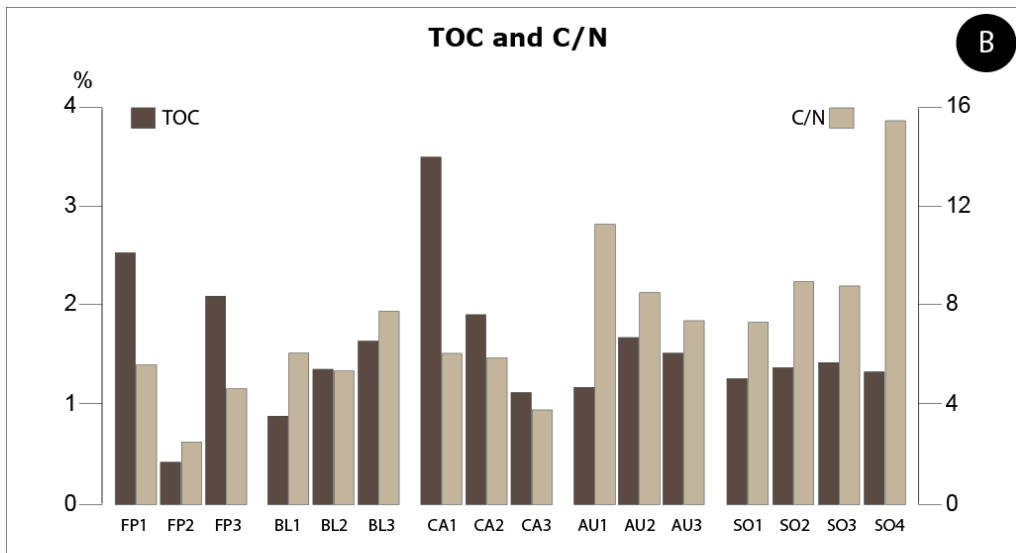
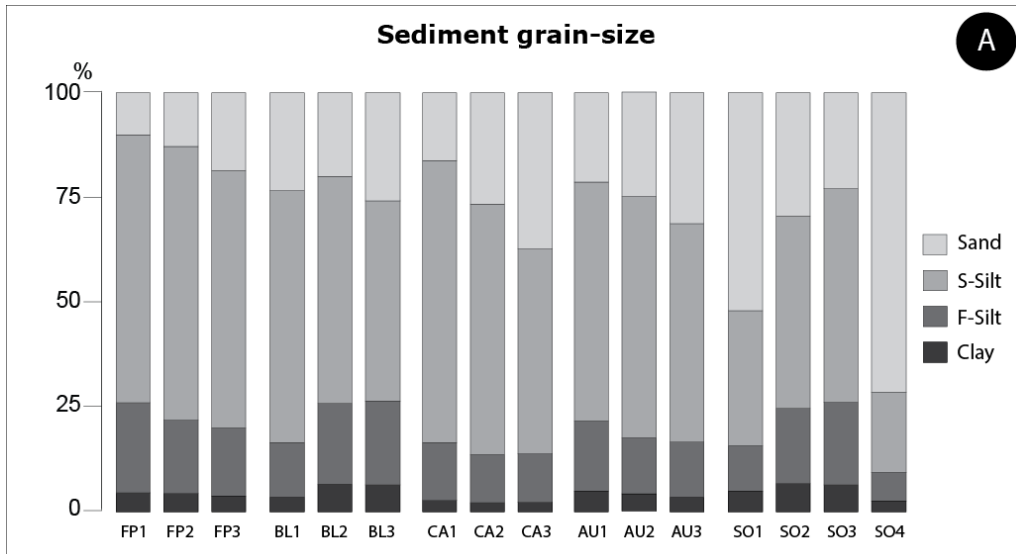
290 showed a relative abundance >2% in at least one sample were selected as dependent variables for  
291 analysis. The selection of variables was done in order to have the best and the clear model.  
292 Statistical significance ( $p$ -value<0.05) was tested using a chi-squared test (number of  
293 permutations: 999).

294 The R software was used for all the calculations by using the following packages: base  
295 (descriptive statistics) (R-Core-Team, 2014), entropy (diversity) (Hausser and Strimmer, 2014),  
296 corrplot (correlation plot) (Wei and Simko, 2016), factoMineR (CA) (Husson et al., 2017), vegan  
297 (CCA) (Oksanen et al., 2016).

## 298 **3 Results**

### 299 **3.1 Sediment characteristics**

300 Silt is the most abundant sediment fraction in all the sampling sites (78%, on average) (Figure  
301 2a). In particular, sortable silt (10-63  $\mu$ m) contributes to 19%-67% and fine silt (4-10  $\mu$ m) to 7%-  
302 21% of the sediment. Sand varying between 10 and 72% is the second most abundant fraction  
303 (28%, on average) (Figure 2a). Clay (4.4%, on average) ranges from 2.2 to 6.8% (Figure 2a).  
304 The highest contents of mud (silt + clay) are found in Grand-Fort-Philippe (86%, on average),  
305 while the sampling stations in the Somme Estuary have the highest contents of sand (44%, on  
306 average). The TOC ranges between 0.44 and 3.7% (Figure 2b). The highest values are recorded  
307 in the Canche Estuary and Grand-Fort-Philippe (2.3% and 1.8%, on average respectively), while  
308 the lowest is in the port of Boulogne-sur-Mer (1.4% on average). The C/N ranges from 2.5 to 15  
309 (7.2, on average) (Figure 2b). The highest values are documented in the Somme Estuary. The  
310 OSI varies between 0.12 and 2.1 (Figure 2c). The highest values are found in all the station in  
311 Grand-Fort Philippe and in CA1.



313 Figure 2. Distribution of environmental parameters in the 16 sampling stations within the 5  
314 sampling areas: A) Sediment grain-size: sand, S-Silt (sortable silt), F-Silt (fine silt) and clay; B)  
315 TOC and C/N in and C) Pollution Load Index (PLI) and Organic Sediment Index (OSI)

### 316 3.2 Trace metals

317 The concentrations of analyzed elements (Al, Co, Cr, Cu, Fe, Mn, V, and Zn) are presented in  
318 supplementary materials, as are the background references used for the calculation of the  
319 enrichment factors (Environmental parameters-EF). The highest enrichment factors (EFs) occur  
320 in the harbor of Boulogne-sur-Mer, with moderate from severe contaminations for Co, Cr and Cu  
321 ( $3 < EF_s < 10$ ), high contamination for Zn ( $10 < EF_{Zn} < 25$ ), and extremely high contamination Mn  
322 ( $EF_{Mn} > 25$ ), specifically in BL2 and BL3 (Table 2). In the Authie Estuary a moderate  
323 contamination is recorded in the AU1 station only with respect to Cr ( $EF_{Cr}=3$ ). No significant  
324 contaminations are recorded in Grand-Fort-Philippe and the Canche and Somme Estuaries. The  
325 highest values of PLI (Figure 2c) are found in Boulogne-sur-Mer, confirming the metals'  
326 enrichments in the sediments of this area, specifically in BL2 and BL3.  $PLI_{local}$  (based on local  
327 geochemical background) shows the same trend but the values have, overall, less intensity  
328 (Supplementary materials\_EF and PLI).

Stations	EF Co	EF Cr	EF Cu	EF Fe	EF Mn	EF V	EF Zn
AU1	1.2	<b>3.2</b>	1.0	1.3	1.8	1.3	1.4
AU2	1.4	2.4	1.1	1.3	1.9	1.4	1.6
AU3	1.3	2.2	1.1	1.3	2.0	1.4	1.6
BL1	1.4	<b>6.6</b>	1.7	1.3	1.6	1.4	2.1
BL2	1.6	2.2	<b>5.7</b>	1.6	1.7	<b>1.7</b>	<b>3.5</b>
BL3	<b>3.5</b>	1.9	2.4	1.5	<b>30.7</b>	<b>1.7</b>	<b>12.6</b>
CA1	1.7	2.0	1.2	1.4	1.8	1.7	2.1
CA2	1.3	2.5	1.1	1.4	1.6	1.6	1.9
CA3	1.2	2.0	0.9	1.3	1.8	1.3	1.5
FP1	1.4	1.9	1.4	1.4	1.5	1.9	2.1
FP2	1.3	2.5	1.1	1.3	1.5	1.6	1.5
FP3	1.3	2.0	1.1	1.4	1.6	1.8	1.7
SO1	1.6	2.8	1.3	1.7	2.2	1.7	1.8
SO2	1.6	2.5	1.3	1.6	2.0	1.6	1.9
SO3	1.5	2.3	1.4	1.6	2.1	1.7	1.8
SO4	1.7	2.2	1.3	1.9	2.4	1.8	2.6

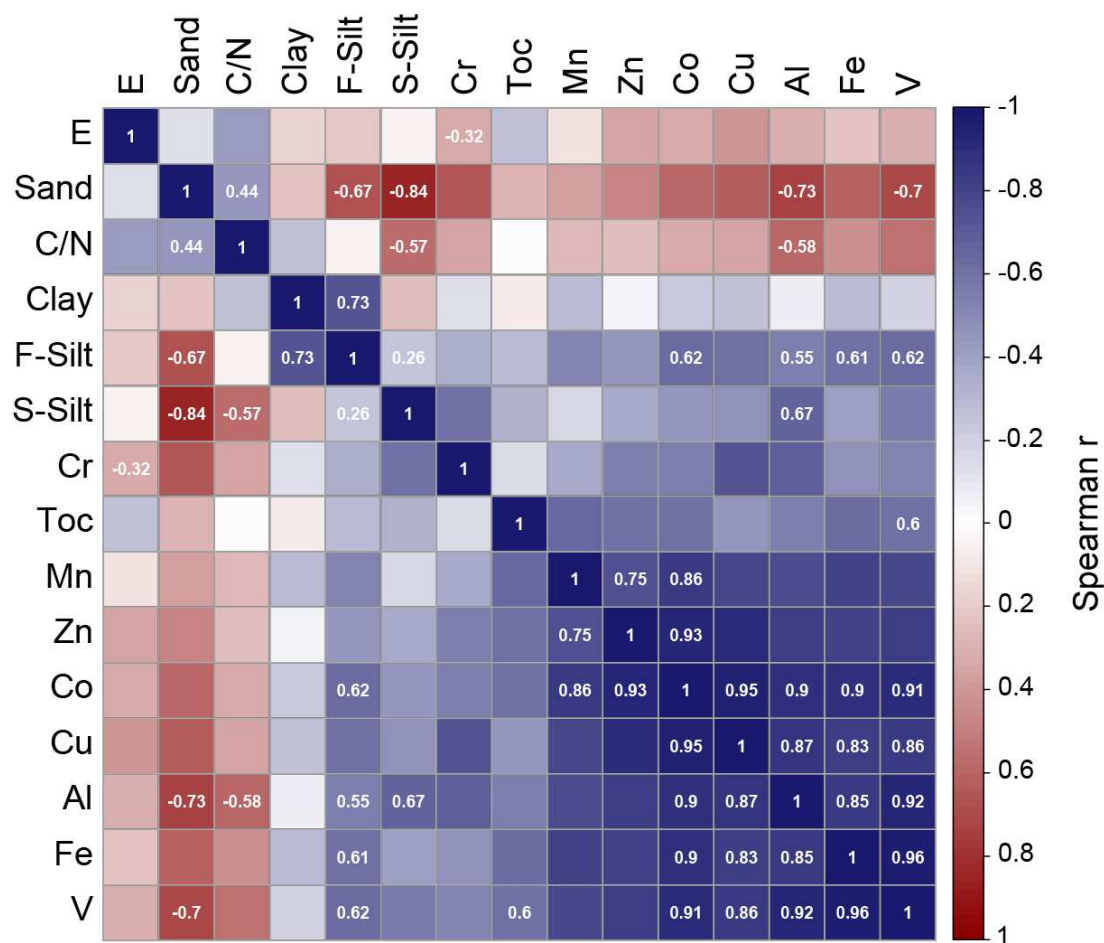
329

330 Table 2. Enrichment factors (EF) for the measured geochemical elements. In bold, values higher  
331 than 3.

332

333 In the Spearman's matrix, the coefficients display strong significant correlations mainly among  
334 metals ( $R < -0.7$  and  $R > 0.7$ ;  $p\text{-value} < 0.05$ ) (Figure 3). Al shows significant positive correlations  
335 with Co, Cu, Fe and V (correlations with Mn and Zn are not significant) and negative with sand.

336 Silt (fine and sortable silt) is the only parameter to show strong significant correlations with  
337 metals. The other parameters show either weak or not significant correlations.



338

339 Figure 3. Spearman's correlation matrix showing the relationships between abiotic parameters  
 340 (E=Elevation; F-Silt=Fine Silt; S-Silt=Sortable Silt). R-values (positive=blue and negative=red)  
 341 are reported only for significant correlations ( $p$ -value<0.05). Environmental variables are ordered  
 342 by a hierarchical clustering.

343

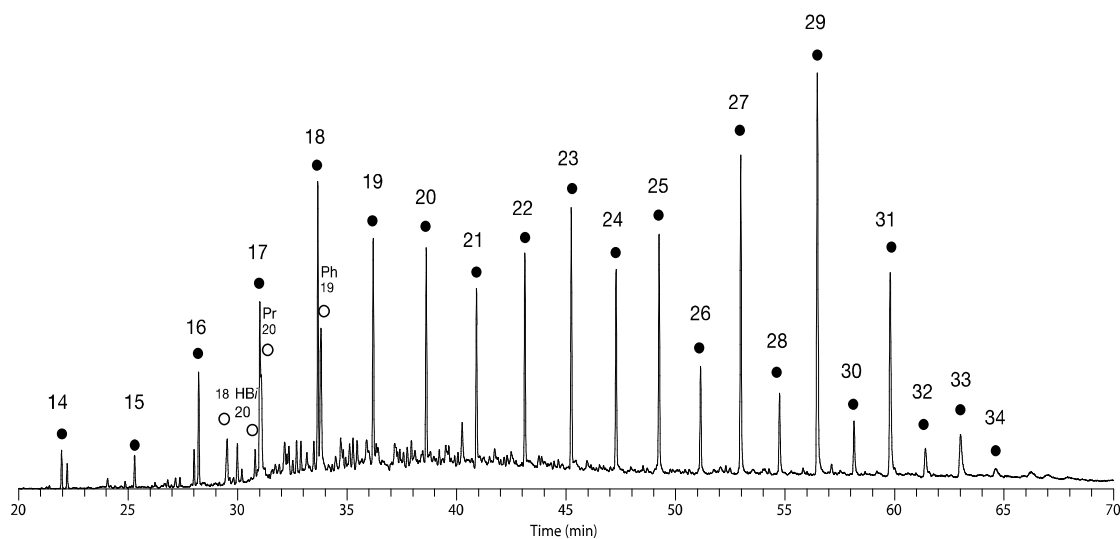
### 344 3.3 Biomarkers

#### 345 3.3.1 Organic matter origin

346 The aliphatic fractions of the analyzed sediment samples consist of *n*-alkanes ranging from C<sub>14</sub> to  
 347 C<sub>32</sub>, presenting a bimodal distribution in most cases excepting in the Canche Estuary and Grand-

348 Fort-Philippe, where light hydrocarbons ( $C_{14}$ - $C_{16}$ ) are not observed (Figure 4). The two modes  
349 present maxima at  $C_{18}$  and  $C_{29}$ , and odd/even predominance is observed in *n*-alkanes with carbon  
350 number higher than  $C_{23}$  (supplementary materials). Isoprenoid alkanes are also observed  
351 consisting of regular isoprenoids, pristane ( $C_{19}$ ) and phytane ( $C_{20}$ ) are among the most abundant  
352 isoprenoids in all samples excepting in Grand-Fort-Philippe. Several homologs of highly  
353 branched isoprenoids (HBI's) are observed in all samples where the  $C_{20}$  homolog is the most  
354 abundant, especially in the Grand-Fort-Philippe where this compound is particularly enriched. In  
355 the samples studied, the TARs indicate that the Authie and Canche Estuaries have the most  
356 important input of terrestrial organic matter, followed by the Somme Estuary where the two  
357 samples present similar values. Finally, Boulogne harbor and the Grand-Fort-Philippe receive the  
358 least abundant terrestrial influence.

359 The  $C_{20}$  HBI /  $C_{16}$  *n*-alkane clearly shows that Grand-Fort-Philippe receives the most abundant  
360 contribution from diatoms. This information is consistent with the presence of brassicasterol  
361 (exclusively produced by diatoms) in the samples from Grand-Fort-Philippe.



362

363 Figure 4. Mass chromatograms of aliphatic fractions a representative sample (AU1), showing the  
 364 distribution of n-alkanes, isoprenoids and branched alkanes. Numbers above symbols indicate  
 365 carbon number. Open circle = n-alkanes; Closed Circle = isoprenoids; Pr = Pristane; Ph =  
 366 Phytane; HBI = C<sub>20</sub> Highly Branched Isoprenoid.

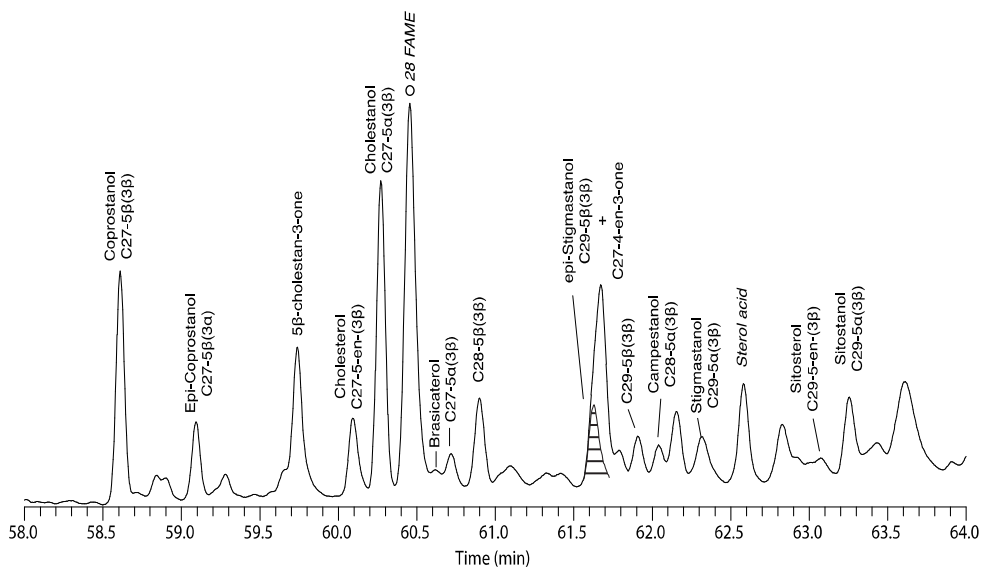
### 367 3.3.2 Biomarkers as indicators of contaminations

#### 368 Polycyclic aromatic hydrocarbons (PAHs)

369 Several polycyclic aromatic compounds are observed in the samples studied. The aromatic  
 370 fractions are dominated by triaromatic compounds: phenanthrene, alkylphenantrenes and  
 371 anthracene, followed by bicyclic compounds including naphthalene, and their alkylated isomers.  
 372 Some tetra- and pentacyclic aromatic compounds are also identified including  
 373 benz(a)anthracene, fluoranthene, pyrene, benzopyrene, and perylene. Based on the total PAH  
 374 ratio, the Boulogne harbor presents the higher values of heavy aromatic compounds followed by  
 375 the Somme and Canche Estuaries. The lower values are observed in Grand-Fort-Phillippe.

376 *Steroids and fecal contamination*

377 All steroid compounds are identified by their mass spectra and retention time. Figure 5 shows the  
378 distributions of sterols and stanols. The fraction contained a number of non-steroidal compounds,  
379 including long-chain fatty alcohols. The dominant peak in the extract is *n*-hexacosanol (C<sub>26</sub>). The  
380 sterol/ stanol content is dominated by the 5-cholestan-3-ol (C<sub>27</sub>) in all cases derived from the  
381 unsaturated natural precursor Cholest-5-en-3-ol also present in lower relative amount. 5-  
382 cholestan-3-ol (coprostanol) and 5-cholestan-3-ol (epicoprostanol) are also present in different  
383 proportions along the sites studied. Plant derived steroids also occur in significant amounts  
384 dominated by 5-sterol (sitosterol) and its reduction product epi-5-stigmastanol (C<sub>29</sub>).



385

386 Figure 5. Total ion current gas chromatogram of the alcohol fraction from a representative  
387 sample (BL3) showing the distribution of steroids.

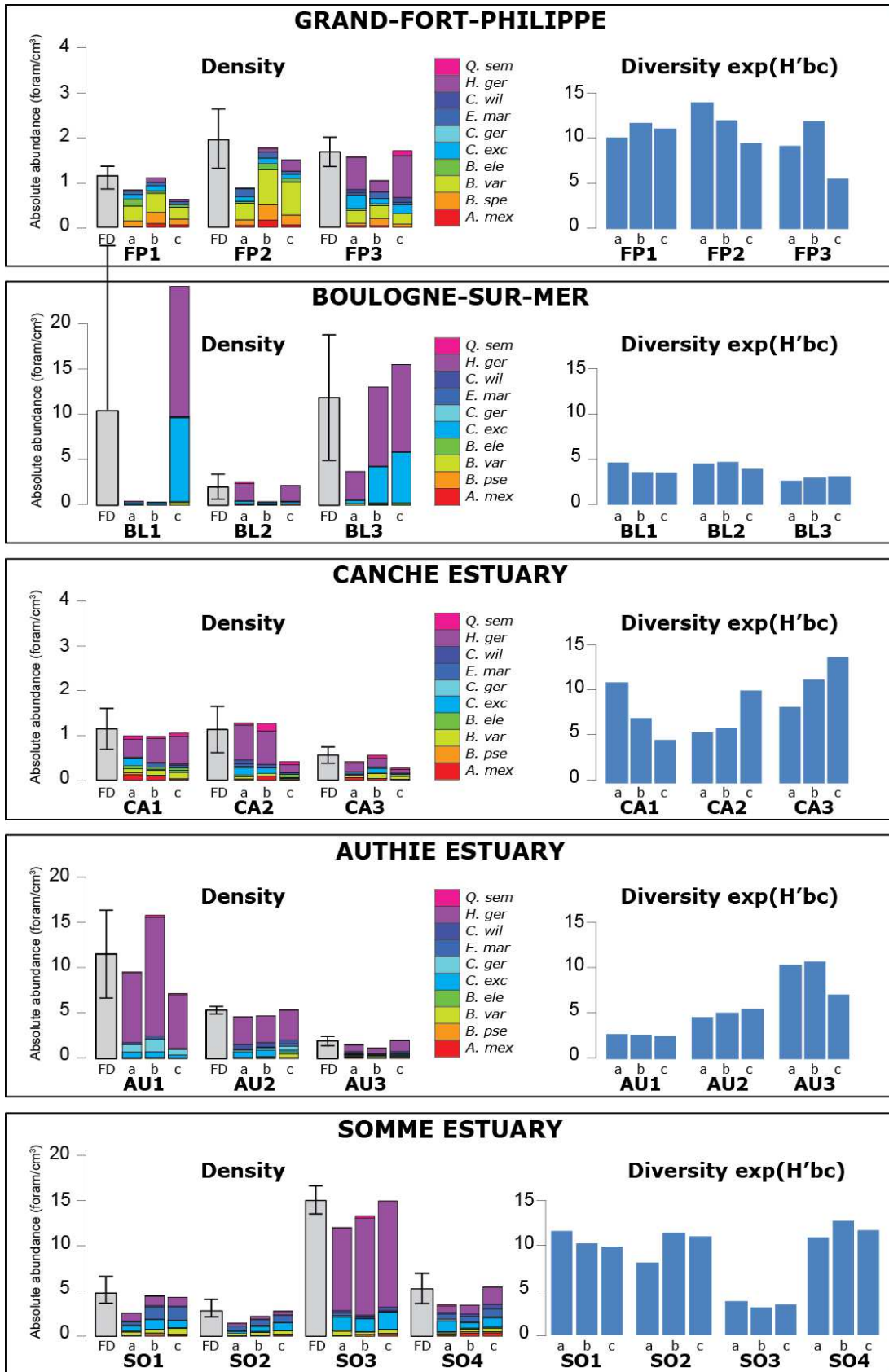
388

389 **3.4 Living foraminiferal analysis**



390 Thirty-eight living (stained) benthic foraminiferal species are found after the identification of ca.  
391 13,000 specimens (Supplementary materials - Counting Table). Twenty-nine of them are hyaline,  
392 seven agglutinated and three porcelaneous. Diversity  $\exp(H'_{bc})$  varies between 2.4 and 13.9  
393 (Figure 6). The highest values of diversity are identified in Grand-Fort-Philippe and the lowest  
394 ones in Boulogne-sur-Mer (10.4 and 3.6 on average, respectively). The foraminiferal density  
395 (FD) ranges between 0.36 and 30.4 (specimens/cm<sup>3</sup>) (Figure 6). The highest values are  
396 encountered in the port of Boulogne-sur-Mer and the lowest ones in the Canche Estuary (8.1 and  
397 0.95 on average, respectively). The living benthic foraminiferal assemblages are dominated by  
398 calcareous taxa. Agglutinated specimens only occur in areas adjacent to tidal marshes, constantly  
399 in low percentages. *Haynesina germanica* is the most abundant taxon (2.5 specimens/cm<sup>3</sup>, on  
400 average). This taxon dominates all the study sites, except for Grand-Fort-Philippe.  
401 *Criboelphidium excavatum* is the second most abundant species (0.73 specimens/cm<sup>3</sup>, on  
402 average), its highest abundances being found in the harbor of Boulogne-sur-Mer (2.02  
403 specimens/cm<sup>3</sup>, on average). In the harbor of Boulogne-sur-Mer, these two taxa represent more  
404 than 80% (on average) of the total abundance. Overall they represent, on average, 54% of the  
405 assemblages and are associated with minor species (i.e. *Arenoparrella mexicana*, *Bolivina*  
406 *variabilis*, *B. pseudoplicata*, *Buliminella elegantissima*, *C. williamsoni*, *Elphidium*  
407 *margaritaceum*, *C. gerthi*, *Quinqueloculina seminula*; these species have, on average, relative  
408 abundance >1% of the assemblage). Bolivinids are dominant (38%) in Grand-Fort-Philippe (*B.*  
409 *variabilis* 0.42 and *B. pseudoplicata* 0.17 specimens/cm<sup>3</sup> on average). Elphidiidae are more  
410 abundant in the Somme Estuary. Even though not very abundant, we record *Ammonia tepida*  
411 (max 3%, the third most abundant taxa), in the Harbor of Boulogne-sur-Mer and of the

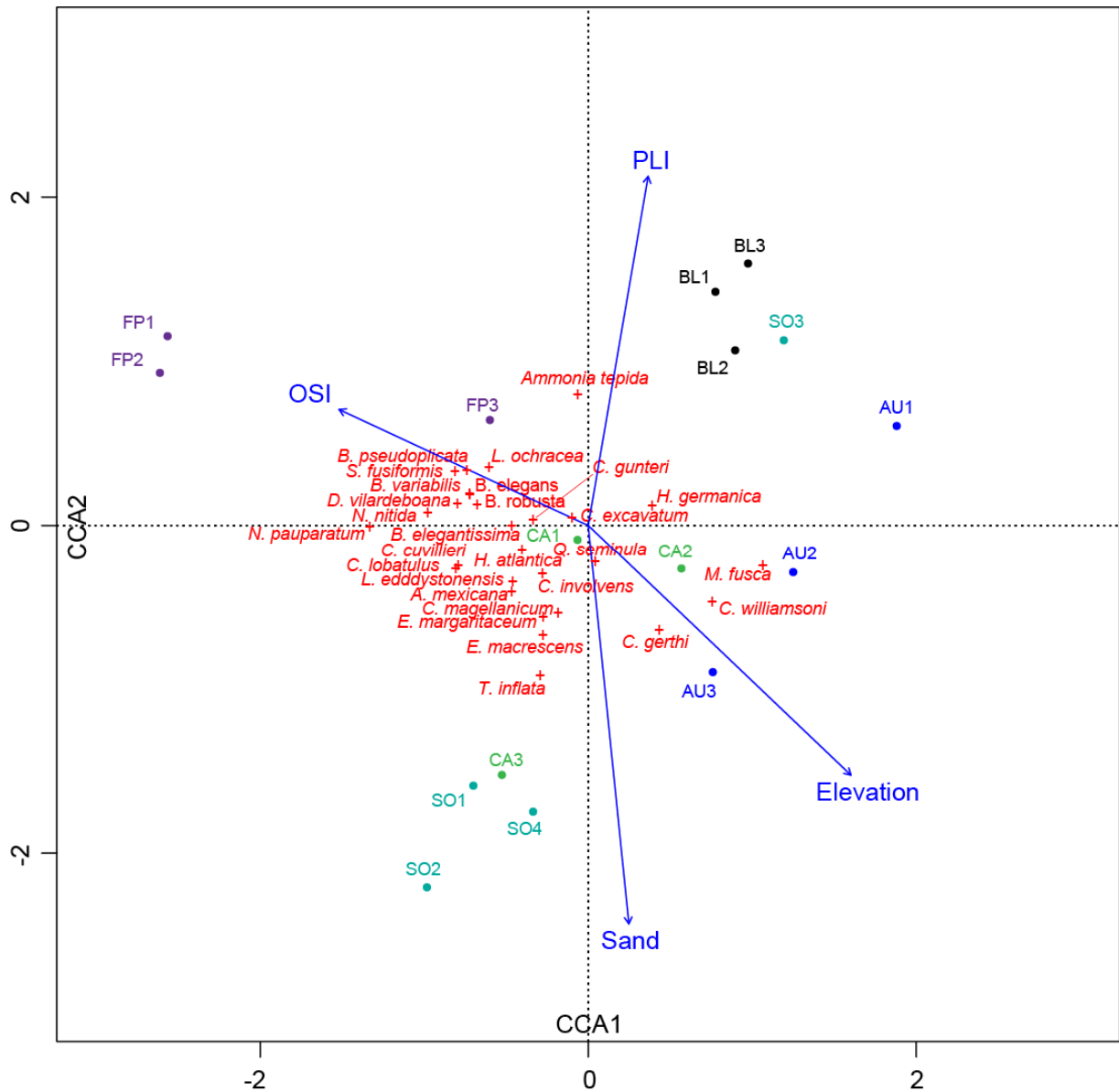
412 agglutinated taxa, *Entzia macrescens* (max 12%) and *Trochammina inflata* (max 5%) in the  
413 Canche Estuary.



415 Figure 6. On the left, the gray bar-plots represent the average of foraminiferal density (FD) at  
416 each sampling station; colored stacked bar-plots are the density (absolute abundance) of the most  
417 abundant species for the replicated samples (a, b, c) at each station. On the right, diversity of  
418 benthic foraminiferal assemblages in the study area. The blue bar-plots represent  $\exp(H'_{bc})$  for  
419 the replicated samples (a, b, c) at each station. Abbreviations of species name are *A. mex:*  
420 *Arenoparrella mexicana*, *B. var:* *Bolivina variabilis*, *B. pse:* *B. pseudoplicata*, *B. ele:*  
421 *Buliminella elegantissima*, *C. exc:* *C. excavatum*, *C. will:* *C. williamsoni*, *E. mar:* *E.*  
422 *margaritaceum*, *C. ger:* *C. gerthi*, *Q. sem:* *Quinqueloculina seminula*.

### 423 3.5 Environmental parameters and benthic foraminiferal relationships

424 The results from the CCA (Figure 7) are statistically significant ( $p$ -value<0.05). In the CCA, the  
425 total inertia is 0.81 (Constrained=0.35, Unconstrained=0.47). The first and second axes explain  
426 0.21 and 0.06 of the constrained variance. Sampling stations from the Authie, Canche and  
427 Somme estuaries are mainly influenced by the type of substrate (Sand) and Elevation (except  
428 SO3 and AU1); stations from the port of Boulogne-sur-Mer are related to the metal  
429 contaminations (PLI); and station from Grand-Fort-Philippe are influenced by the organic loads  
430 (OSI). Elphidiidae (*E. margaritaceum* *C. magellanicum*, *C. williamsoni*, and *C. gerthi*) and  
431 agglutinated species (*E. macrescens*, *T. inflata*, *M. fusca* and *A. mexicana*) are positively related  
432 to sandy substrate and Elevation. *Haynesina germanica* and *A. tepida* are positively related to  
433 metals contaminations, while the infaunal species (namely *B. variabilis*, *B. pseudoplicata*, *S.*  
434 *fusiformis*, *Bulimina. elegans*, *B. robusta* and *Buliminella. elegantissima*) are positively related to  
435 OSI.



436

437 Figure 7. Correspondence canonical analysis (CCA) based on substrate (Sand), Elevation,  
 438 Organic Sediment Index (OSI) and Pollution Load Index (PLI) as constrained parameters and  
 439 benthic foraminiferal abundances (>2% in at least in one sample) as secondary variables.

440

## 441 **4 Discussion**

442 Three foraminiferal-type assemblages are identified in the intertidal areas of the Hauts-de-France  
443 reflecting contrasting ecological conditions at regional scale: 1) *H. germanica* associated to  
444 Elphidiidae in low impacted macrotidal estuaries ; 2) *H. germanica* and *C. excavatum* in  
445 industrial-perturbed harbors; and 3) infaunal species *B. variabilis* and *B. pseudoplicata* in  
446 embankment areas (Figure 8).

### 447 **4.1 Estuarine macrotidal assemblages**

448 The intertidal areas of the Hauts-de-France along the Eastern English Channel show  
449 contrasting environmental conditions with respect to the different degree of human influence  
450 (Figure 8). The Canche, Authie and Somme estuaries are extended macro-tidal estuarine zones,  
451 characterized by the absence of major industrialization and considered as natural areas (Henry et  
452 al., 2004). However, low concentrations of chemical contaminants, such as Zn and PAHs, have  
453 been documented in surface sediments, probably due to municipal and agricultural activities in  
454 the catchment areas of these estuaries (Amara et al., 2007). Our results are quite in accordance  
455 with these findings, although we record only a moderate contamination of Cr at one station in the  
456 Authie Estuary (AU1). In the Somme and Canche Estuary, PAHs show concentrations much  
457 lower than in the port of Boulogne-sur-Mer, probably in relation to the circulation of steamboats  
458 in the bays (Nacci et al., 2002).

459 In response to these almost unpolluted conditions, foraminiferal assemblages are typical of  
460 macrotidal estuaries from temperate Atlantic areas. The assemblages are more diversified than in  
461 the anthropogenized harbor of Boulogne-sur-Mer, and the overall diversity is comparable to that  
462 from the same regions (Armynot du Châtelet et al., 2018a). Based on the CCA, the assemblages

463 seem to be influenced by the type of substrate (muddier or sandier) and the elevation. They are  
464 dominated by *H. germanica* associated to high percentage of Elphidiidae (*C. excavatum*, *E.*  
465 *margaritaceum* and *C. williamsoni*), these being taxa common in transitional marine  
466 environments (Alve and Murray, 1994; Bouchet et al., 2007; Thomas and Schafer, 1982). In  
467 natural intertidal settings, benthic foraminifera are commonly distributed along the elevation  
468 gradient (Leorri et al., 2010). Although *H. germanica* is widespread in intertidal environments  
469 along the eastern North Sea coasts, it tends to dominate in the upper part of the tidal gradient,  
470 while *C. excavatum* and *E. margaritaceum* have been mainly found in non-marsh and subtidal  
471 environments (Armynot du Châtelet et al., 2018a; Armynot du Châtelet et al., 2018b).  
472 *Criboelphidium excavatum* prefers the lowest part of the intertidal gradient (Armynot du  
473 Châtelet et al., 2005), where more marine conditions prevail (Debenay and Guillou, 2002).  
474 *Criboelphidium excavatum* and *E. margaritaceum* occur principally in tidal flat areas of the  
475 Somme Estuary, close to the main channel associated with the highest sand contents. This  
476 reflects a higher hydrodynamic regime. Similarly, Francescangeli et al. (2017) revealed the  
477 presence of *E. margaritaceum* in the sandy tidal flat area of the Canche Estuary. This could  
478 suggest that grain-size (sandy fraction) drives the distribution of these taxa. In the coastal  
479 sediment of the Arabian Gulf, Arslan et al. (2016) reported that species belonging to this genus  
480 can better survive in quartz sandy substrates. *Criboelphidium gerthi* and *C. williamsoni* have  
481 been documented in salt marsh areas and tidal flat areas (Armynot du Châtelet et al., 2009;  
482 Debenay et al., 2000). In accordance with Francescangeli et al. (2017) and Müller-Navarra et al.  
483 (2016), elphidiids could also indicate the occurrence of tidal channels (and/or small pounds) with  
484 a local fluctuation of water dynamics. *Entzia macrescens* and *T. inflata* are found in very low  
485 abundance only in upper-tidal areas close to the limit to the salt marsh. These agglutinated taxa

486 are the most widespread and abundant salt marsh foraminifera (Scott et al., 2001; Scott and  
487 Leckie, 1990; Scott and Medioli, 1978). As in most of the intertidal areas in temperate European  
488 tidal flats, *H. germanica* results to be the dominant species within the benthic foraminiferal  
489 assemblages. However, the hydrodynamic variability of the estuarine system seems to favor the  
490 Elphidiidae.

#### 491 4.2 Industrial and anthropogenic-perturbed assemblages

492 The harbor of Boulogne-sur-Mer, along the Liane Estuary, is the most polluted study area,  
493 showing strong enrichments in trace metals as previously reported (Amara et al., 2007; Berthet et  
494 al., 2003; Kerambrun et al., 2012) (Figure 8). The harbor of Boulogne-sur-Mer experienced an  
495 intense industrialization phase during the 1970s-1990s (such as handcrafted and industrial  
496 fishing; activities connected to freight transport; metallurgic and chemical factories). Among  
497 these, a steel plant (ferromanganese transformation) was placed close to BL3 (southwestern zone  
498 of Boulogne-sur-Mer) and played a pivotal role in the pollution of the harbor (1967-2003)  
499 (Francescangeli et al., 2016).

500 The highest contents of PAHs are also found in the harbor of Boulogne-sur-Mer. These organic  
501 pollutants have carcinogenic and mutagenic properties commonly related with shipping traffic,  
502 combustion of fossil fuels and petroleum spillage (Dhananjayan and Muralidharan, 2012; Nacci  
503 et al., 2002). The differences in 5 $\beta$ -stanols distributions permit comparison with the degree of  
504 fecal contamination.

505 Comparing our results with others previously reported (Biache and Philp, 2013; Jardé et al.,  
506 2007; Zocatelli et al., 2017), higher values of fecal contaminations are observed in the Boulogne  
507 harbor, followed by the Canche and Authie estuaries. However, the Sr1 ratio, herein used to  
508 highlight fecal contents, is lower than the observed in highly sewage-contaminated sites



509 (Sr1>0.7; (Grimalt et al., 1990)), indicating that the levels of fecal contaminations are reduced  
510 from the critical levels at the time of the sampling. Finally, Boulogne harbor shows the lowest  
511 values of Sr2 (0.33), indicating human sewage contaminations (Bull et al., 2002; Emrich et al.,  
512 2017; Zocatelli et al., 2017).

513 Impacted by the presence of pollution, foraminiferal assemblages are the less diversified in the  
514 harbor of Boulogne-sur-Mer. It has been shown that an increase of industrial pollution (metals  
515 and PAHs) limits benthic foraminiferal diversity and leads to the dominance of tolerant and  
516 opportunistic taxa (Alve, 1995; Armynot du Châtelet et al., 2004; Romano et al., 2016). Benthic  
517 foraminiferal assemblages are largely dominated by *H. germanica* associated to *C. excavatum*.  
518 Although these taxa are common taxa in naturally intertidal environments (like in the previous  
519 areas), these species have been considered as tolerant to industrial pollution (Armynot du  
520 Châtelet and Debenay, 2010; Armynot du Châtelet et al., 2011; Debenay et al., 2001; Romano et  
521 al., 2008). In a recent reconstruction of the paleoecological evolution of harbor of Boulogne-sur-  
522 Mer, Francescangeli et al. (2016) reported, however, that *C. excavatum* is less tolerant to trace  
523 metal contamination than is *H. germanica*. *Ammonia tepida* is, on average, the third most  
524 abundant species in the harbor of Boulogne-sur-Mer, being scarcely present in the other studied  
525 sites. This taxon has been generally considered as a bioindicator of stressed conditions in coastal  
526 environments (e.g., Vilela et al., 2011). Hence, in the harbor of Boulogne-sur-Mer, the extremely  
527 low diversity of the living assemblages and the large dominance of opportunistic species are the  
528 response of the biota to human-induced stress conditions.

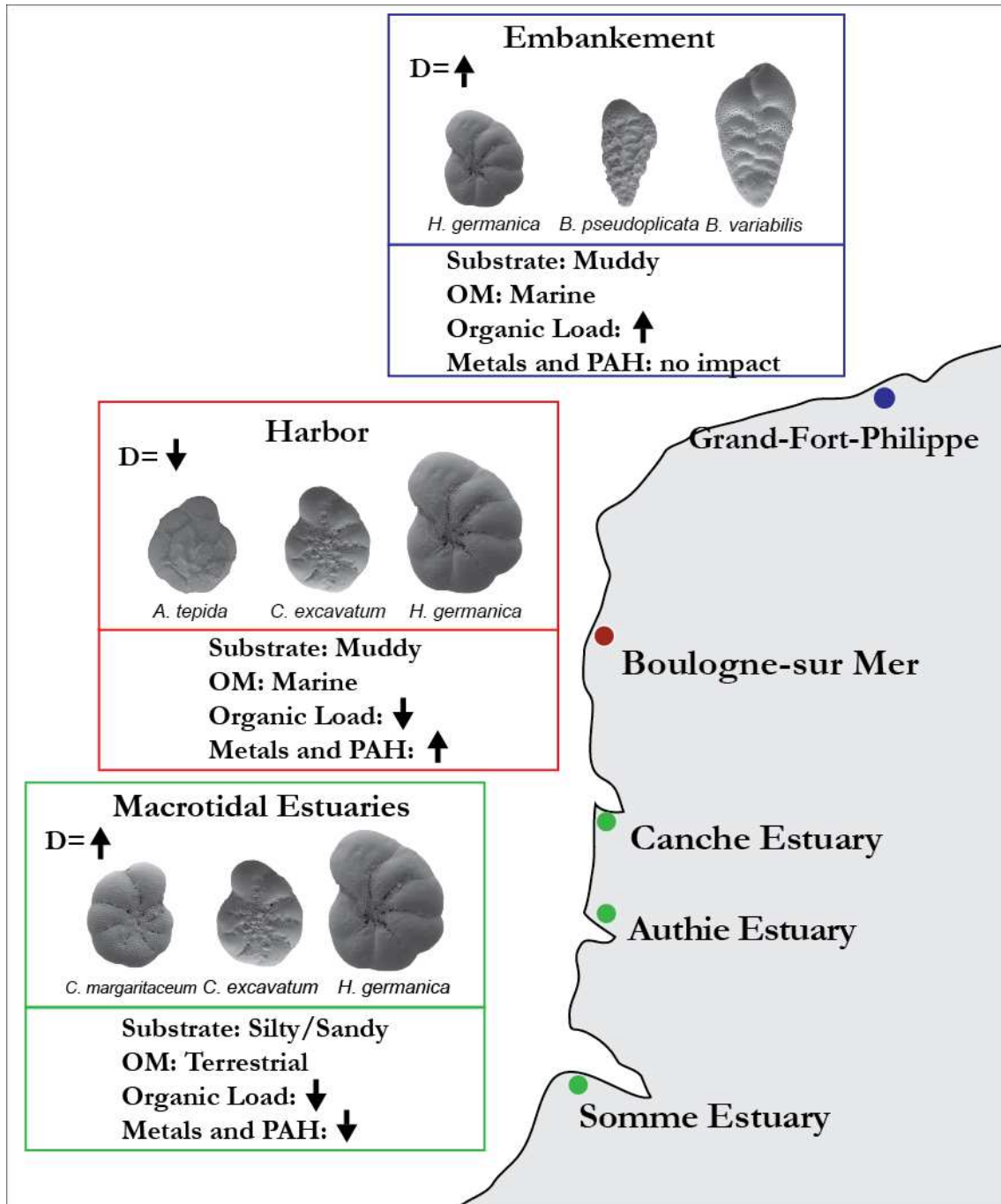
#### 529 4.3 Infaunal-dominant assemblages

530 The embankment and urbanization of Aa river in Grand-Fort-Philippe led to the degradation  
531 of the salt marshes habitat (Romont, 1996). Although this site is close to the city and down drift

532 from the nuclear power plant of Gravelines, it is the least impacted in terms of trace metals,  
533 PAHs and human-derive faeces (Figure 8). Studies on benthic fauna indicate that the global 1°C  
534 temperature increase linked to the power plant thermal plume does not change the communities,  
535 except some minor changes on sand worms (Ifremer, 2010; 2011). The highest organic loads are  
536 in Grand-Fort-Philippe. The C/N (4-10) shows a marine-derived origin of the OM, due to the  
537 probably occurrence of diatoms bloom. Furthermore, biomarkers (C<sub>20</sub> HBI / C<sub>16</sub> n-alkane)  
538 indicate the most abundant contributions from diatoms (Meyers, 1994).

539 On average, foraminiferal assemblages in the embankment area of Grand-Fort-Philippe  
540 exhibit the lowest FD values and the highest values diversity of all the studied sites.  
541 Assemblages are dominated by *Bolivina variabilis* and *B. pseudoplicata*. Bolivinids include  
542 infaunal species that are common in intertidal environments (Murray, 2006); some of them are  
543 tolerant to oxygen-depleted conditions (Alve, 1990; Debenay et al., 2001). They have an  
544 elongated and flattened morphology (commonly largely perforated) that seems to be an  
545 adaptation to a lack of oxygen (Bernhard, 1986). Higher organic loads associated to low oxygen  
546 tolerant species might suggest the occurrence of oxygen deficiency in the sediments of Grand-  
547 Fort-Philippe. Hence it might be hypothesized that bolivinids moved up from the deep sediments  
548 layer to cope with low oxygen conditions, as mentioned in the Jorissen et al. (1995) and de  
549 Stigter (1996) TROX model. The modification of the river flowing could have led to an  
550 accumulation of OM in Grand-Fort-Philippe, as testified by the highest OSI. This change might  
551 have favored *B. variabilis* and *B. pseudoplicata* rather than *H. germanica*, which was dominant  
552 at the other study sites. In addition, Grand-Fort-Philippe receives the most abundant  
553 contributions from marine-derived OM (mainly diatoms). Marzocchi et al. (2018) showed that an  
554 input of fresh OM (such as diatoms) can stimulate bacterial activity measured as oxygen and

555 nitrate consumption (denitrification) in surface sediment. Hence, it could be that there was a  
556 downward transport of diatoms due to mixing sediment factors (such as bioturbation or burying  
557 by sediment deposition after a storm). This could have led to a downward decrease of oxygen in  
558 the sediment reinforcing the hypothesis of the uprising of bolivinids.



559

560 Figure 8. Synthetic scheme of the foraminiferal communities in relation to the different  
 561 environmental characteristics in the Hauts-de-France. (D=Diversity, OM=Organic Matter,  
 562 PAH=Polycyclic aromatic hydrocarbons).

563

## 564 **5 Conclusions**

565 Along the intertidal areas of the Hauts-de-France we document the occurrence of three main  
566 type of foraminiferal assemblages reflecting contrasting ecological conditions at regional scale:  
567 1) estuarine macrotidal assemblages (*H. germanica* associated to Elphidiidae) in low impacted  
568 estuaries; 2) industrial-perturbed assemblages (*H. germanica* and *C. excavatum*) in harbor areas;  
569 and 3) infaunal-dominant assemblages (*B. variabilis* and *B. pseudoplicata*) in embankment areas.  
570 Our study shows that a series of environmental factors needs to be accounted for to properly  
571 characterize intertidal environments. In these highly stressed areas, where a multiproxy approach  
572 must be used to improve environmental interpretations, foraminifera represent a "shortcut" to  
573 evidence environmental conditions. This quantitative study constitutes a baseline for the  
574 planning of future foraminiferal-based biomonitoring programs at least in northern part of France  
575 and in the southern English Channel. It further contributes to the international effort of getting a  
576 better understanding of benthic foraminiferal response along organic and chemical gradient of  
577 pollution in order to implement a foraminiferal sensitivity-based biotic index in intertidal and  
578 transitional waters.

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## Embankment

D=↑



*H. germanica* *B. pseudoplicata* *B. variabilis*

Substrate: Muddy

OM: Marine

Organic Load: ↑

Metals and PAH: no impact

## Harbor

D=↓



*A. tepida* *C. excavatum* *H. germanica*

Substrate: Muddy

OM: Marine

Organic Load: ↓

Metals and PAH: ↑

## Macrotidal Estuaries

D=↑



*C. margaritaceum* *C. excavatum* *H. germanica*

Substrate: Silty/Sandy

OM: Terrestrial

Organic Load: ↓

Metals and PAH: ↑