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. Introduction

Intertidal areas are fragile environments that are naturally stressed by high spatial and temporal variability of physico-chemical parameters (Elliott and Quintino, 2007). In conjunction with it, anthropogenic activities (e.g., sewage, dredging, urbanization, aquaculture) strongly contribute to the degradation of these critical ecotones. The distribution of benthic organisms (e.g., foraminifera, nematodes, macro-invertebrates, macro-algae) in intertidal areas is the result of a complex interplay among these natural and human-induced features (Armynot du Châtelet et al., 2016; Müller-Navarra et al., 2019; Rolet et al., 2015; Semprucci et al., 2016). Hence, disentangling human impacts from natural stresses can be difficult and remains controversial in these areas (Dauvin, 2007; Elliott and Quintino, 2007). Agricultural and urban run-off are among the most important sources of contaminants to intertidal areas. Run-off includes organic compounds like pesticides, pharmaceutical products and fossil fuel-by-products (aromatic polycyclic hydrocarbons and fecal materials), and inorganic ones such as nutrients and trace elements including toxic ones (i.e., Pb, Hg, Cd). Some of these contaminants can be accumulated in coastal soft sediments over long-time periods, affecting benthic and epibenthic fauna and inducing major ecological damages (Frontalini and Coccioni, 2011; Levin et al., 2001). Hence, the use of multidisciplinary approaches combining sedimentological, geochemical and biological proxies is recommended for unravelling environmental features in intertidal systems. Such an approach will allow for a better interpretation of the
The use of biological indicators to monitor the environmental pollution has been enforced worldwide by marine legislations and, hence, has kindled the interest of numerous researchers suggesting different biological quality elements to assess the health of marine systems such as macroalgae, macrofauna and meiofauna (e.g. Ar Gall et al., 2016; Borja et al., 2006; Bouchet et al., 2012; Rosenberg et al., 2004; Semprucci et al., 2016). The benthic macrofauna is currently the most widely used biological quality element in monitoring the ecological quality status of marine environments (Birk et al., 2012; Blanche et al., 2008; Bouchet and Sauriau, 2008; Daunin, 2018; Pinto et al., 2009). Within benthic meiofauna, foraminifera (protozoans) represent a promising tool for detecting the environmental quality of water bodies and for assessing its deviation from natural reference conditions (Alve et al., 2019; Dimiza et al., 2016; El Kateb et al., 2020; Hess et al., 2020; Jorissen et al., 2018). Their sensitivity and rapid response to environmental stress, as well as specific ecological requirements of benthic foraminifera, make them an efficient environmental proxy (Schäfer et al., 2012) in intertidal areas (e.g. Alve, 1995; Bouchet et al., 2007, 2018; Debenay et al., 2001; Martins et al., 2015). These studies confirmed the reliability of benthic foraminifera in biomonitoring programs. However, there is still a lack of knowledge on foraminiferal ecology and a need for further quantitative studies to improve the application of foraminifera in environmental biomonitoring, particularly in coastal and intertidal areas.

The northern part of the intertidal areas of the Hauts-de-France (northern France) has experienced strong human-induced modifications over the last two centuries. Since the second industrial revolution, numerous polluting activities (i.e., metallurgical, textile and chemical factories, harbors of Boulogne-sur-Mer, Calais and Dunkirk, nuclear power plant of Gravelines) have developed, leading to a large degradation of environmental quality (Amara et al., 2007; Francescangeli et al., 2016; Rolet et al., 2015). Conversely, the coast of Picardy, in the southern part of the Hauts-de-France, includes mostly natural areas that are protected by natural parks and less impacted by pollution (Henry et al., 2004). A general littoral drift that drives the coastal pollutants northward from the English Channel to the North Sea enhances this North/South distinction (Anthony, 2000). The differential degree of disturbance makes the coastlines of the region an excellent laboratory for observing the response of benthic foraminifera to environmental variations both in the natural and human-affected zones. So far, three studies have focused on the response of living benthic foraminifera to environmental conditions in the Hauts-de-France (Armynot du Châtelet et al., 2009, 2011; Francescangeli et al., 2017). These studies were, however, very local (Boulogne-sur-Mer Harbor and Canche Estuary), and were not sufficient to give a comprehensive overview on the distribution of benthic foraminifera and their response to environmental conditions. Two other studies provided a wider view on the foraminiferal communities in the intertidal areas of the northern France and the English Channel (Armynot du Châtelet et al., 2018a; Armynot du Châtelet et al., 2018b).

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

2. Materials and methods

2.1. Study area

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

The area is drained by five main rivers (Fig. 1a). The northernmost one is the Aa River that opens into the English Channel in Grand-Fort-Philippe – an embankment area where the natural flowing of the river channel was modified by human action during the 17th centuries. The estuary presently consists of a 4-km-long artificial channel. The Liane River has been heavily modified since the 18th century with the building of Boulogne-sur-Mer harbor, the biggest of the area (Armynot du Châtelet et al., 2017). The Canche, Authie and Somme rivers open onto the English Channel at Le Touquet-Paris-Plage, Berck-sur-Mer and Saint-Valéry and Le Crottoy, respectively. These estuaries were weakly modified compared to the two former ones, with only littoral defences.

2.2. Sampling strategy

Sixteen sampling stations were selected from five estuaries along the intertidal zones of the Hauts-de-France region. These stations are from north to south (Fig. 1B-F): the Aa Estuary, in the embanked area of Grand-Fort-Philippe (FP1, FP2, FP3), the Liane Estuary in the harbor of Boulogne-sur-Mer (BL1, BL2, BL3) then the Canche (CA1, CA2, CA3), Authie (AU1, AU2, AU3) and Somme Estuaries (SO1, SO2, SO3, SO4). Information on regional pollution levels were retrieved from Billon (2001), Berthet et al. (2003), Henry et al. (2004), Amara et al. (2007) and Kerambrun et al. (2012). Overall, analyses of the sediment indicate that Bay of Somme is the least contaminated in the region, while the harbour of Boulogne-sur-Mer is the most impacted in terms of trace metals. At each station, four replicated sediment samples were collected (10 dm³, the uppermost 1 cm over a surface of 1 m²). One replicate was used to measure sediment properties (grain-size, calcium carbonate, organic matter, major and trace elements). The other three replicates were used for the benthic foraminiferal analyses. The 48 foraminiferal replicated samples were stored in transparent graduated containers and stained with buffered Rose Bengal dye (2 g of Rose Bengal in 1000 mL of ethyl alcohol) to distinguish living (stained) from dead (unstained) tests at time of collection (Lutze and Altenbach, 1991; Walton, 1952). Six additional samples were collected for biomarkers analysis in glass vials at time of collection (FP1, BL3, CA3, AU3, SO1 and SO3). Sampling station positions and elevations were measured using a Trimble GeoXT dGPS allowing a precision higher than 10–15 cm (in...
elevation) (Table 1). We used the WGS 84 coordinate system to position the sampling stations. Elevations were referenced to the mean sea level (MSL) calculated after elevation reference system IGN69.

### Table 1

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2.3. Sedimentological analyses

Sediment grain-size analyses were performed using the principle of diffraction and diffusion of a monochromatic laser beam on suspended
particles (Malvern Mastersizer 2000; red He–Ne laser). The method is based on near-forward scattering of a laser beam by particles in suspension (Trentesaux et al., 2001). Measurements can range from 0.02 to 2000 μm, with an obscuration ranging between 10 and 20%. Four grain-size fractions were considered: clay (<4 μm), fine silt (4–10 μm), sortable silt (10–63 μm), and sand (63–2000 μm). Silt fraction and motility are discussed in Mc Cave et al. (1995).

The C, H, N and S for Total Organic Carbon (TOC) estimation was determined using a Flash EA 1112 Elemental Analyzer (Thermo) equipped with a auto-sampler. The analysis was performed on 1.5–2 mg of dried and crushed sediment, added to approximately 5 mg of vanadium pentoxide, used as a combustion catalyst. 2.5-Bis (5-tert-butyloxazol-2-yl) thiophene (BBOT) was used as standard. The TOC was determined by subtracting carbonate carbon from total carbon concentration. Calcium carbonate content was determined using a Ber-nard calcmeter. Triplicated measurements were carried out for each sample using 0.5 g of finely crushed dry sediment (Armynot du Châtelet et al., 2009). The C/N ratios were used to distinguish the origin of sedimentary organic matter (Meyers, 1994). To evaluate the organic load, the Organic Sediment Index (OSI) (Ballinger and McKee, 1971) was calculated as follows (Eq. (1)):

\[ OSI = \frac{\text{TOC wt} \%}{\text{ON wt} \%} \]  

(1)

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

### 2.4. Trace metal analysis

#### 2.4.1. Total concentration

Total concentrations of Al, Co, Cr, Cu, Fe, Mn, V and Zn were analyzed at each sampling station. After drying the sediment particles at room temperature, the raw samples were gently crushed to limit the destruction of soft particles. Then 200 mg of the fine fraction recovered after grinding at 63 μm were attacked first with a mixture of 5 mL of Suprapur® nitric acid (65%) and 10 mL of a concentrated HF boiling solution over 48 h. After the acids’ evaporation, 10 mL of a freshly prepared HNO₃/HCl mixture (1/2 v:v) was added in order to eliminate the remaining solid grains. The recovered solutions were subsequently diluted in a known volume of ultrapure water and analyzed using ICP-AES (inductively coupled plasma – atomic emission spectroscopy; Varian Vista-PRO, axial view) and ICP-MS (inductively coupled plasma – mass spectroscopy; Thermo Elemental X Series). This attack procedure was validated and the accuracy of the analytical procedure was checked by means of the following sediment standard reference materials (Canadian International Standards): HISS-1, MESS-3 and PACS-2.

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

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

\[ EF = \frac{[X_i] / [Y_i]}{([X_0] / [Y_0])} \]  

(2)

where [X_i] and [Y_i] are the concentrations of the target element and a conservation element, respectively in the sample; [X_0] and [Y_0] are the local geochemical background concentrations the target element and a conservation element, respectively. The EF is normalized against aluminum (conservation element) following for instance Delgado et al. (2010).

Fine particles (silts and clays), can partly overcome the bias bound to the nature of the sediment. The following EF categories, estimated by previous studies (Birth, 2003), are used to evaluate the degrees of pollution: EF < 3: no relevant contamination or minor contamination; 3 < EF < 5: moderate contamination; 5 < EF < 10 severe contamination; 10 < EF < 25 high contamination; EF > 25 extremely high contamination.

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

\[ PLI = \sqrt{CF_1 \times \ldots \times CF_n} \]  

(3)

\[ CF_X = \frac{[X_i]}{[X_0]} \]  

(4)

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

#### 2.5. Biomarkers

##### 2.5.1. Samples treatment

Between 5 and 10 g of dry sediments were extracted using an azeotropic mixture of dichloromethane (DCM) and methanol (MeOH) 2:1 (v:v) by means of a Soxhlet extractor for 48 h. Subsequent separation of the total lipids extracts (TLE’s) into apolar, oxygenated and polar fractions was achieved via silica column chromatography using solvent mixtures of 9:1 (v:v) hexane/DCM, 8:2 (v:v) DCM/ethyl acetate, and 1:1 (v:v) DCM/MeOH, respectively. All the resulting fractions were dried using a rotary evaporator. The oxygenated and polar fraction containing sterols and acids, respectively, were derivatized using BSTFA-TMCS 99:1, Supelco (N,O-Bis(trimethylsilyl)) trifluoroacetamide with trimethylchlorosilane) to improve the chromatographic resolution. Briefly, 200 μL of BSTFA-TMCS 99:1 were added to the dry extracts and ultrasonicated for 10 min. The remaining solution was dried under N₂ atmosphere. All alcohols and acids were later identified and semi quantified as trimethylsilyl-derivatives.

##### 2.5.2. Gas chromatography - mass spectrometry analysis

One mL of extract was injected into a gas chromatograph (Perkin Elmer 680) coupled with a mass spectrometer (Perkin Elmer 600C). Chromatographic conditions were as follow: inlet heated at 250 °C; DB-MS-UI column initially at 40 °C for 1 min, then heated to 320 °C and maintained 10 min at 320 °C; helium column flow of 1 mL min⁻¹, split ratio of 10. Mass spectrometer conditions were as follow: scan time 0.2 s, inter-delay scan 0.1 s, and ionization energy 70 eV. Identification of compounds was based on a comparison with the NIST mass spectra database and/or on the comparison of retention times of standards. For the purpose of product semi-quantification, standard solutions of androstanol, anthracene D₁₀ and nonadecane C₂₉ were used.

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

#### 2.6. Foraminiferal analysis

In the laboratory, wet samples were gently washed with tap water,
through 315 and 63 μm mesh sieves, and dried at 40 °C. Foraminiferal tests of the intermediate fraction were concentrated by flotation on trichloroethylene (Horton and Murray, 2006; Semensatto and Dias-Brito, 2007). Although heavy liquid separation should be avoided, it may be used in environments with low foraminiferal density, due to a high sedimentation rate as in estuarine areas resulting in a strong dilution of foraminifera within mineral grains. Only specimens containing dense, brightly red-stained protoplasms were considered as living (Alve and Murray, 1999; de Stigter et al., 1999). All living specimens were counted and identified following the generic classification Loeblich and Tappan (1987), and the specific ones of Debenay (2012) and Debenay et al. (2001). The observations were carried out under a binocular microscope, model Olympus SZX16. The foraminiferal density (FD; absolute abundance; specimens/cm²) and the relative abundances of living taxa were calculated for each station. For diversity, the exp(H’) contributes to 19%–35% of the Shannon diversity index (H’).

3. Results

3.1. Sediment characteristics

Silt is the most abundant sediment fraction in all the sampling sites (78%, on average) (Fig. 2a). In particular, sortable silt (10–63 μm) contributes to 19%–67% and fine silt (4–10 μm) to 7%–21% of the sediment. Sand varying between 10 and 72% is the second most abundant fraction (28%, on average) (Fig. 2a). Clay (4.4%, on average) ranges from 2.2 to 6.8% (Fig. 2a). The highest contents of mud (silt + clay) are found in Grand-Fort-Philippe (86%, on average), while the relative abundances of living taxa were calculated for each station. For diversity, the exp(H’) contributes to 19%–35% of the Shannon diversity index (H’). The OSI varies between 0.12 and 1.15; the lowest is in the port of Boulogne-sur-Mer (1.4% on average). The C/N ratios range from 2.5 to 15 (7-2, on average) (Fig. 2b). The highest values are documented in the Somme Estuary. The OSI varies between 0.12 and 2.1 (Fig. 2c). The highest values are found in all the station in Grand-Fort-Philippe and in CA1.

3.2. Trace metals

The concentrations of analyzed elements (Al, Co, Cr, Cu, Fe, Mn, V, and Zn) are presented in supplementary materials, as are the background references used for the calculation of the enrichment factors (Environmental parameters-EF). The highest enrichment factors (EFs) are observed in the harbor of Boulogne-sur-Mer, with moderate to severe contaminations for Co, Cr and Cu (3 < EFs < 10), high contamination for Zn (10 < EFs < 25), and extremely high contamination Mn (EFs > 25), specifically in BL2 and BL3 (Table 2). In the Authie Estuary a moderate contamination is recorded in the AU1 station only with respect to Cr (EFcr = 3). No significant contaminations are recorded in Grand-Fort-Philippe and the Canche and Somme Estuaries. The highest values of PLI (Fig. 2c) are found in Boulogne-sur-Mer, confirming the metals’ enrichments in the sediments of this area, specifically in BL2 and BL3.

3.3. Biomarkers

3.3.1. Organic matter origin

The aliphatic fractions of the analyzed sediment samples consist of n-alkanes ranging from C14 to C32, presenting a bimodal distribution in most cases excepting in the Canche Estuary and Grand-Fort-Philippe, where light hydrocarbons (C14–C16) are not observed (Fig. 4). The two modes present maxima at C18 and C29, and odd/even predominance is observed in n-alkanes with carbon number higher than C23 (supplementary materials). Isoprenoid alkanes are also observed consisting of regular isoprenoids, pristane (C29) and phytane (C30) are among the most abundant isoprenoids in all samples excepting in Grand-Fort-Philippe. Several homologs of highly branched isoprenoids (HBIs) are observed in all samples where the C30 homolog is the most abundant, especially in the Grand-Fort-Philippe where this compound is particularly enriched. In the samples studied, the TARs indicate that the Authie and Canche Estuaries have the most important input of terrestrial organic matter, followed by the Somme Estuary where the two samples present similar values. Finally, Boulogne harbor and the Grand-Fort-Philippe receive the least abundant terrestrial influence.

The C30 HBI/C16 n-alkane clearly shows that Grand-Fort-Philippe receives the most abundant contribution from diatoms. This information is consistent with the presence of brassicasterol (exclusively produced by diatoms) in the samples from Grand-Fort-Philippe.

3.3.2. Biomarkers as indicators of contaminations

3.3.2.1. Polycyclic aromatic hydrocarbons (PAHs).

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

3.3.2.2. Steroids and fecal contamination.

All steroid compounds are
Fig. 2. Distribution of environmental parameters in the 16 sampling stations within the 5 sampling areas: A) Sediment grain-size: sand, S-Silt (sortable silt), F-Silt (fine silt) and clay; B) TOC and C/N in and C) Pollution Load Index (PLI) and Organic Sediment Index (OSI).
identified by their mass spectra and retention time. Fig. 5 shows the distributions of sterols and stanols. The fraction contained a number of non-steroidal compounds, including long-chain fatty alcohols. The dominant peak in the extract is \( n \)-hexacosanol (C\(_{26}\)). The sterol/stanol content is dominated by the 5-cholestan-3-ol (C\(_{27}\)) in all cases derived from the unsaturated natural precursor Cholest-5-en-3-ol also present in lower relative amount. 5-Cholestan-3-ol (coprostanol) and 5-cholestan-3-ol (epicoprostanol) are also present in different proportions along the sites studied. Plant derived steroids also occur in significant amounts dominated by 5-sterol (sitosterol) and its reduction product epi-5-stigmastanol (C\(_{29}\)).

### 3.4. Living foraminiferal analysis

Thirty-eight living (stained) benthic foraminiferal species are found after the identification of ca. 13,000 specimens (Supplementary materials - Counting Table). Twenty-nine of them are hyaline, seven agglutinated and three porcelaneous. Diversity exp(H\(_b\)) varies between 2.4 and 13.9 (Fig. 6). The highest values of diversity are identified in Grand-Fort-Philippe and the lowest ones in Boulogne-sur-Mer (10.4 and 3.6 on average, respectively). The foraminiferal density (FD) ranges between 0.36 and 30.4 (specimens/cm\(^3\)) (Fig. 6). The highest values are encountered in the port of Boulogne-sur-Mer and the lowest ones in the Canche Estuary (8.1 and 0.95 on average, respectively). The living benthic foraminiferal assemblages are dominated by calcareous taxa. Agglutinated specimens only occur in areas adjacent to tidal marshes, constantly in low percentages. Haynesina germanica is the most abundant taxon (2.5 specimens/cm\(^3\), on average). This taxon dominates all the study sites, except for Grand-Fort-Philippe. Cribroelphidium excavatum is the second most abundant species (0.73 specimens/cm\(^3\), on average), its highest abundances being found in the harbor of Boulogne-sur-Mer (2.02 specimens/cm\(^3\), on average). In the harbor of Boulogne-sur-Mer, these two taxa represent more than 80% (on average) of the total abundance. Overall they represent, on average, 54% of the assemblages and are associated with minor species (i.e. Arenoparrella mexicana, Bolivina variabilis, B. pseudoplicata, Buliminella elegantissima, C. williamsoni, Elphidium, margaritaceum, C. gerthi, Quinqueloculina seminula; these species have, on average, relative abundance >1% of the assemblage). Bolivinids are dominant (38%) in Grand-Fort-Philippe (B. variabilis 0.42 and B. pseudoplicata 0.17 specimens/cm\(^3\) on average). Elphidiidae are more abundant in the Somme Estuary. Even though not very abundant, we record Ammonia tepida (max 3%, the third most abundant taxon), in the Harbor of Boulogne-sur-Mer and of the agglutinated taxa, Entzia macrocrescens (max 12%) and Trochammina inflata (max 5%) in the Canche Estuary.

### 3.5. Environmental parameters and benthic foraminiferal relationships

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

4. Discussion

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

4.1. Estuarine macrotidal assemblages

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

In response to these almost unpolluted conditions, foraminiferal assemblages are typical of macrotidal estuaries from temperate Atlantic areas. The assemblages are more diversified than in the anthropogenized harbor of Boulogne-sur-Mer, and the overall diversity is comparable to that from the same regions (Armynot du Châtelet et al., 2018a). Based on the CCA, the assemblages seem to be influenced by the type of sub-strate (muddier or sandier) and the elevation. They are dominated by *H. germanica* associated to high percentage of Elphidiidae (*C. excavatum*, *C. gerthi*, *Q. sem*: *Quinqueloculina seminula*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 6. On the left, the gray bar-plots represent the average of foraminiferal density (FD) at each sampling station; colored stacked bar-plots are the density (absolute abundance) of the most abundant species for the replicated samples (a, b, c) at each station. On the right, diversity of benthic foraminiferal assemblages in the study area. The blue bar-plots represent exp(H’bc) for the replicated samples (a, b, c) at each station. Abbreviations of species name are *A. mex*: *Arenoparella mexicana*, *B. var*: *Bolivina variabilis*, *B. pse*: *B. pseudoplicata*, *B. ele*: *Buliminella elegantissima*, *C. exc*: *C. excavatum*, *C. will*: *C. willami*, *E. mar*: *E. margaritaceum*, *C. ger*: *C. gerthi*, *Q. sem*: *Quinqueloculina seminula*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
E. margaritaceum and C. williamsoni), these being taxa common in transitional marine environments (Alve and Murray, 1994; Bouchet et al., 2007; Thomas and Schafer, 1982). In natural intertidal settings, benthic foraminifera are commonly distributed along the elevation gradient (Leorri et al., 2010). Although H. germanica is widespread in intertidal environments along the eastern North Sea coasts, it tends to dominate in the upper part of the tidal gradient, while C. excavatum and E. margaritaceum have been mainly found in non-marsh and subtidal environments (Armony et al., 2018a; Armynot du Châtelet et al., 2018b).

Cribroelphidium excavatum prefers the lowest part of the intertidal gradient (Armony du Châtelet et al., 2005), where more marine conditions prevail (Debenay and Guillou, 2002). Cribroelphidium excavatum and E. margaritaceum occur principally in tidal flat areas of the Somme Estuary, close to the main channel associated with the highest sand contents. This reflects a higher hydrodynamic regime. Similarly, Francescangeli et al. (2017) revealed the presence of E. margaritaceum in the sandy tidal flat area of the Canche Estuary. This could suggest that grain-size (sandy fraction) drives the distribution of these taxa. In the coastal sediment of the Arabian Gulf, Arslan et al. (2016) reported that species belonging to this genus can better survive in quartz sandy substrates. Cribroelphidium gerthi and C. williamsoni have been documented in salt marsh areas and tidal flat areas (Armony du Châtelet et al., 2009; Debenay et al., 2000). In accordance with Francescangeli et al. (2017) and Müller-Navarra et al. (2016), elphidiids could also indicate the occurrence of tidal channels (and/or small ponds) with a local fluctuation of water dynamics. Entzia macrescens and T. inflata are found in very low abundance only in upper-tidal areas close to the limit to the salt marsh. These agglutinated taxa are the most widespread and abundant salt marsh foraminifera (Scott et al., 2001; Scott and Leckie, 1990; Scott and Medioli, 1978). As in most of the intertidal areas in temperate European tidal flats, H. germanica results to be the dominant species within the benthic foraminiferal assemblages. However, the hydrodynamic variability of the estuarine system seems to favor the Elphidiidae.

Fig. 7. Correspondence canonical analysis (CCA) based on substrate (Sand), Elevation, Organic Sediment Index (OSI) and Pollution Load Index (PLI) as constrained parameters and benthic foraminiferal abundances (>2% in at least in one sample) as secondary variables.
4.2. Industrial and anthropogenic-perturbed assemblages

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

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

Comparing our results with others previously reported (Biache and Philp, 2013; Jardé et al., 2007; Zocatelli et al., 2017), higher values of fecal contaminations are observed in the Boulogne harbor, followed by the Canche and Authie estuaries. However, the Sr1 ratio, herein used to highlight fecal contents, is lower than the observed in highly sewage-contaminated sites (Sr1>0.7 (Grimalt et al., 1990)); indicating that the levels of fecal contaminations are reduced from the critical levels at the time of the sampling. Finally, Boulogne harbor shows the lowest values of Sr2 (0.33), indicating human sewage contaminations (Bull et al., 2002; Emrich et al., 2017; Zocatelli et al., 2017). Impacted by the presence of pollution, foraminiferal assemblages are the less diversified in the harbor of Boulogne-sur-Mer. It has been shown that an increase of industrial pollution (metals and PAHs) limits benthic foraminiferal diversity and leads to the dominance of tolerant and opportunistic taxa (Alve, 1995; Armynot du Châtelet et al., 2004; Romano et al., 2016). Benthic foraminiferal assemblages are largely dominated by *H. germanica* associated to *C. excavatum*. Although these taxa are common taxa in naturally intertidal environments (like in the previous areas), these species have been considered as tolerant to industrial pollution (Armynot du Châtelet and Debenay, 2010; Armynot du Châtelet et al., 2011; Debenay et al., 2001; Romano et al., 2008). In a recent reconstruction of the paleoecological evolution of harbor of Boulogne-sur-Mer, Francescangeli et al. (2016) reported, however, that *C. excavatum* is less tolerant to trace metal contamination than is *H. germanica*. Ammonia tepida is, on average, the third most abundant species in the harbor of Boulogne-sur-Mer, being scarcely present in the other studied sites. This taxon has been generally considered as a bio-indicator of stressed conditions in coastal environments (e.g., Vilela et al., 2011). Hence, in the harbor of Boulogne-sur-Mer, the extremely low diversity of the living assemblages and the large dominance of

![Fig. 8. Synthetic scheme of the foraminiferal communities in relation to the different environmental characteristics in the Hauts-de-France. (D = Diversity, OM=Organic Matter, PAH=Poly cyclic aromatic hydrocarbons).](image)
opportunistic species are the response of the biota to human-induced stress conditions.

4.3. Infaunal-dominant assemblages

The embankment and urbanization of Aa river in Grand-Fort-Philippe led to the degradation of the salt marshes habitat (Romont, 1996). Although this site is close to the city and down drift from the nuclear power plant of Gravelines, it is the least impacted in terms of trace metals, PAHs and human-derive faeces (Fig. 8). Studies on benthic fauna indicate that the global 1 °C temperature increase linked to the power plant thermal plume does not change the communities, except some minor changes on sand worms (Hermer, 2010, 2011). The highest organic loads are in Grand-Fort-Philippe. The C/N (4–10) shows a marine-derived origin of the OM, due to the probably occurrence of diatoms bloom. Furthermore, biomarkers (C20 HBI/C16 n-alkane) indicate the most abundant contributions from diatoms (Meyers, 1994).

On average, foraminiferal assemblages in the embankment area of Grand-Fort-Philippe exhibit the lowest FD values and the highest values diversity of all the studied sites. Assemblages are dominated by Bolivina variabilis and B. pseudoplicata. Bolivinids include infaunal species that are common in intertidal environments (Murray, 2006); some of them are tolerant to oxygen-depleted conditions (Alve, 1990; Debenay et al., 2001). They have an elongated and flattened morphology (commonly largely perforated) that seems to be an adaptation to a lack of oxygen (Bernhard, 1986). Higher organic loads associated to low oxygen tolerant species might suggest the occurrence of oxygen deficiency in the sediments of Grand-Fort-Philippe. Hence it might be hypothesized that bolivinids moved up from the deep sediments layer to cope with low oxygen conditions, as mentioned in the Jorissen et al. (1995) and de Stigter (1996) TROX model. The modification of the river flowing could have led to an accumulation of OM in Grand-Fort-Philippe, as testified by the highest OSI. This change might have favored B. variabilis and B. pseudoplicata rather than H. germanica, which was dominant at the other study sites. In addition, Grand-Fort-Philippe receives the most abundant contributions from marine-derived OM (mainly diatoms). Marzocchi et al. (2018) showed that an input of fresh OM (such as diatoms) can stimulate bacterial activity measured as oxygen and nitrate consumption (denitrification) in surface sediment. Hence, it could be that there was a downward transport of diatoms due to mixing sediment factors (such as bioturbation or burying by sediment deposition after a storm). This could have led to a downward decrease of oxygen in the sediment reinforcing the hypothesis of the uprising of bolivinids.

5. Conclusions

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

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

F. Francescangeli: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing.
M. Quijada: Methodology, Formal analysis, Visualization, Writing - review & editing. E. Armynot du Châtelet: Formal analysis, Writing - review & editing, Resources, Supervision. F. Frontalini: Validation, Investigation, Writing - review & editing. A. Treantesaux: Validation, Writing - review & editing, Supervision, Project administration. G. Billon: Methodology, Formal analysis, Writing - review & editing, Visualization. V.M.P. Bouchet: Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

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Appendix A. Supplementary data

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