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Benthic foraminiferal metabarcoding and morphology-based assessment around three offshore gas platforms: Congruence and complementarity

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ABSTRACT

Since the 1960 s, there has been a rapid expansion of drilling activities in the central and northern Adriatic Sea to meet the increasing global energy demand. The discharges of organic and inorganic pollutants, as well as the alteration of the sediment substrate, are among the main impacts associated with these activities. In the present study, we evaluate the response of benthic foraminifera to the activities of three gas platforms in the north-western Adriatic Sea, with a special focus on the Armida A platform for which extensive geochemical data (organic matter, trace elements, polycyclic aromatic hydrocarbons, other hydrocarbons, and volatile organic compounds) are available. The response to disturbance is assessed by analyzing the foraminiferal diversity using the traditional morphology-based approach and by 18S rDNA-based metabarcoding. The two methods give congruent results, showing relatively lower foraminiferal diversity and higher dominance values at stations closer to the platforms (< 50 m). The taxonomic compositions of the morphological and metabarcoding datasets are very different, the latter being dominated by monothalamous, mainly soft-walled species. However, compositional changes consistently occur at 50 m from the platform and can be related to variations in sediment grain-size variation and higher concentrations of Ni, Zn, Ba, hydrocarbons and total organic carbon. Additionally, several morphospecies and Molecular Operational Taxonomic Units (MOTUs) show strong correlations with distance from the platform and with environmental parameters extracted from BIOENV analysis. Some of these MOTUs have the potential to become new bioindicators, complementing the assemblage of hard-shelled foraminiferal species detected through microscopic analyses. The congruence and complementarity between metabarcoding and morphological approaches support the application of foraminiferal metabarcoding in routine biomonitoring surveys as a reliable, time- and cost-effective methodology to assess the environmental impacts of marine industries.

1. Introduction

The exploration and exploitation of non-renewable resources, particularly oil and gas, have rapidly increased to meet global energy demand (Manoukian et al., 2010). The extraction activity results in the discharge of produced water and associated pollutants such as metals, polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) (i.e., benzene, toluene, ethylbenzene, and xylenes, commonly refereed as BTEX) and other chemicals (e.g., phenols and additives) that have the potential to spread many kilometers away from the platform (De Biasi et al., 2007; Gomiero et al., 2011). The presences of platforms can also alter local water flow and sediment grain-size, thereby coupled with their related drilling and production activities negatively affecting the marine environment and posing serious threat to marine communities in the area (Manoukian et al., 2010). A rapid expansion of drilling activities has taken place in the central and northern Adriatic Sea since the 1960 s with the construction of more than 110 offshore gas platforms representing approximately 90% of the offshore platforms

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present in the Mediterranean Sea (Fabi et al., 2004; Scarcella et al., 2011). These intensive gas-related activities might have detrimental effects on biota including benthic organisms (e.g., Gomiero et al., 2013; Punzo et al., 2015a). The impact depends on the number of platforms in the area and their depths and dimensions, the sediment type, and the geographic and oceanographic conditions (Manoukian et al., 2010). Several investigations have evaluated the impacts of platforms along the Italian coast using a wide range of bioindicators such as fish and macrofauna (e.g., Manoukian et al., 2010; Punzo et al., 2015a, 2015b; Scarcella et al., 2011; Spagnolo et al., 2014), as well as biomarkers and bioassays (e.g., Gomiero et al., 2015, 2013, 2011) and multi-marker eDNA metabarcoding (Cordier et al., 2019).

Benthic foraminifera are single-celled organisms that have been extensively used as bioindicators of pollution in a wide range of marine and transitional marine environments (e.g., Alve, 1995; Armynot du Châtelet and Debenay, 2010; Frontalini and Coccioni, 2011; Martins et al., 2016). Traditional morphology-based foraminiferal biomonitoring is laborious, time-consuming and requires highly trained specialists for taxonomical identifications, making it costly and unpractical, particularly for large-scale surveys. The introduction of high-throughput sequencing (HTS) technologies has opened new prospect for the development of foraminiferal metabarcoding (Pawlowski et al., 2014b, 2014a). The HTS of environmental DNA and RNA (eDNA and eRNA) metabarcodes has presented an alternative method to assess the quality of marine environments (e.g., Pawlowski et al., 2016b). Foraminiferal metabarcoding has been successfully applied for the environmental monitoring of fish farms (Pawlowski et al., 2016a, 2014a; Keeley et al., 2012; Pochon et al., 2015; He et al., 2019), oil spills (Moss et al., 2016), mercury (Hg) pollution (Frontalini et al., 2018), and oil and gas drilling activity and production sites (Cordier et al., 2019; Laroche et al., 2018, 2016). It has been reported that macrofaunal and benthic foraminiferal molecular (eDNA/eRNA) data exhibit similar responses to drilling activities (Laroche et al., 2016). A multi-trophic levels evaluation based on bacterial, foraminiferal, and other eukaryote metabarcoding was performed to assess the impact of three offshore oil and gas drilling and production sites and was compared to macrofaunal morphological data (Laroche et al., 2018). Laroche et al. (2018) found that bacteria appear to be the most responsive to impact, followed by foraminifera, macrofauna and general eukaryotes (mostly metazoans). Additionally, Laroche et al. (2018) suggested that eDNA metabarcoding outperforms the traditional morphological-based biomonitoring for oil and gas drilling and production operations.

The aims of the present study are i) to define the potential impact zone around three gas platforms (Armida A, Garibaldi A, and Agostino B) in the northwestern Adriatic Sea (Italy); ii) to compare the performance of metabarcoding and morphological analyses in assessing the impact of Armida A platform on benthic foraminiferal communities; and iii) to identify new potential bioindicators of pollution in the foraminiferal metabarcoding dataset.

2. Study area

The northern sector of the Adriatic Sea is very shallow and gently sloping, with an average water depth of approximately 35 m (Frontalini and Coccioni, 2008). A cyclonic gyre is present in front of the Po River mouth (Russo and Artegiani, 1996) and the northern Adriatic Sea is influenced by the high nutrient input of the Po River. The study area is located in the north-western part of the Adriatic Sea (Italy) in front of the city of Ravenna (Fig. 1a). With 35 gas extraction platforms, this area is characterized by the highest density of platforms in Italy. Among them, three offshore gas production platforms: Armida A (hereafter named as AA), Garibaldi A (hereafter named as GA) and Agostino B (hereafter named as AB) are placed (Fig. 1b). The 6-leg AA (48 × 25 m, 6 wells) was installed in 1985 and is located 13 km from the coast (lat. 44°28′46″, long. 12°27′12″) at 18.3 m water depth. The 8-leg GA (46 × 90 m, 11 wells) was deployed in 1969 and is placed 19 km from the coast (lat. 44°31′20″, long. 12°30′38″) at 24.5 m water depth. The 8-leg AB (46 × 22 m, 11 wells) was installed in 1971 and is located 15 km from the coast (lat. 44°33′13″, long. 12°28′18″) at 21.5 m water depth.

3. Materials and methods

3.1. Sampling strategy

Samples were collected around three offshore gas platforms in July and August 2017. A total of thirty-two sites along four axes at 0 m, 25 m, 50 m, 125 m, 250 m, 500 m, 1000 m and 2000 m from the platforms were sampled with a box-corer. Samples were labeled as the platform (Armida - AA, Garibaldi A - GA and Agostino B - AB) (Fig. 1c), axis (A1: north, A2: west, A3: south, and A4: east), and distance from the structure (0, 25, 50, 125, 250, 500, 1000, and 2000 m) (Fig. 1d). The sampling locations were determined with Global Position System and indicated with WGS84 datum (Table S1).

At each station, the temperature, pH, salinity, oxidation–reduction state (Eh) and dissolved oxygen (DO, expressed as mg/l) of seawater were measured in a vertical profile. Additionally, surface (PR0), intermediate (PR1), and bottom (PR2) water samples were collected using Niskin bottles (2–3 L) at stations 0, 25, 50, 125, and 500 m from the AA platform and assessed for nutrients (i.e., ammonia as N, chlorides, sulfate, N nitrous as NO_2, N nitrous as N, nitrate as N, soluble orthophosphate) and water pollutants (i.e., benzene and ethylbenzene).

For each station, three replicates (labeled as I, II, and III) of sediment samples from independent box-corer deployments were collected, resulting in 288 sediment samples. For the foraminiferal morphological analyses, only the uppermost part (1 cm) of sediment was considered. Immediately after sampling, sediments were placed in Falcon tubes and treated with a rose Bengal solution (2 g of rose Bengal in 1000 ml of ethanol) for at least 14 days to distinguish between living and dead foraminifera and gently mixed. Approximately 10 g of surface sediment (1 cm) was collected using a sterile spoon, placed in a tube with LifeGuard Soil Preservation Solution (MoBio) and immediately frozen at −20 °C. Additional aliquots of sediment from the AA platform only were collected and immediately frozen for subsequent grain-size, organic matter, and geochemical analyses.

3.2. Water and sediment analyses

Water, grain-size, and geochemical analyses were performed at the SGS laboratories for samples collected at AA only. Sediments for grain-size analysis were treated with an H_2O_2 solution, sieved, and dried at 40 °C. Grain-size analysis was conducted to determine the percentage of clay, fine silt, medium silt and coarse silt by sedigraph (Micrometrics 5100) and the contents of very fine sand, fine sand, medium sand, coarse sand, very coarse sand and gravel by ASTM micro-sieve. The total organic carbon (TOC) analysis was performed following the European Standard UNI EN 13137 (2002). For inorganic geochemistry analysis, trace elements (Al, As, B, Cd, Cr, Fe, Cu, Hg, Li, Mn, Ni, Pb, Se, V, Zn) were totally digested (acid-leachable) and then analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) following the U.S. EPA. 6020B (2014) methods, while the mercury analysis was performed in accordance with U.S. EPA 7474 (2007). The Pollution Load Index (PLI) (Tomlinson et al., 1980) was calculated according to Martins et al. (2014). Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*a*) anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenzo(*a,h*) anthracene, benzo(*g,h,i*)perylene, and indeno(1,2,3-*c,d*)pyrene were analyzed according to the U.S. EPA 8270D (2014) method. The analyses of VOCs and the sum of aliphatic hydrocarbons nC6–nC12, n-decane, n-dodecane, n-heptane, n-hexane,

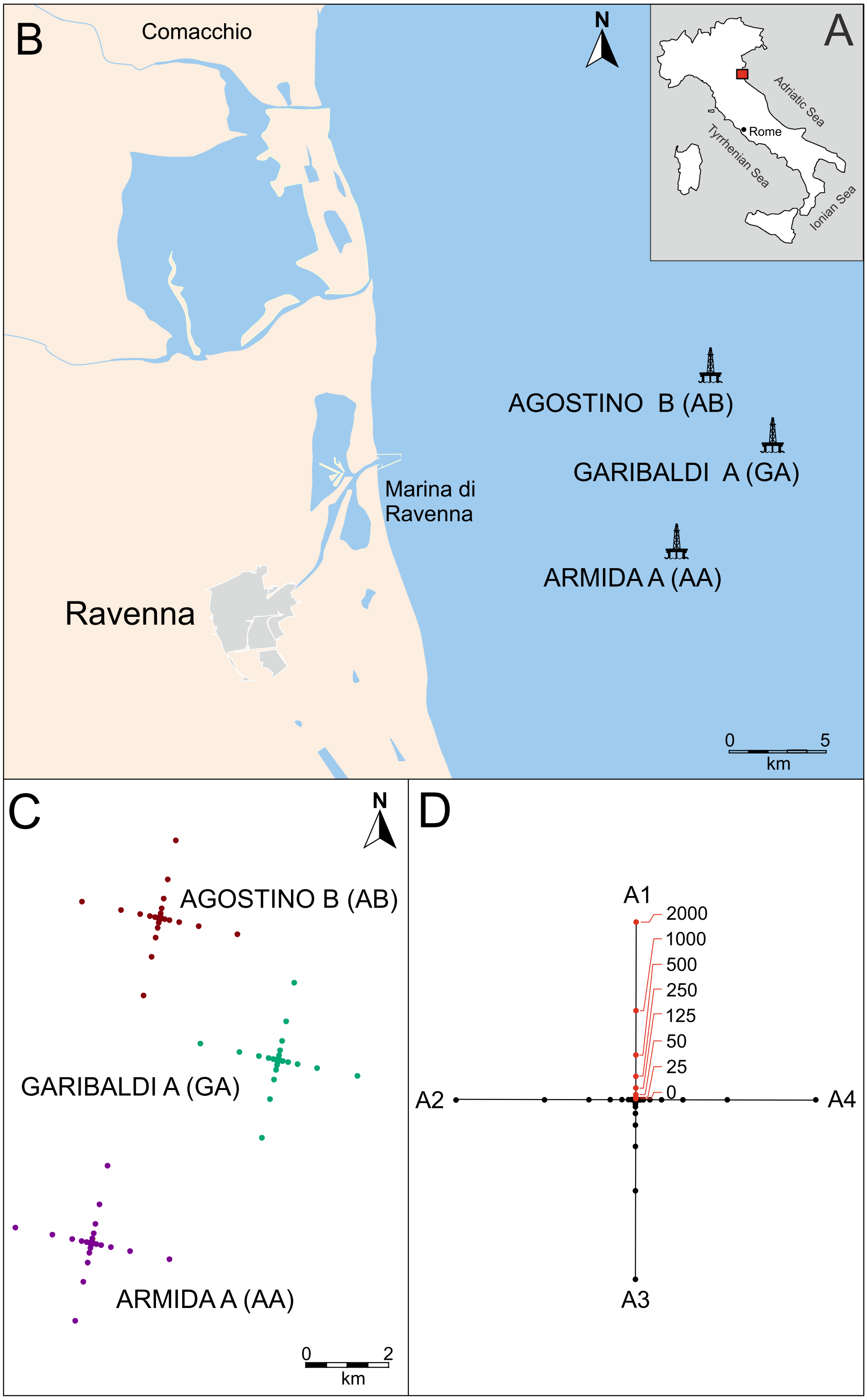

Fig. 1. Site map indicating the geographic position of: (A) the study area within the Adriatic Sea; (B) the three offshore gas platforms (Armida – AA, Garibaldi A - GA and Agostino B - AB) in front of Ravenna coast; (C) sampling stations; and (D) axes (A1: north, A2: west, A3: south, and A4: east), and distance from the platform (0, 25, 50, 125, 250, 500, 1000, and 2000 m).

n-nonane, n-octane, n-undecane, benzene, ethylbenzene, toluene, o-xylene and BTEX were conducted according to the U.S. EPA 8260C (2006) method. Hydrocarbon (C10–C40, n-C12, n-C13, n-C14, n-C15, n-C16, n-C17, n-C18, n-C19 and n-C20) analyses were based on ISO 16703 (2004). The analytical detection limits are reported in Table S2.

3.3. *Benthic foraminifera*

3.3.1. *Morphological analyses*

Sediment samples were dried at 50 °C and weighed, then gently washed through a 63 μm sieve with tap water to remove clay, silt, and any excess dye. Quantitative analyses were performed on the fractions > 63 µm from a single replicate. All living benthic foraminifera were hand-picked and placed in microslides for taxonomical classification. Foraminiferal specimens were taxonomically identified according to the classification references of Frontalini and Coccioni (2008 and references therein). Several foraminiferal parameters including the foraminiferal density (FD), Fisher's α index, Simpson's Dominance (D), and the Shannon index (H'), were calculated using the PAST – PAlaeontological Statistics (Hammer et al., 2001) analysis package (version 1.68) and plotted against distance from the platform.

3.3.2. Metabarcoding analyses

The eDNA extraction, PCR amplification, and high-throughput sequencing (HTS) protocols are described in Cordier et al. (2019). Briefly, three extractions per sediment sample were performed with a DNeasy Power Soil Kit (Qiagen) according to the manufacturer's instructions from 500 µl of sediment (suspended in LifeGuard preservation buffer). The three extractions per sediment sample were then pooled. We PCR amplified the hypervariable region of nuclear 18S rRNA gene (37 + 41f) using foraminiferal-specific primers (forward F1 5′-AAGG GCACCACAAGAACGC-3′ and reverse 17 – 5′-CGGTCACGTTCGT TGC-3′), generating amplicons ranging from 230 to 380 bp. The PCR conditions are described in Table S3. The PCR products were checked by agarose gel electrophoresis, then quantified by high-resolution capillary electrophoresis using the QIAxcel System (Qiagen) and pooled in equimolar concentration. The sequencing libraries were then prepared using the Illumina TruSeq® DNA PCR-Free Library Preparation Kit. The libraries were quantified by qPCR using the KAPA Library Quantification Kit. The sequencing was performed on an Illumina MiSeq instrument using paired-end sequencing for 600 cycles with a V3 kit. The raw data is available from the Sequence Read Archive public database under the accession PRJEB29469.

To retain only high-quality data, stringent parameters were applied to filter out sequence reads with a mean quality score below 30 and those with any ambiguous bases or mismatches in the tagged primers. Chimeras originating from the artificial recombination of different sequences during the PCR cycles were excluded using the *de novo* mode in UCHIME (Edgar et al., 2011). We then clustered the reads into Molecular Operational Taxonomic Units (MOTUs) using the SWARM v2.1.8 algorithm (Mahé et al., 2015) and the default *d* parameter (i.e., 1). The representative reads of each MOTU (the most abundant read of each MOTU) were then compared against curated reference sequence database for taxonomic assignment (http://forambarcoding.unige.ch) using the *assign_taxonomy.py* script from the QIIME v1.9.1 toolkit (Caporaso et al., 2010) with default option (*uclust* method, Edgar, 2010). Annotations were done using the Last Common Ancestor approach from up to three candidate reference sequences with above 95% of similarity with the queries. In the absence of a universal solution for the HTS filtering and to improve the congruence in terms of alpha diversity, and because most of the rarest MOTUs were unassigned, the MOTUs represented by less than 1000 reads were excluded. Similar to the morphological dataset, D, Fisher's α, and the H' indexes were calculated for the molecular dataset to examine their variation against distance from the platforms.

3.4. Statistical analyses and distributional maps

Replicate samples from each station for metabarcoding data were summed to match the morphology sampling design. An additive logarithmic transformation was used to normalize the environmental and the biotic data (log-transformed relative abundances). On the basis of the abiotic sediment data (i.e., grain-size, TOC, metals, Hg, PAHs and hydrocarbons), a Q-mode cluster analysis (CA) was performed using Ward's linkage method and the Euclidean distance. Additionally, a Principal Component Analysis (PCA) was carried out on the same abiotic parameters as primary variables for the AA platform, then additional variables (diversity indexes, relative abundance of species and MOTUs) were computed as secondary variables. All of these statistical analyses were performed using Statistica 8.0 (StatSoft, Inc., 2007). Detrended Correspondence Analysis (DCA) based on the morphospecies (relative abundance > 5%) and MOTUs (relative abundance > 3%) distributions along the transect was performed using the package vegan (Oksanen et al., 2019). Graphs were created using the R package Rioja (Juggins, 2017; R Core Team, 2017).

Differences in the beta-diversity of the molecular and morphological datasets were tested by permutational multivariate analysis of variance (PERMANOVA) based on normalized composition tables (log-transformed relative abundances) using Bray–Curtis dissimilarity matrices with 999 permutations. The tests were performed using the *adonis* function in the vegan R package (Oksanen et al., 2016). The PERMANOVA analysis was based on nested models with three hierarchical spatial factors: platform (i.e., AA, GA, and AB), axis (i.e., A1, A2, A3, and A4) and distance (i.e., 0 m, 25 m, 50 m, 125 m, 250 m, 500 m, 1000 m, and 2000 m). The significance of the Spearman correlation between foraminiferal eDNA MOTUs and morphology Bray–Curtis dissimilarity matrices was tested using the *mantel* function (vegan) for Mantel's test with 999 permutations. A BIO-ENV procedure (Clarke and Ainsworth, 1993) was used to identify the set of explanatory environmental parameters (i.e., grain-size, TOC, PAHs, and trace elements) that produced a Euclidean matrix that best correlated (best R^2 in Mantel Spearman test) with the morphological and molecular communities as determined by the Bray–Curtis dissimilarities matrix for the AA platform. In untransformed data, a Spearman's rank correlation was used ($\alpha = 0.05$) to relate the most abundant MOTUs, morphospecies, and diversity indexes with the extracted environmental parameters and distance. Distributional maps of selected abiotic parameters (e.g., sediment grain-size classes, trace elements, TOC, PAHs and hydrocarbons) were prepared with ArcMap v10.2 software. Inverse Distance Weighting (IDW) was used as an interpolation method.

4. Results

4.1. Environmental parameters for Armida platform

4.1.1. Physicochemical parameters of water

The chloride ion concentration varies between 18,200 and 22,000 mg/L and sulfate concentrations range from 2,500 to 3,000 mg/L. The concentration of ethylbenzene shows only minor variations, ranging from 0.02 to 0.03 µg/L, whereas benzene fluctuates between 0.01 and 0.83 µg/L (Table S4).

4.1.2. Sediment grain-size

The coarser fraction (> 2 mm), when present, is mainly represented by shells or shell fragments. On average, the sand and gravel fractions represent only 3.9% and 6.2%, respectively (Table S5). Mud (< 63 µm) is the dominant fraction (89.9 ± 18%). The fine fraction is mainly represented by silt (68.1 ± 14.5%) and secondarily by clay (21.8 ± 4.9%). Relatively lower values of mud contents are documented at stations closer to the platform (0–25 m) (Fig. 2).

4.1.3. Geochemistry

The concentrations of TOC, metals, Hg, PAHs and hydrocarbons vary greatly around the AA platform (Fig. 2, Fig. S1, Tables S6 and S7). The TOC ranges between 0.7% and 1.8% with a mean value of 0.96 ± 0.21%. Cadmium and Hg vary between 0.14 and 0.26 mg/kg and 0.08–0.22 mg/kg, respectively. Nickel (36–97 mg/kg), Ba (55–1300 mg/kg) and Zn (100–850 mg/kg) show quite high values, particularly at stations close to the platform. Similarly, Cu (20–50 mg/kg) exhibits relatively higher values at stations close to the platforms. The minimum, maximum, mean and standard deviation of all the considered elements are reported in Table S6. The PLI varies over a narrow range from 1.17 to 1.98; this implies the presence of pollution at

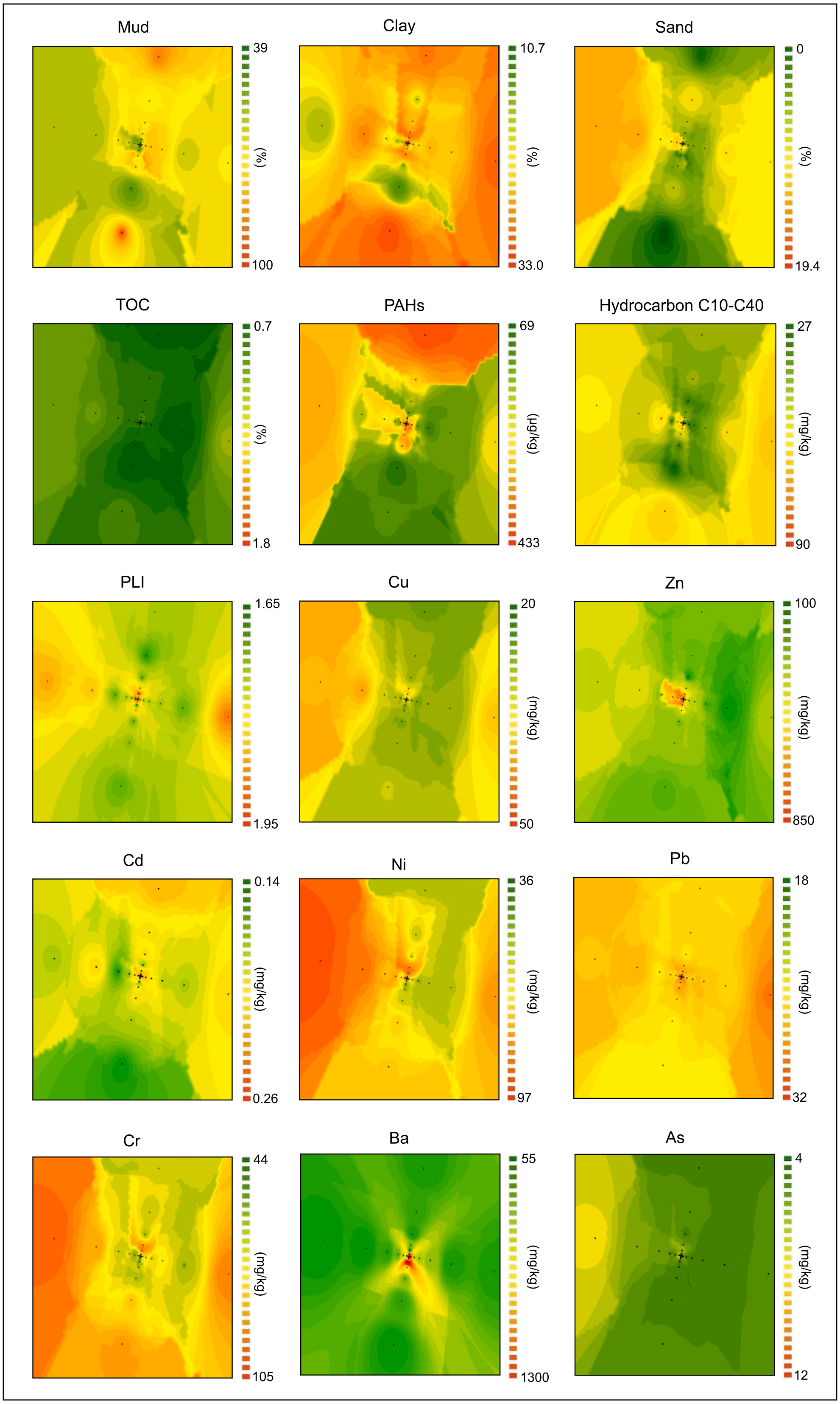

Fig. 2. Interpolation maps for Armida A platform based on Inverse Distance Weighting of selected abiotic variables (grain-size classes, TOC, PLI, PAHs, hydrocarbons C10-C40, and trace elements).

all the studied stations because the values are > 1 (Fig. 2). The total PAH concentrations vary between 69 and 433 µg/kg. The highest concentrations of individual PAH compounds are mostly located close to the platform and at a station 2 km north of the platform.

4.2. *Benthic foraminifera*

4.2.1. *Taxonomic composition*

4.2.1.1. Molecular data. The total number of raw sequences is 17,177,281, from which 5,067,597 are retained in the downstream analysis after stringent quality filtering. Overall, 22,886 MOTUs are produced by the SWARM clustering algorithm. This number has been reduced to 345 by removing MOTUs represented by less than 1000 reads (Table S8). Among them, 84 and 27 MOTUs are assigned to single-chambered, organic-walled or agglutinated Monothalamea and multichambered calcareous or agglutinated Globothalamea, respectively, whereas 234 MOTUs remain unassigned. The MOTUs assigned to the same morphospecies have been combined, reducing the total number of the assigned MOTUs to 43. The number of reads per sample (i.e. the pooled replicates per station) varies from 9614 to 278682.

The assigned MOTUs are mainly represented by monothalamous taxa. The most common monothalamous species are *Bathysiphon flexilis*, *Bathysiphon* sp., *Micrometula* sp., *Cylindrogullmia alba*, *Psammophaga magnetica*, *Vellaria pellucidus*, *Vellaria* sp., *Flexammina islandica*, *Cedhagenia saltatus*, *Saccammina* sp., *Cylindrogullmia* sp., *Hippocrepinella* sp., *Tinogullmia* sp., *Nemogullmia longevariabilis*, and *Globipelorhiza* sp.. The most common MOTUs assigned to Globothalamea are *Ammonia*

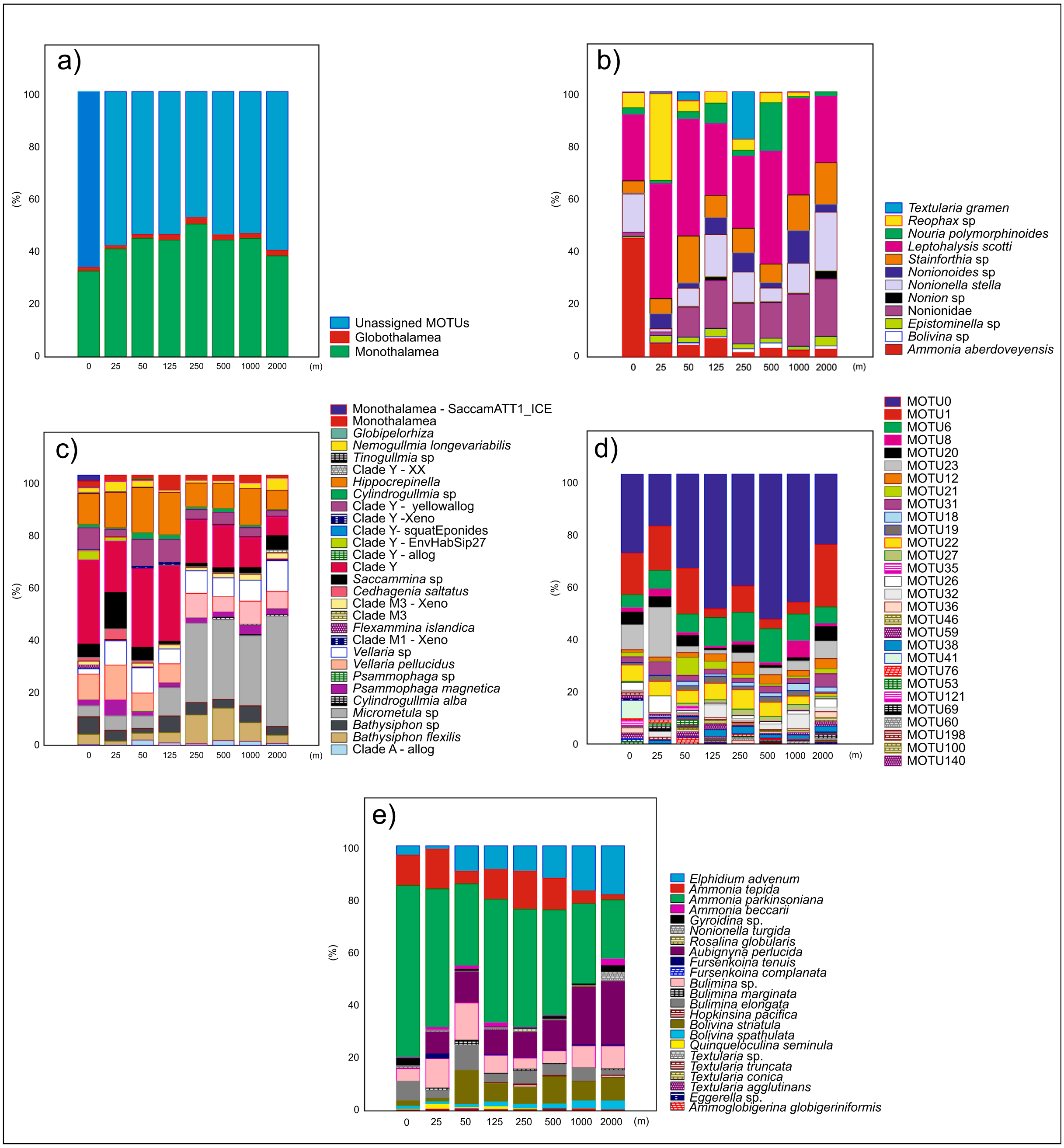

Fig. 3. Histograms showing the relative abundance of (a) Globothalamea, Monothalamea and unassigned MOTUs; (b) MOTUs assigned to Globothalamea; (c) MOTUs assigned to Monothalamea; (d) unassigned MOTUs along a distance gradient from the Armida A platform. morphospecies; and (e) morphospecies. All bars are rescaled to 100% for each histogram.

aberdoveyensis, *Bolivina* sp., *Epistominella* sp., *Nonion* sp., *Nonionella stella*, *Nonionoides* sp., *Stainforthia* sp., *Leptohalysis scotti*, *Nouria polymorphinoides*, *Reophax* sp., and *Textularia gramen*.

Variations in the relative abundance of MOTUs along a distance gradient from the AA platform have been identified (Fig. 3a–d). Monothalamea increases in proportion along a distant gradient from the platform (i.e., the highest values are found between 50 and 1000 m) as does Globothalamea, but the latter represents only a minor component of the molecular dataset (Fig. 3a). Among Globothalamea, *A. aberdoveyensis* shows the highest abundances close to the platform, whereas *L. scotti* records the lowest one. *Bolivina* sp., *Epistominella* sp., Nonionidae, *Nonionoides* sp., *N. stella*, *Nonion* sp., and *Stainforthia* sp. increase in proportion as the distance from the platform increases (Fig. 3b). Among Monothalamea, the most striking shift in relative abundance is observed in the case of *Micrometula* sp., which is less than 1–2% close to the platform (0–50 m) but greater than 10% at the stations situated more than 250 m from the platform. The same pattern is observed for *Bathysiphon flexilis*, *Vellaria* sp. and in a MOTU assigned to clade M3 – Xeno. The opposite is observed in *Saccammina* sp., *V. pellucidus*, and a MOTU assigned to clade Y, whose abundance decreases at distances > 250 m (Fig. 3c). Among unassigned MOTUs, several (e.g., MOTUs 0, 6, 12, 18, 27, 38, 46) are found to increase in abundance at stations farther from the platform (> 50 m), whereas MOTUs 20, 22, 23, 26, 31, 121, 140, 198, 224) show higher abundance close to the platform (< 50 m), and MOTU 224 is present at the 0 m station only (Fig. 3d).

4.2.1.2. Morphological assemblages. All the studied samples, except AB-A1-0 m and AB-A1-25 m, contain living (stained) benthic foraminifera that are commonly associated with fragments of mollusks and ostracods. A total of thirty-two species (7 agglutinated, 3 porcelaneous and 22 hyaline) belonging to twenty genera is identified (Table S9). The relative abundances of morphologically identified species vary from station to station around the three platforms with only twelve species showing relative abundances exceeding 1%, on average, of the assemblage (*Ammonia parkinsoniana, Aubignyna perlucida, Bulimina* sp.*, Bulimina elongata, Bolivina striatula, Elphidium advenum, Ammonia tepida, Bolivina spathulata, Nonionella turgida, Ammonia beccarii, Bulimina marginata* and *Gyroidina* sp.).

In particular, the foraminiferal assemblages around the AA platform are dominated, on average, by *A. parkinsoniana* (41.6%), *A. perlucida* (12.0%), *E. advenum* (10.1%), *A. tepida* (9.5%), *Bulimina* sp. (7.8%), *B. striatula* (7.0%) and *B. elongata* (4.9%) (Table S9). *Bolivina striatula*, *H. pacifica*, *A. perlucida*, and *E. advenum* increase in abundance in stations more distant from the AA platform (25–50 m). By contrast, *A. parkinsoniana* shows the opposite trend, accounting for over 60% of the assemblages at the 0 m station and decreasing in relative abundance to only ~20% at 2000 m (Fig. 3e).

4.3. Statistical analyses

4.3.1. Alpha diversity

A comparison of molecular and morphological data shows very congruent results despite of the different scales (Fig. 4). In the molecular data, the diversity plots show an overall lower diversification (Fisher's α and H' index) and higher D at stations close to the platforms (0 m, 25 m, and 50 m) (Fig. 4). Similarly, lower values of diversity (Fisher's α and H' index) and higher D values are found in the morphological dataset at stations closer to the platforms. Higher diversity values are commonly encountered between the 125 and 2000 m

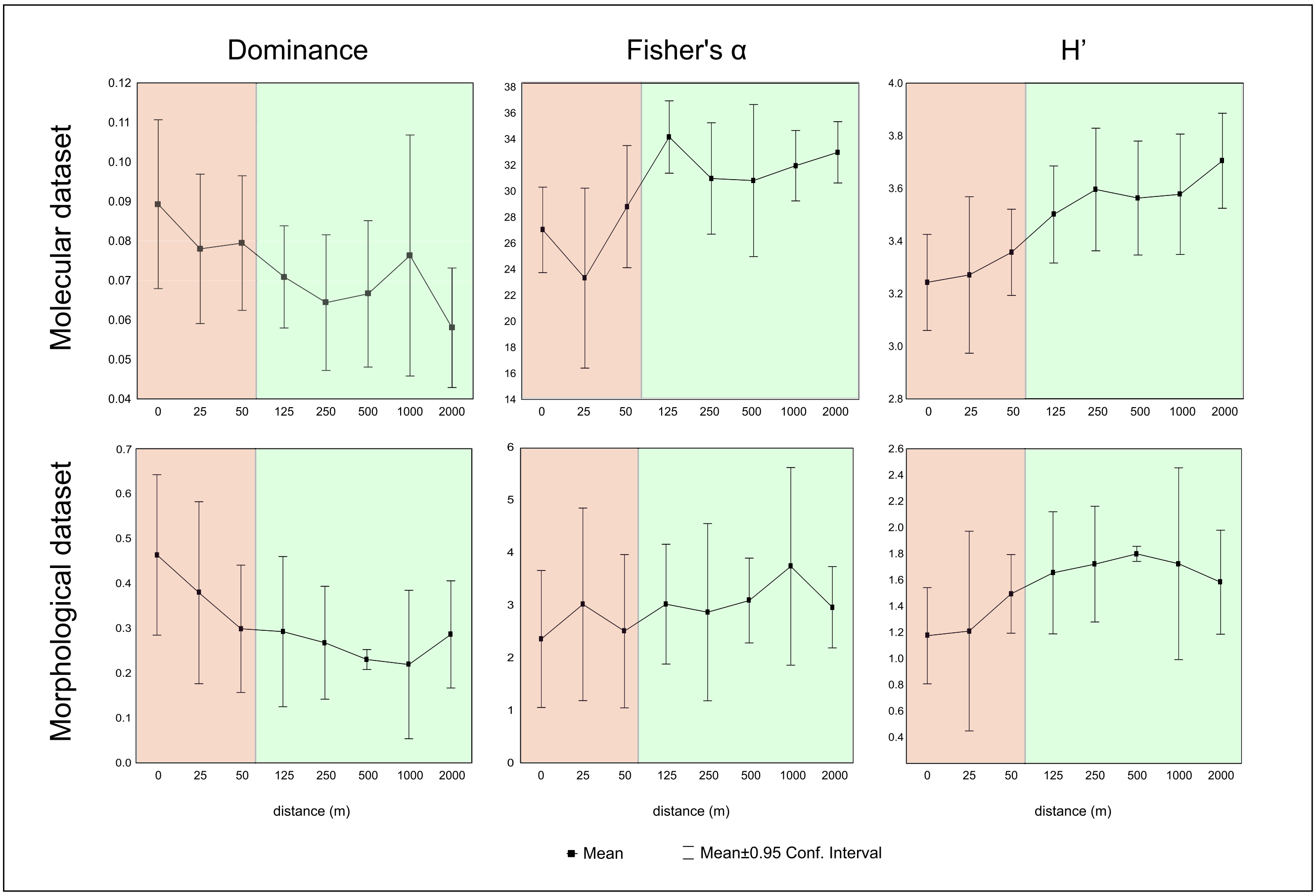

Fig. 4. Averaged values (mean and confidence interval) of dominance (D: Simpson's Dominance), Fisher's α and H' indexes calculated for the molecular and morphological datasets along a distance gradient from the Armida A platform.

stations, with somewhat lower values for the 1000 and 2000 m stations. The other platforms, AB and GA, show great variability along a distance gradient, often with higher values of D and lower values of diversity (Fisher's α and H' index) at stations close to the platforms (Figs. S2 and S3). These trends are evident for the AB platform for stations at > 500 m from the platform, which exhibits an increase in D and a decrease of the diversity index values for both the molecular and morphological datasets (Fig. S2). By contrast, the highest D and lowest diversity values for the molecular dataset are found at 0 m for the GA platform, with an evident increase in diversity for stations located 25 and 50 m away (Fig. S3). However, the morphological dataset of the GA platform shows clear trends that are similar to those of the AA platform data.

4.3.2. *Beta diversity*

Mantel's test finds a significant positive correlation ($r = 0.3$; p-value < 0.001) between the molecular and morphological beta diversity matrices (Fig. S4). The PERMANOVA results show that platform, axis, and distance have a significant effect on the observed compositional changes in both molecular and morphological communities (Table S10). However, these factors only partly explain these changes; in fact, the unexplained variations (residuals) are 0.51 and 0.59 for the molecular and morphological communities, respectively (Table S10). The PERMANOVA results reveal that the highest R^2 value corresponds to the platform for the molecular dataset and to the platform/axis/distance for the morphological dataset (Table S10). On the basis of the BIOENV, the most important environmental parameters are sand, As, Ba, Pb, PAHs, and hydrocarbons C10–C40 for the morphological dataset, and Ni, Zn, and TOC for the molecular dataset (Table S11).

4.3.3. *Cluster analysis*

The Q-mode cluster analysis (based on the abiotic data of AA) resulted in the grouping of samples into two main clusters, A and B. Cluster B can be further subdivided into two sub-clusters, B1 and B2 (Fig. S5). Cluster A includes all the stations located around the platform (0 m) and AA-A2-25. Sub-cluster B1 includes all stations located at an intermediate distance from the platform (25–50 m) and AA-A2-125, whereas sub-cluster B2 comprises stations located at a distance > 50 m from the platform. All stations at 1000 and 2000 m are independently grouped within sub-cluster B2.

4.3.4. *Detrended Correspondence analyses profiling*

The DCA is independently computed for both foraminiferal morphological and molecular datasets and reveals a clear break between 25 and 50 m from the platforms (Fig. 5). This trend is confirmed for all three platforms but the GA for the molecular dataset where the break is at 125 m (Fig. 5) and for each axis (break < 25–125 m) (Figs. S6 and S7) in both datasets.

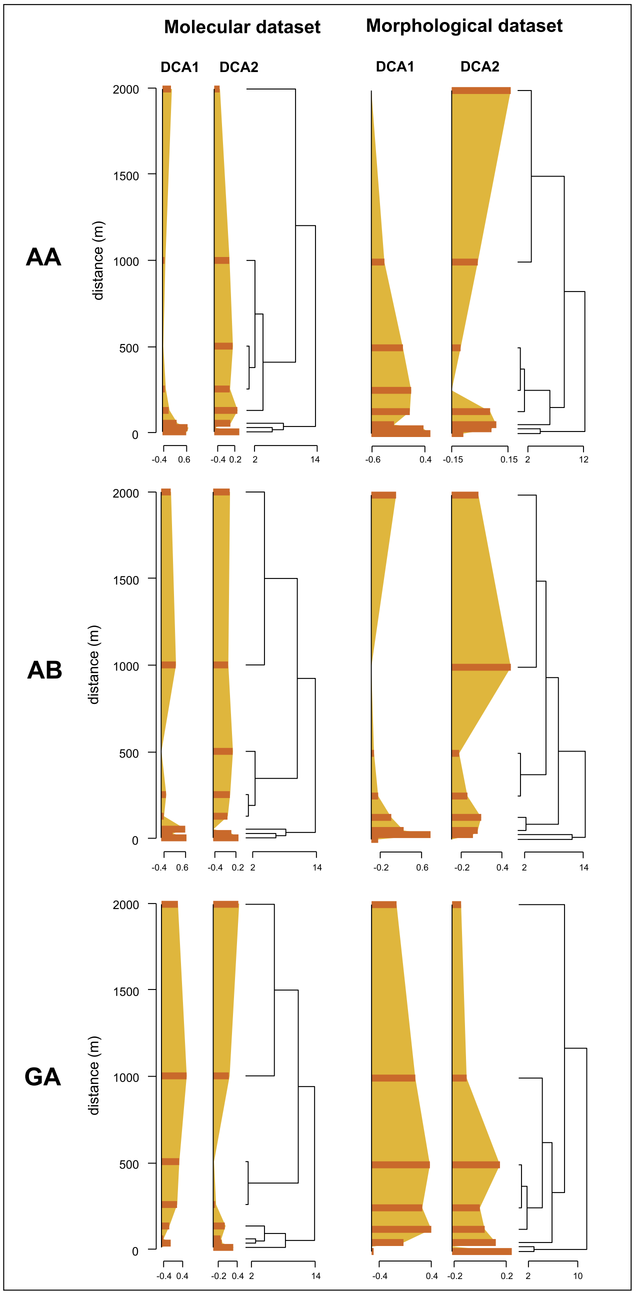

Fig. 5. DCA profiling comparison of the first two extracted components for molecular and morphological dataset of all three platforms (AA, AB and GA).

4.3.5. *Relationship between environmental parameters and benthic foraminiferal datasets*

The PCA shows that ~72.4% of data variance can be explained by the first two principal components (Fig. 6). In particular, the eigenvalues of component 1 (~59.9% of inertia) and component 2 (12.5% of inertia) are 11.9 and 2.5, respectively. Sediment grain-size distributions, TOC, hydrocarbons and some trace elements (Al, V, Ni, Cr, Fe, Ba and Zn) are the predominant elements in the first component, while the contributions to the second component are mainly due to Pb, Cd, Ba, and PLI (Fig. 6). By projecting the diversity indexes (Fig. 6a,b), the morphospecies (Fig. 6c), and the MOTUs (Fig. 6d–f) on the PCA plane, there is an increase in the morphological and molecular diversity and in the abundances of some morphospecies and MOTUs along positive values of the first component. This component is related to the distance from the platform. In particular, the diversity indexes of the molecular and morphological datasets point in the same direction, that is, a decrease of diversity and an increase in dominance at stations closer to the platform (Fig. 6a,b).

Some morphospecies show a distance-dependent trend. *Bolivina striatula*, *A. perlucida*, and *N. turgida* are positively related to the positive values of component 1, while *A. parkinsoniana* exhibits the opposite trend, where it increases in abundance at closer stations (Fig. 6c). Similar patterns are observed in the MOTUs assigned to Globothalamea, with *A. aberdoveyensis* showing affinity towards increasing levels of TOC and gravel, while all other globothalamid MOTUs are positively related to positive values of component 1 that imply an increase in abundance at stations more distant from the platform (Fig. 6d).

Micrometula sp., *B. flexilis*, *Vellaria* sp., *Hippocrepinella* sp., *C. alba* and *Cylindrogullmia* sp. are positively related to the component 1,

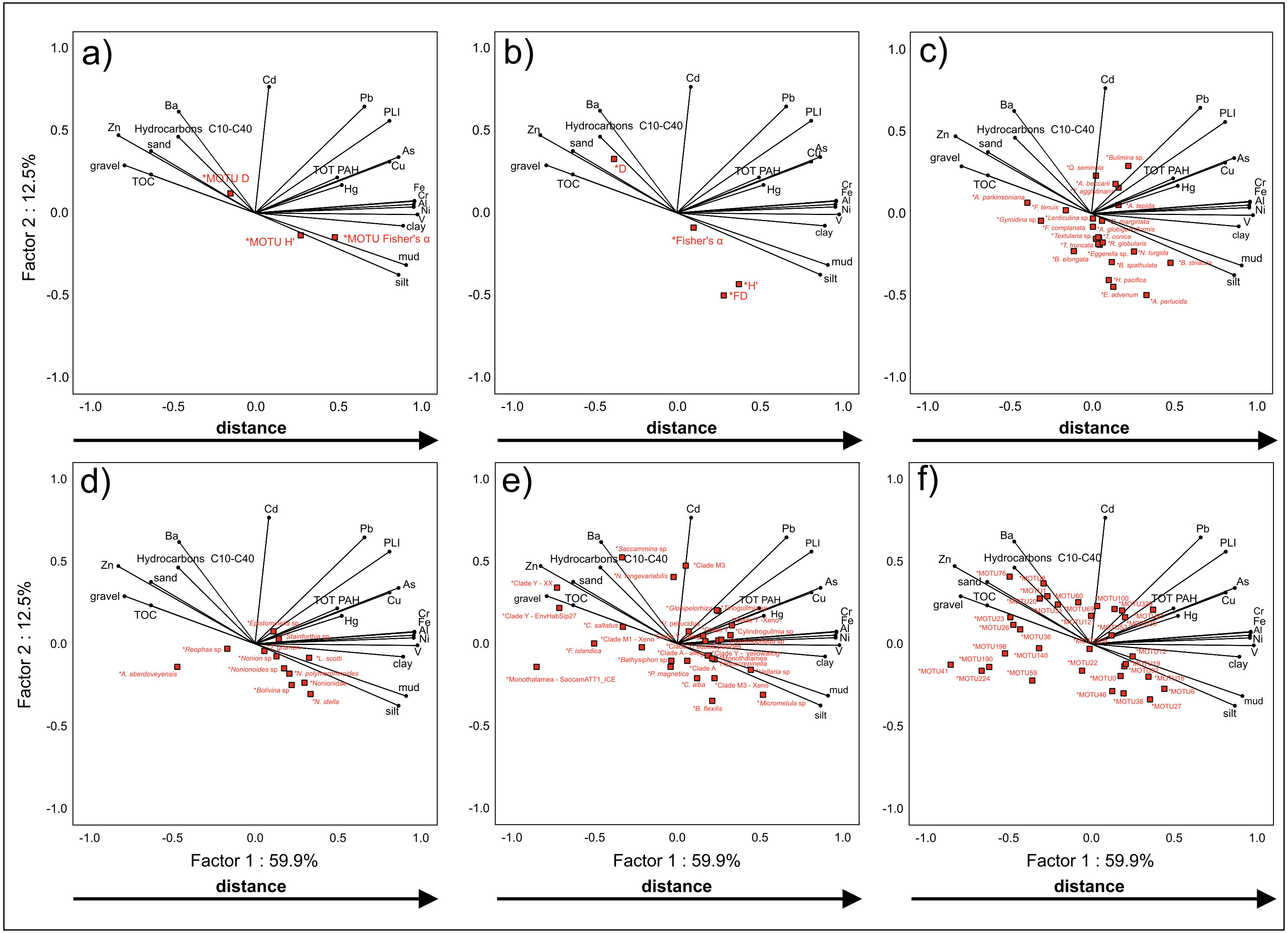

Fig. 6. PCA ordination diagram of sampling based on selected abiotic variables. Secondary biotic variables are also plotted (a) diversity indexes calculated on the molecular dataset; (b) diversity indexes calculated on the morphological dataset; (c) morphospecies; (d) Globothalamea MOTUs; (e) Monothalamea MOTUs and f) Unassigned MOTUs. Stars mark the secondary variables (diversity indexes, morphospecies, and Globothalamea, Monothalamea and Unassigned MOTUs).

whereas Clade Y, *F. islandica*, and SaccamATT1_ICE show an opposite trend (Fig. 6e). An increase in abundance along either positive or negative values of component 1 is also observed for some unassigned MOTUs (Fig. 6e).

Variations in the relative abundance of MOTUs and morphospecies and in the diversity indexes for both datasets have been correlated with the distance from the platform and with the BIOENV extracted environmental parameters (Table S12). The identified significant correlations confirm the findings of the PCA in which distance was not considered but rather used as an underling factor. Positive correlations are found between distance and H’ and Fisher's α for both morphological and metabarcoding data (Table S12). Positive correlations with the distance are also found for MOTUs assigned to *Bolivina* sp., *Epistominella* sp., Nonionidae, *Nonion* sp., *N. stella*, *Stainforthia* sp., *Micrometula* sp., *C. alba*, *Vellaria* sp., Monothalamea Clade M3 - Xeno, MOTU46, MOTU27, MOTU32, MOTU18, and for morphospecies (*B. striatula*, *H. pacifica*, *A. perlucida,* and *E. advenum*). By contrast, significant negative correlations with distance are documented for Monothalamea CladeY, Monothalamea Clade Y - yellowallog, Monothalamea - SaccamATT1_ICE, MOTU140, MOTU22 and for morphospecies *A. parkinsoniana*.

5. Discussion

5.1. Environmental characterization of the impact zone

The extensive available environmental data of the area around the Armida A gas platform enables the assessment of the zone of impact. These acquired data have been compared to the Italian Sediment Quality Guidelines (SQGs), namely, the chemical base level (LCB, absent or low ecotoxicological effect) and chemical limit level (LCL, probable ecotoxicological effects) (APAT-ICRAM, 2007). In most of the samples, several trace elements, Hg, PAHs, hydrocarbons, and BTEX concentrations are below the detection limits (Tables S2, S4 and S6) and below the national and international SQGs levels. There are, however, some exceptions including Ni, Zn, Cu, Cr, acenaphthene and benzo(b)fluoranthene. Copper, Cr, acenaphthene, and benzo(*b*)fluoranthene are detected at concentrations higher than the Italian LCB levels, but only occasionally. Nickel and Zn show instead concentrations commonly above the LCB and sometimes even above the LCL. At some stations, Zn is detected at concentrations five times higher (e.g., 850 mg/kg) than the LCL (i.e., 170 mg/kg). Compared to the sediment guidelines promulgated by the National Oceanic and Atmospheric Administration (NOAA) and the United States Environmental Protection Agency (US-EPA) (Ligero et al., 2002; Long et al., 1995), and the Ontario Ministry of Environment (Burton and Allen, 2002 for a review), Cr and Hg are found at concentrations higher than effect range-low (ER-L) in 25 and 15 sites, respectively, out of the 32 analyzed sites. The concentrations of Zn are even higher than the severe effect level (SEL) and the effect range-median (ER-M) at 1 site and the ER-L at 7 sites. The Ni concentrations exhibit higher values than the SEL at 10 sites and above the ER-M at 22 sites.

According to Tomlinson et al. (1980), a PLI value of 0 indicates pristine conditions, a value of 1 suggests baseline levels of pollutants, and values greater than 1 indicates the progressive deterioration of environmental quality. The PLI values are constantly higher than 1 (mean 1.7 ± 0.2, $n = 32$), suggesting that the environment, in general, is degraded at the analysed stations, including those distant from the platform. The highest PLI values are found at stations within 50–125 m from the platform; the only exceptions are two stations at 0 m along A2 and A3 likely owing to their high sand content. These two stations (i.e., 0 m along A2 and A3) even show the lowest mud percentages (i.e., < 40%). On the other hand, relatively high PLI values (> 1) at distant stations (i.e., > 1000 m) can be ascribed to the higher contents of fine fractions (i.e., clay, Spearman's ρ 0.48, $p < 0.01$, $n = 32$). Indeed, fine sediments have an enhanced capability of retaining trace elements and this could explain the higher values of PLI at more distant stations. Although the concentrations of Ba cannot be compared to national SQGs, its concentrations show a very peculiar distribution around the AA platform, where it is detected at up to 1,300 mg/kg. Significant anomalous concentrations of Ba and Zn have been reported around platforms (De Biasi et al., 2007 and reference therein). Barium is a component of barite ($BaSO_4$), which is widely used in drilling muds as a weighting agent (Hartley, 1996). Barite-enriched muds might contain other elements including As, Cr, Cd, Ni, Pb, Cu, and Zn (Gomiero et al., 2011). Zinc might be partly related to the sacrificial anodes for the corrosion protection of the platform (De Biasi et al., 2007). Based upon the concentrations of the analyzed elements and their comparison with international SQGs, the area can be considered as having low levels of pollution, and the stations with detections exceeding the SQGs values (i.e., Zn) are mostly close to the platform (within 50 m).

5.2. Metabarcoding and morphology provide congruent diversity metrics

A similar trend is documented for the diversity indexes of both the molecular and morphological datasets at all platforms. Specifically, lower values of diversity (Fisher's α and H’) and higher values of dominance are associated with stations close to the Armida platform, in the area within 50 m. This is in agreement with the environmental characteristics of this platform. On the basis of changes in the macrozoobenthic communities, Manoukian et al. (2010) and Spagnolo et al. (2014) defined an impact area of 120 m from the platform during the installation and initial production stages. Three years later, the impact area changed to 30 m, which is comparable to our results. Manoukian et al. (2010) also suggested that the effect on soft-bottom benthic communities might be influenced by the geographical position of the platforms (i.e., the distance from the coast might affect the sediment grain-size and the water circulation and characteristics). The lack of environmental parameters for the other two platforms (Garibaldi A and Agostino B) does not allow us to interpret the results obtained for them. Remarkably, the shift in diversity indexes in relation to the distance is more pronounced for the metabarcoding data than for the morphological data as also supported by the correlation values. This is particularly visible in the case of Fisher's α index, but other indexes show a similar trend. Large variations in the alpha-diversity scales are found between morphological and metabarcoding datasets. Indeed, the number of MOTUs are higher as naked and tiny foraminifera normally overlooked in the morphological analyses are included (Pawlowski et al., 2014b). Although the beta diversities of the two communities (i.e., morphological and molecular) are positively correlated, it seems that the signal contained in the metabarcoding data is stronger, which could be explained by higher number of taxa detected by molecular approach or their higher sensitivity to environmental changes (see below). Notably, the morphological analyses were performed on only one replicate and the interpretations and comparisons with the molecular dataset should be done with care.

The PERMANOVA results show that both the platform and the distance might play significant roles in shaping both molecular and morphological communities. Differences in macrofaunal compositions between platforms in the northern Adriatic Sea were also noted by Manoukian et al. (2010) and Spagnolo et al. (2014). However, a primary control of distance on macrofaunal composition emerges when platforms are individually analyzed (Manoukian et al., 2010). BIOENV analyses independently performed on the two datasets reveal that the most important variables structuring the foraminiferal communities are Ni, Zn, and TOC for the molecular community and sand, As, Ba, Pb, PAHs and hydrocarbons C10–C40 for the morphological community. Nickel and Zn are the only two elements that consistently show values higher than the LCB, and frequently higher than LCL. Barium could not be compared to any SQG, but its highest concentrations are commonly associated with stations within 25–50 m from the platform. These three elements, along with Cr and Pb, might be present at relevant high concentrations in drilling fluids (e.g., Neff, 1987). TOC is one of the most widely used environmental parameters in benthic foraminiferal studies (e.g., Alve et al., 2016) and is known to affect the structure of the foraminiferal assemblages (e.g., Jorissen et al., 2018). Sand represents another important parameter shaping benthic communities, including foraminiferal assemblages. Higher contents of sand are commonly associated with lower TOC values and higher oxygen availability and are characterized by higher occurrence of epiphytic/epifaunal species (e.g., Barras et al., 2014).

Our results are in agreement with previous metabarcoding studies that have observed lower diversity in the vicinity of offshore platforms. The multi-marker analysis of water and sediment samples in the same platforms shows a very limited impact of platforms on the benthic and pelagic communities and is mainly restricted to stations close to the platforms (i.e., within 50 m) (Cordier et al., 2019). Cordier et al. (2019) documented a significant increase in the AZTI marine biotic index (AMBI) calculated from the eDNA sequences of benthic macrofauna at sites located within 50 m distance from the platforms. This finding is supported by an evident change in the foraminiferal morphospecies and MOTU compositions, as revealed by DCA profiling for all platforms. In another metabarcoding study that targeted benthic foraminifera and assessed the impact of two oil platforms off the coast of Taranaki (New Zealand), Laroche et al. (2016) showed a shift in diversity indexes along an impact gradient at approximately 250 m. This difference in the extension of the impact zone could be ascribed to the type of platform and level of platform activity. The foraminiferal alpha- and beta-diversity changes were related to the variations of sediment grain-size, which was coarser close to the platforms, while changes in macrofaunal assemblages were related to hypoxia (Laroche et al., 2016). In our study, the lower diversity index values identified around Armida might be related to either more pollution impact (i.e., Ba, Zn and hydrocarbons) or to a change towards coarser grain-sizes as a results of the altered physical substrate due to the presence of the platform. Similarly, differences in the composition of the benthic communities close to two gas platforms (Calipso and Barbara NW in the northwestern Adriatic Sea) were related to sediment grain-size (Manoukian et al., 2010).

5.3. Foraminifera as bioindicators

The taxonomic composition of the foraminiferal metabarcoding and morphological data are very different. This is mainly due to the different methodologies, which limit the analyses to hard-shelled foraminiferal species (morphology) or comprise all species (metabarcoding). In the latter approach, the hard-shelled taxa are less well represented probably because the DNA of soft-walled or naked species is easier to isolate. The predominance of soft-walled and naked, monothalamous taxa in metabarcoding studies, is well documented (Cordier et al., 2019; Lecroq et al., 2011; Pawlowski et al., 2014a, 2014b). It makes the comparison of these approaches difficult, but it also considerably expands the range of foraminiferal taxa that could become potential bioindicators.

The results of our study perfectly illustrate the complementarity of both types of data. In the morphological dataset, *A. parkinsoniana* is found at increased abundance at stations close to the platform. *Ammonia parkinsoniana* is a common species all along the Adriatic Sea coast at water depths of 10–20 m (Jorissen, 1988). It has been documented as the only morphospecies in the sandy bottoms in the northern Adriatic Sea (Jorissen, 1988) and as the dominant morphospecies in shallow water depths of the central Adriatic Sea (Frontalini and Coccioni, 2008). This taxon, along with *Elphidium* species, was reported to prefer nutrient-poor and oxygen-rich environment (Jorissen, 1988). This taxon has recently been assigned to Ecological Group I as a “Sensitive species” (Jorissen et al., 2018). In our study, the increasing abundance of this species might better reflect the sediment characteristics (i.e., coarsening of the grain-size) rather than the increase of TOC. In fact, TOC shows only minor variations, with a mean of 0.96 ± 0.21% around the AA platform. On the opposite side of the PCA plane, increasing abundances of *N. turgida*, *B. striatula*, *H. pacifica*, and *A. perlucida* are well documented. Such increases are associated with the increased content of finer sediments further away from the platform. Accordingly, *N. turgida* and *Bulimina denudata* have been found to increase along the clay belt in front of the Po River and in the zone most influenced by run-off products (Jorissen, 1988). *Nonionella turgida* has recently been included in the Ecological Group V as a “First-order opportunist” (Jorissen et al., 2018). Another species found in the morphological datasets, *Bolivina striatula*, is regarded as particularly resistant to oxygen-depleted environments and has opportunistic and pollution-tolerant behavior. Hence, it has been included in Ecological Group III, comprising 3rd order opportunists or “tolerant” species (Jorissen et al., 2018). This ecological group also contains *A. perlucida*, which is reported to be “tolerant” in low polluted environment (Frontalini and Coccioni, 2008) and has a preference for environments with high food availability (Jorissen, 1988). Recently, this species was also found to tolerate relatively high organic matter concentrations and prefers muddy substrates (Barbieri et al., 2019).

Among the potential bioindicator species identified in the morphological data, only a few have been found in the metabarcoding data. One of them, *A. parkinsoniana*, is not present in molecular assemblage but most probably this morphospecies corresponds to *Ammonia aberdoveyensis*, which did appear in metabarcoding data, showing exactly the same distribution pattern (Fig. 6d). The molecular systematics of foraminifera are not always in agreement with morphological classification, and it is possible that other species are also identified differently. This is probably for the case of species belonging to the genus *Nonionella* with *N. stella* in the metabarcoding data showing the same trend as the morphospecies *N. turgida*, both increasing in abundance far from the platform at stations characterized by fine sediment. Another species occurring in the metabarcoding dataset but not recognized in our morphological data is, *L. scottii*, which was positively related to the distance and the finer sediment fractions (i.e., silt, clay and mud).

Compared to these few hard-shelled morphospecies that are underrepresented in the metabarcoding data, the common single-chambered monothalamous foraminifera comprise a much larger number of candidate bioindicator species. Among the monothalamids that occurred close to the platform are Monothalamea clade Y, *Saccammina* sp., and several unassigned MOTUs. The most common monothalamids, whose abundances increased with distance, are *Micrometula* sp. and *Bathysiphon flexilis*. Both species have been reported to thrive further away from salmon farm cages in Scotland (Pawlowski et al., 2014a). They also have been shown to be more sensitive to increased Hg compared to the others in a Hg-spiked sediment laboratory experiment (Frontalini et al., 2018). Many potential opportunistic species have also been found among the unassigned MOTUs (Fig. 6f). The majority of prevalent foraminiferal MOTUs detected in the metabarcoding study of offshore platforms in New Zealand (Laroche et al., 2018, 2016) were also unassigned, suggesting that the group of non-identified foraminifera could constitute a valuable reservoir of potential bioindicator species.

5.4. Strengths and weaknesses of foraminiferal metabarcoding applied to biomonitoring

Our study presents several pieces of evidence that support the use of foraminiferal metabarcoding in environmental biomonitoring. First, using metabarcoding to investigate the response to environmental impacts provides very similar results as the traditional approach based on morphological identification of foraminiferal species. For the Armida platform, the approaches show a congruent trend of increasing diversity along the distance gradient, with alpha diversity values significantly changing in the metabarcoding analyses, as well as a marked change in the MOTUs and morphospecies composition. The high congruence of morphological and molecular trends in diversity and biotic indexes has been observed in both marine and freshwater environments when studying diatoms (Visco et al., 2015) and benthic invertebrates (e.g., Lejzerowicz et al., 2015). Second, the metabarcoding datasets comprise a much broader range of taxa, including not only hard-shelled foraminifera but also soft-walled and naked species that are much more abundant and diversified. Therefore, metabarcoding provides a more holistic view of foraminiferal diversity. Many of the species revealed by metabarcoding seem to express specific adaptations to environmental variables considerably increasing the sensitivity of the approach compared to morphological analyses. Third, the taxonomic identification of species based on DNA sequence data overcomes the uncertainties of morphological identification based on personal expertise and increases the taxonomic resolution by taking into consideration genetic variations that might be related to specific ecological adaptations. Fourth, metabarcoding offers the opportunity to process large amounts of samples, in a faster, easier, and relatively cheaper alternative to conventional biomonitoring (Pawlowski et al., 2018, 2016b; Taberlet et al., 2012).

In addition to these advantages, the foraminiferal metabarcoding also has some limitations that should be taken into consideration when interpreting the data it produces. First, the presence of unassigned MOTUs might represent an important part of the molecular dataset; barcoding efforts are therefore required to close this gap. Indeed, the ecology of monothalamous species that predominate in metabarcoding datasets is poorly known, which impedes the direct assessment of ecological status based on a species list, as can be done for many morphospecies. To overcome this limitation, it would be necessary to assign indicator values to particular species or MOTUs based on their occurrences in the metabarcoding data, as it has been done in the case of the taxonomy-free molecular diatoms index (Apothéloz-Perret-Gentil et al., 2017). Accordingly, this taxon-free approach allows inferring the diatom index based on much higher percentages of MOTUs (up to 95%) compared to the morphotaxonomy (Apothéloz-Perret-Gentil et al., 2017). Alternatively, it has been proposed to predict the ecological status using a machine learning approach (Cordier et al., 2018; 2017). However, this approach requires extensive training datasets that are not always available. Other limitations of foraminiferal metabarcoding as a biomonitoring tool are specifically related to the particularities of the method. One of the major issues is the separation of the signal that originates from the extracellular “free” DNA molecules preserved in the sediments from the DNA present in the living cells. To improve the distinction of living cells, some authors have proposed to analyze environmental RNA that presumably has shorter life span than eDNA (Laroche et al., 2017). However, because RNA molecules are more unstable, working with RNA is far more difficult and costly, reducing its attractiveness for routine biomonitoring. Here, we overcome this issue by considering only MOTUs that are represented by more than 1000 reads.

Other issues specific to metabarcoding are various biases related to DNA extraction and PCR amplification that can influence the produced data. The underrepresentation in our datasets of globothalamids compared to monothalamids can be related to the more difficult isolation of DNA from cells covered by hard shells (Pawlowski et al., 2014a). The absence of some species or the overrepresentation of others can be related to the specificity of PCR primers, which might differently amplify some DNA molecules. In general, the relative abundance of species in metabarcoding data is only distantly related to their abundance in morphological analyses expressed by the number of specimens. In fact, large variations in the alpha-diversity scales are found between the morphological and metabarcoding datasets. Despite the technical factors, such as specificity of PCR primers, the relative abundance of foraminiferal metabarcodes can also be biased by the number of rRNA genes copies, the variable number of nuclei, or variations in ploidy related to alternation of generation in foraminiferal life cycle (Weber and Pawlowski, 2013). These different technical and biological biases have to be taken in consideration when interpreting metabarcoding data. Several protocols, guidelines and recommendations have been defined for both ancient (Armbrecht et al., 2019) and recent (Pawlowski et al., in prep) sediment DNA analyses. It is anticipated that the standardization of these methodological procedures and technological advances will optimize and further develop the application of eDNA metabarcoding in biomonitoring and make the results more readily comparable (Ruppert et al., 2019).

6. Conclusions

To conclude, our study supports and reinforces the application of benthic foraminiferal metabarcoding as a quick and reliable methodology for biomonitoring surveys. In particular, our study demonstrates the congruence of molecular and morphological data illustrated by the congruent patterns of alpha diversity and compositional changes along the distance from the platform and according to the environmental variables. Moreover, our research reveals numerous potential bioindicator monothalamous species that could complement those traditionally used in the morphological approach. Albeit very promising, our results raised several questions relative to the potential of foraminiferal metabarcoding. Its wider application in routine biomonitoring will require much more extensive study focusing on the ecology of monothalamous species that are common in metabarcoding data and their sensitivity to particular pollutants and other environmental variables. It is essential to analyze many more samples from impacted areas in order to establish and validate new molecular foraminiferal indexes and to develop predictive models that can infer these indexes from metabarcoding data. We advocate that benthic foraminifera might represent an important proxy for the assessment of ecological status of marine sediments, and therefore we prone their integration into routine DNA based environmental biomonitoring of the impact of marine industries.

CRediT authorship contribution statement

Fabrizio Frontalini: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Tristan Cordier:** Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Eszter Balassi:** Formal analysis. **Eric Armynot du Chatelet:** Formal analysis. **Kristina Cermakova:** Formal analysis. **Laure Apothéloz-Perret-Gentil:** Formal analysis. **Maria Virginia Alves Martins:** Formal analysis, Writing - original draft. **Carla Bucci:** Visualization. **Enrico Scantamburlo:** Formal analysis. **Mauro Treglia:** Formal analysis. **Vladimiro Bonamin:** Formal analysis. **Jan Pawlowski:** Conceptualization, Methodology, Data curation, Formal analysis, Resources, Supervision, Writing - original draft, Writing - review & editing.

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.

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Appendix A. Supplementary material

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