Multi-benthic size approach to unveil different environmental conditions in a Mediterranean harbor area (Ancona, Adriatic Sea, Italy)

Elisa Baldrighi [Corresp., 1, 2], Sarah Pizzini [3, 4], Elisa Punzo [1], Angela Santelli [3], Pierluigi Strafella [3], Tommaso Scirocco [5], Elena Manini [3], Daniele Fattorini [6, 7], Claudio Vasapollo [Corresp. 3]

[1] 1Institute for Biological Resources and Marine Biotechnologies—IRBIM, National Research Council—CNR, Italy, ANCONA, MARCHE, Italy

[2] 2Department of Biology, University of Nevada-Reno, Reno, Nevada, United States of America, Reno, Nevada, USA

[3] 1Institute for Biological Resources and Marine Biotechnologies—IRBIM, National Research Council—CNR, Italy, Ancona, Italy

[4] 3Fano Marine Center, The Inter-Institute Center for Research on Marine Biodiversity, Resources and Biotechnologies, Fano, Italy, Fano, Italy

[5] 1Institute for Biological Resources and Marine Biotechnologies—IRBIM, National Research Council—CNR, Italy, Lesina, Italy

[6] 4Dipartimento di Scienze della Vita e dell'Ambiente (Disva), Università Politecnica delle Marche (Univpm), Ancona, Italy, Ancona, Italy

[7] 5Consorzio Nazionale Interuniversitario per le Scienze del Mare (Conisma), Unità di Ricerca di Ancona (Italy)., ANCONA, MARCHE, Italy

Corresponding Authors: Elisa Baldrighi, Claudio Vasapollo
Email address: elisa.baldrighi@irbim.cnr.it, claudio.vasapollo@cnr.it

Harbors are hubs of human activity and are subject to the continuous discharge and release of industrial, agricultural, and municipal waste and contaminants. Benthic organisms are largely known to reflect environmental conditions they live in. Despite meio- and macrofauna interacting within the benthic system, they are ecologically distinct components of the benthos and as such may not necessarily respond to environmental conditions and/or disturbances in the same way. However, in a few field studies the spatial patterns of meio- and macrofauna have been simultaneously compared. In the present study, we assess the response and patterns in the abundance, diversity, and distribution of the two benthic size classes to the different environmental conditions they live in (*i.e.,* sediment concentrations of selected trace metals and Polycyclic aromatic hydrocarbons (PAHs); organic matter contents and grain size) characterizing the Ancona harbor (Adriatic Sea). Meio- and macrofauna provided partially similar types of information depending on the indices used (univariate measures or community structure/ species composition) and the different 'response-to-stress'. The community structure (*i.e.,* taxa composition) of both benthic size components clearly showed differences among sampling stations located from inside to outside the harbor, reflecting the marked environmental heterogeneity and disturbance typically characterizing these systems. Notwithstanding, the univariate measures (*i.e.,* meio- and macrofauna total abundance, diversity indices and equitability) didn't show similar spatial patterns. Meiofauna were likely to be more sensitive to the effects of environmental features and contaminants than macrofauna. Overall, trace

metals and PAHs affected the community composition of the two benthic components, but only the meiofauna abundance and diversity were related to the environmental variables considered (*i.e.,* quantity and quality of organic matter). Our results pinpoint the importance of studying both meio- and macrofauna communities, which could provide greater insight into the processes affecting the investigated area and reveal different aspects of the benthic ecosystems in response to harbor conditions.

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5 Tommaso Scirocco[1], Elena Manini[1], Daniele Fattorini[4,5], Claudio Vasapollo[1]*

7 [1]Institute for Marine Biological Resources and Biotechnology—IRBIM, National Research Council—
8 CNR, Italy
9 [2]Department of Biology, University of Nevada-Reno, Reno, Nevada, United States of America
10 [3]Fano Marine Center, The Inter-Institute Center for Research on Marine Biodiversity, Resources and
11 Biotechnologies, Fano, Italy
12 [4]Dipartimento di Scienze della Vita e dell'Ambiente (Disva), Università Politecnica delle Marche
13 (Univpm), Ancona, Italy
14 [5]Consorzio Nazionale Interuniversitario per le Scienze del Mare (Conisma), Unità di Ricerca di Ancona
15 (Italy).

17 Corresponding Author:
18 Elisa Baldrighi[1,2]
19 Largo Fiera della Pesca 2, Ancona, 60125, Italy
20 Email address: elisa.baldrighi@irbim.cnr.it
21
22 Corresponding Author:
23 Claudio Vasapollo[1]
24 Largo Fiera della Pesca 2, Ancona, 60125, Italy
25 Email address: claudio.vasapollo@cnr.it

Abstract

28 Harbors are hubs of human activity and are subject to the continuous discharge and release of
29 industrial, agricultural, and municipal waste and contaminants. Benthic organisms are largely
30 known to reflect environmental conditions they live in. Despite meio- and macrofauna
31 interacting within the benthic system, they are ecologically distinct components of the benthos
32 and as such may not necessarily respond to environmental conditions and/or disturbances in the
33 same way. However, in a few field studies the spatial patterns of meio- and macrofauna have
34 been simultaneously compared. In the present study, we assess the response and patterns in the
35 abundance, diversity, and distribution of the two benthic size classes to the different
36 environmental conditions they live in (*i.e.,* sediment concentrations of selected trace metals and
37 Polycyclic aromatic hydrocarbons (PAHs); organic matter contents and grain size) characterizing
38 the Ancona harbor (Adriatic Sea).
39 Meio- and macrofauna provided partially similar types of information depending on the indices
40 used (univariate measures or community structure/ species composition) and the different

41 'response-to-stress'. The community structure (*i.e.,* taxa composition) of both benthic size
42 components clearly showed differences among sampling stations located from inside to outside
43 the harbor, reflecting the marked environmental heterogeneity and disturbance typically
44 characterizing these systems. Notwithstanding, the univariate measures (*i.e.,* meio- and
45 macrofauna total abundance, diversity indices and equitability) didn't show similar spatial
46 patterns. Meiofauna were likely to be more sensitive to the effects of environmental features and
47 contaminants than macrofauna. Overall, trace metals and PAHs affected the community
48 composition of the two benthic components, but only the meiofauna abundance and diversity
49 were related to the environmental variables considered (*i.e.,* quantity and quality of organic
50 matter). Our results pinpoint the importance of studying both meio- and macrofauna
51 communities, which could provide greater insight into the processes affecting the investigated
52 area and reveal different aspects of the benthic ecosystems in response to harbor conditions.

54

Introduction

55

56 Coastal waters are widely recognized as marine areas of high ecological and economic value, but
57 also as highly threatened zone, exposed to multiple human activities (*e.g.*, harbors) and their
58 negative impacts (Travizi *et al.*, 2019).
59 Harbors are essential to the economic growth of coastal regions, where maritime traffic,
60 shipping, international trade, and fishing are continuously increasing (Simonini *et al.*, 2005;
61 Franzo *et al.*, 2022). Harbors are usually characterized by high sediment pollution levels due to
62 heavy metals and hydrocarbons caused by intense maritime traffic and huge organic matter loads
63 (Covazzi Harriague *et al.*, 2007; Baldrighi *et al.*, 2019). The high concentrations of contaminants
64 and the relevant inputs of organic matter represent a persistent and ongoing threat, especially for
65 the biota living in the sediment (Travizi *et al.,* 2019; Franzo *et al.*, 2022). In addition, the
66 exposure of the innermost part of harbors to both wind and waves is limited and may create
67 conditions of reduced water circulation, favoring sedimentation processes, anoxia, and trapping
68 pollutants (Guerra-García & García-Gómez, 2004a; Spagnolo *et al.*, 2011). The EU Water
69 Framework Directive (WFD; Directive 2000/60/EC) considers harbors as 'heavily modified
70 water bodies', which cannot meet the common criteria of good ecological quality status.
71 Therefore, their effective management is crucial for the sustainable use of these maritime spaces
72 and for the protection of the adjacent coastal habitats (Chatzinikolaou *et al.*, 2018; Franzo *et al.*,
73 2022).
74 Benthic organisms are largely known to reflect environmental conditions they live in. Among
75 benthic components, species of meio- and macrofauna are widely recognized as good ecological
76 indicators (Schratzberger *et al.*, 2003; Patricio *et al.*, 2012). Benthic meiofaunal and macrofaunal
77 communities are regularly utilized in impact assessment, but very few studies are carried out
78 taking into account both communities (Whomersley *et al.*, 2009; Frontalini *et al.*, 2011; Covazzi-
79 Harriague *et al.,* 2012; Xu *et al.,* 2014). The meio- and macrofauna are ecologically distinct

80 components of the benthos and as such may not necessarily respond to environmental conditions
81 and/or disturbances in the same way (Schratzberger *et al.,* 2003; Patrício *et al.*, 2012).
82 Aside from differences in body size, meio- and macrobenthos each have a series of distinctive
83 ecological and evolutionary characteristics supporting an expectation of differences in their
84 response to environmental conditions. Meiofauna is characterized by small size, high abundance
85 in marine sediments, ubiquitous distribution, rapid generation times (i.e., months), fast metabolic
86 rates, and absence of a planktonic phase, resulting in a short response time and high sensitivity to
87 different environmental conditions and certain types of disturbance (Giere, 2009). Meiofauna are
88 often classified as *permanent* (species spending their whole lives as meiofauna)
89 or *temporary* (animals that start off as meiofauna but grow into macrofauna) (Giere, 2009). In
90 some environments (e.g., open slope systems), temporary meiofauna (e.g., Bivalvia, Oligochaeta,
91 Amphipoda, Nemertea, Priapulida, Holothuroidea, Ascidiacea Cnidaria and Decapoda larvae)
92 have been found to constitute a high percentage of the meiobenthic community (Bianchelli *et al.,*
93 2010). All these organisms are expected to grow into macrofauna and become part of the
94 macrobenthic population of the slope systems. According to modern phylogenetic approaches,
95 seven phyla belong exclusively to meiofauna, while seventeen phyla are accommodated between
96 meio- and macrofauna (Giere and Schratzberger, 2023).
97 Meiobenthic organisms cover different ecological roles according to their trophic group, living
98 mode, locomotion adaptation to move and digging in different kind of sediment grains and they
99 comprise both unicellular (e.g., Foraminifera, Ciliata) a metazoan organism (Giere, 2009).
100 Moreover, because of their high density even small volumes of samples can be analyzed to
101 assess meiofaunal changes over different spatial scales and environmental conditions (*e.g.*,
102 Schratzberger & Ingels, 2018; Ridall & Ingels, 2021). Consequently, meiofaunal communities
103 have generated considerable interest as potential indicators of anthropogenic disturbances in
104 aquatic ecosystems (*e.g.*, Ridall & Ingels, 2021; Semprucci *et al.*, 2022).
105 There are some advantages in using macrofauna, strictly linked to the functional traits of this
106 group: 1) relative longevity, with many species having life spans in excess of 2 yr, allows the
107 macrofauna to integrate responses to environmental pressures over extended time periods
108 (Simboura & Zenetos, 2002); 2) sedentary or sessile mode of life, therefore organisms are not
109 able to escape stress and integrate the environmental quality in a given area; 3) relatively easy to
110 sample quantitatively, even if larger amount of sediment are needed compared to the meiofauna;
111 4) relatively easy taxonomic identification and available taxonomic keys for most groups and 5)
112 well-documented and predictive response to a number of environmental stressors (thus,
113 community changes can be interpreted with a degree of confidence) (Grey *et al.,* 1988;
114 Somerfield *et al.,* 2006; Todorova *et al.,* 2020). Meio- and macrofauna are ecologically distinct
115 components of the benthos, however both benthic size classes are highly influenced by some
116 main sediment features such as the grain size (Moreno *et al.,* 2008b; Pereira *et al.,* 2018), the
117 quantity and quality of organic matter (Vezzulli *et al.,* 2003; Papageorgiou *et al.,* 2010) and
118 presence of pollutants (McCready *et al.,* 2006; Dauvin *et al.,* 2017). Macrobenthic invertebrates
119 have been identified by the EU WFD as key biological components to assess the ecological

120   status of aquatic ecosystems, due to their important role in ecosystem functioning and to their
121   involvement in food-web nutrient recycling (Punzo *et al.*, 2017).
122   The complementary use of two sets of faunistic groups with contrasting ecological characteristics
123   could provide greater insight into the processes affecting such an area.
124   Up to now, field studies where the spatial patterns of meio- and macrofauna have been
125   simultaneously compared, changes in both assemblages as a response to natural gradients were
126   found to be scattered across habitats (Somerfield *et al.*, 2006; Papageorgiou *et al.*, 2010; Patrício
127   *et al.*, 2012). These investigations have demonstrated the fundamental advantage of a multi-
128   species approach, with the inclusion of many taxonomic and functional groups that have a broad
129   range of sensitivities to any given environmental regime (*e.g.*, Frontalini *et al.*, 2011).
130   Adriatic Sea ecosystem is negatively affected by many kinds of biological and ecological threats
131   *e.g.*, eutrophication, pollution, fragmentation of benthic habitats, invasion of alien species
132   (Katsanevakis *et al.*, 2011; Pećarević *et al.*, 2013; Corriero *et al.*, 2016). In the regional
133   perspective the basin is highly positioned on the list of 'Priority issues in the Mediterranean
134   environment' drawn up by the European Environment Agency (EEA), with 20 (15%) out of the
135   131 hotspot pollution sites identified along the Mediterranean coastline in the frame of the
136   Strategic Action Programme (SAP) of the United Nations Environment Programme (UNEP;
137   EEA, 2006), including many harbors such as the Ancona one (Travizi *et al.*, 2019).
138   Since previous works published on Ancona harbor have always treated the different benthic
139   components separately (Mirto & Danovaro 2004; Spagnolo *et al.*, 2011; 2019; Baldrighi *et al.*,
140   2019; Travizi *et al.*, 2019; Franzo *et al.*, 2022), the aim of the present work is to test whether
141   meio- and macrofaunal assemblages could provide a comparable and/or complementary
142   assessment of its ecological conditions.
143   In the present study, we characterized the meio- and macrofaunal communities (*i.e.,* abundance,
144   diversity, and distribution patterns) in relation to the environmental conditions they live in (*i.e.,*
145   concentration of selected Heavy Metals (HMs) and Polycyclic aromatic hydrocarbons (PAHs);
146   organic matter contents into the sediment and grain size) characterizing the Ancona harbor. We
147   hypothesized that the analysis of two different benthic components may give us more
148   comprehensive information on the environmental features of the harbor, instead of a single
149   benthic size component as is usually done (Xu *et al.,* 2012). In a future perspective, this multi-
150   size approach should be highly recommended in coastal monitoring and management plans.

151

Materials & Methods

152

Sampling area and sampling strategy

153

154

155   The Ancona harbor (water depth range, 4-15 m) is located in the western coast of central
156   Adriatic Sea (Fig. 1), it has a water sheet of 700,000 m^2 and 5,400 m of docks (Spagnolo *et al.*,
157   2011). The harbor is one of the most important of the Adriatic Sea, with intense ferryboat and
158   merchant ship activity. Previous investigations reported that the area is subjected to organic

159   waste dumping derived from fishing boats and is also affected by a strong industrial pollution
160   due to the presence of shipyards. Consequently, a huge organic matter load and high heavy metal
161   and hydrocarbon concentrations are present inside the harbor area (Mirto & Danovaro, 2004;
162   Bianchelli *et al.*, 2016).
163   In the present study, sediment samples were collected in winter 2015 from five sampling stations
164   located from inside to outside the Ancona harbor (Fig. 1). The five sampling stations were
165   chosen according to the different environmental features and anthropogenic activities present in
166   the area: MAN station was located in the inner part of the harbor where small fishing boats dock;
167   PORT station was located in a transition area where work ships are berthed; DS and LR stations
168   were located in a more external position, nearby shipping facilities such as active berths; API
169   station was located outside the harbor where no activity takes place.
170   At each station, sediment samples for characterizing the benthic fauna (meio- and macrofauna)
171   and environmental features were collected with a box-corer (40×30 cm wide and 50 cm high) in
172   three independent replicates (*i.e.,* box-corer deployments), processed and preserved differently
173   according to the analysis to be performed (see below). At all stations, the temperature and
174   salinity at the sea bottom were measured using CTD (Conductivity, Temperature, and Depth)
175   probe equipped with previously calibrated sensors.
176
177   **Environmental variables and microbial component**
178
179   The content of each box-corer was sub-sampled with PVC corers (inner diameter, 4.5 cm) to
180   assess the biochemical composition of the organic matter and grain size. The top 3 cm of
181   sediment from three independent replicates for each parameter were frozen at -20°C, except for
182   the grain size determination, for which samples were kept at *in situ* temperature in single
183   replicate until brought to the laboratory. The biochemical composition of the organic matter
184   (total protein, carbohydrate, and lipid concentration) and chloroplastic pigments (chlorophyll-*a*
185   (Chl-*a*) and phaeopigment (Phaeo) concentration) were determined by standard techniques
186   (Danovaro, 2010). Concentrations were calculated using standard curves and normalized to
187   sediment dry weight after desiccation (60°C, 24 h). Biopolymeric organic carbon (BPC) was
188   calculated as the sum of the carbon equivalents of carbohydrates, proteins, and lipids (Fabiano *et*
189   *al.*, 1995) and was used as a proxy for the available trophic resources. The value of the protein to
190   carbohydrate ratio (PRT/CHO) was utilized as descriptor of the nutritional quality of organic
191   matter in the sediment, with a PRT/CHO ratio >1.0 indicating relatively high quality and high
192   food availability (Pusceddu *et al.*, 2010).
193   For grain size determination, aliquots of fresh sediment were sieved over a 63 µm mesh. The two
194   fractions (>63 µm, sand; <63 µm, silt/mud) were dried in an oven at 60°C and weighed. Data
195   were expressed as a percentage of sediment total dry weight (Pusceddu *et al.*, 2010).
196
197   **Contaminants into the sediment**
198

199 Concentration of selected HMs and PAHs was determined in the surface sediment (0-3 cm)
200 collected at each sampling station and frozen at -20°C. Analyses were conducted following
201 previous validated methods, fully described in Benedetti *et al.* (2014) and Etiope *et al.* (2014).
202 In brief, HMs were determined after digestion under pressure with nitric acid and hydrogen
203 peroxide (7:1), using a Mars 6 Microwave Digestion System (CEM Corporation, Charlotte, NC,
204 USA). As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn were analyzed by Atomic Absorption
205 Spectroscopy (AAS), with flame (SpectrAA 220FS Spectrometer, Varian Inc., Palo Alto, CA,
206 USA) and flameless atomization (240Z AA Spectrometer, Agilent Technologies Inc., Santa
207 Clara, CA, USA), while the Hg content was quantified by Cold Vapor Atomic Absorption
208 Spectroscopy (CVAAS; QuickTrace M-6100 Mercury Analyzer, Teledyne CETAC
209 Technologies Ltd., Omaha, NE, USA).
210 PAHs were determined after KOH-methanol extraction with a Mars 6 Microwave Digestion
211 System (CEM Corporation). Extracts were concentrated using a RC 10.09 Vacuum
212 Concentration System (Jouan SA, Saint-Herblain, France) and purified by J.T.Baker™
213 BAKERBOND™ Octadecyl (C18) Solid Phase Extraction (SPE) cartridges (Avantor Inc.,
214 Radnor, PA, USA). PAHs were analyzed by High-Performance Liquid Chromatography (HPLC)
215 using both Fluorimetric (FLD) and (UV) Diode Array Detection (DAD) (Infinity 1260 Series,
216 Agilent Technologies, Santa Clara, CA, USA).
217 For both HMs and PAHs, appropriated blank solutions and the Standard Reference Material
218 (SRM) 1944: *New York/New Jersey Waterway Sediment* (NIST – National Institute of Standards
219 and Technology), digested as samples, were used to check for accuracy, precision, and
220 recoveries of the employed analytical methodologies; concentrations obtained from SRM
221 analyses were always within the 95% confidence intervals of the NIST certified values.
222 The results obtained in this study were compared with threshold values for chemicals specified in
223 the Ministerial Decree 173/2016, the Italian normative that rules the management of dredged
224 sediments and sets out their quality. Only those values exceeding the upper thresholds values
225 (L2) are defined as alerting values (Table 2.5, Ministerial Decree 173/2016).
226
227 **Meiofauna and macrofauna**
228
229 For meiofauna samples, the content of each box-corer (three independent replicates) was sub-
230 sampled with PVC corers (inner diameter, 4.5 cm). The top 3 cm of sediment, where meiofaunal
231 organisms are typically more abundant, were preserved in 4% buffered formaldehyde. For
232 meiofaunal extraction, sediment samples were sieved through a 500 μm mesh; a 32 μm mesh
233 was used to retain the smallest metazoan organisms. The latter fraction was centrifuged 3 times
234 with LUDOX® HS-40 colloidal silica (diluted with water to a final density of 1.18 g cm^{-3}) and
235 stained with Rose Bengal (0.5 g L^{-1}; Heip *et al.*, 1985). Meiofaunal organisms were counted (no.
236 of individuals 10 cm^{-2}) and identified to the higher taxonomic level (*i.e.,* Order and Class) under
237 a Leica S8APO stereomicroscope.

238 For macrofauna samples, the first 20 cm of three independent box-corer deployments were
239 sieved *in situ* using a 500 μm mesh and all organisms retained were preserved in 5% buffered
240 formaldehyde. Macrofauna was sorted in laboratory using a Leica S8APO stereomicroscope and
241 a Leica DM2500 binocular microscope, identified and classified to the lowest possible
242 taxonomic level, and quantified (no. of individuals m^{-2}). The data collected was subjected to a
243 control and validation process and organized in a dedicated database. The nomenclature of the
244 species was verified and validated using the web portal https://www.marinespecies.org/ and the
245 'WoRMS Taxon Match Tool' (World Register of Marine Species Editorial Board, 2022).
246 Taxon richness (*i.e.,* Orders or Classes and species for meio- and macrofauna, respectively; S),
247 total number of individuals per taxon/species (N), Shannon's diversity index (H', based on log$_2$;
248 Shannon & Weaver, 1949), and Pielou's equitability index (J'; Pielou, 1969) of benthic
249 communities were calculated.
250
251 **Statistical analysis**
252
253 To assess differences in benthic communities among the sampling stations a Permutational
254 Multivariate Analysis of Variance (PERMANOVA; 9999, number of random unrestricted
255 permutations of raw data) was used (Anderson, 2001). The design included one factor: the
256 sampling station (five levels, fixed). The analysis was based on Bray-Curtis' similarity of
257 previously fourth root transformed meio- and macrofaunal data. In case of significant differences
258 obtained by the main test, the pairwise test was performed and, as there was a limited number of
259 unique permutations, the *p* values were obtained from Monte Carlo tests (Anderson & Robinson,
260 2003). Permutational Multivariate Analysis of Dispersion (PERMDISP) test was applied to
261 assess if differences among the sampling stations (between-group) were due to real differences in
262 benthic community composition and not to differences in the multivariate dispersion of replicates
263 (within-group) among their respective centroids. A non-metric Multidimensional Scaling
264 (nMDS) ordination was carried out to visualize similarities among the sampling stations.
265 Similarity Percentages (SIMPER) analysis (cut-off, 90%) was used to identify the meio- and
266 macrofaunal taxa that contributed to the dissimilarity among the sampling stations.
267 One-way Analysis of Variance (ANOVA) was used to explore differences among the sampling
268 stations for organic matter content and microbial component, total abundance, diversity, and
269 equitability indices in meio- and macrofaunal benthic communities. The ANOVA assumptions
270 were tested graphically plotting residuals *vs.* fitted values, normality of residuals and residuals
271 *vs.* covariate (factor station) to assess the variance homogeneity (Zuur *et al.*, 2016). When
272 ANOVA showed significant differences, the Tukey's Honestly Significant Difference (HSD) test
273 was performed to find significant effects between different levels.
274 Since environmental variables have been processed in single replicates, a value of 1σ
275 (corresponding to the 70% of data around the mean in a normal standard curve; Quinn &
276 Keough, 2002) has been subtracted and added to create the missing replicates for which the mean

277 correspond to the original value. Two more values have been created, one lower and one higher
278 than the actual value, and the average of the three values given exactly the observed value.
279 The number of environmental and pollutant-related variables were separated in two groups:
280 pollutants (comprising HMs and PAHs) and environmental variables (comprising the organic
281 matter compounds and silt/mud percentage). Since many compounds had linked each other,
282 multicollinearity (cut-off at Spearman's correlation = |0.8|) among variables was assessed to
283 reduce the dataset and avoid problems in the analysis algorithms. In case of multicollinearity, the
284 variables with a more stringent biological or environmental value were retained acting as proxy
285 for the omitted collinear ones. A summary of the collinear variable and which ones have been
286 chosen is given in Table S1. After normalization, both type of variables was used to characterize
287 the study area by means of a Principal Component Analysis (PCA).
288 Spearman's rank correlation analysis was performed to test relationships between meio- and
289 macrofauna total abundance, taxon richness, Shannon's diversity (Shannon & Weaver, 1949) and
290 Pieolu's equitability (Pielou, 1966) indices, and the environmental features considered.
291 In order to verify the existence of a significant relation between the benthos data matrix and the
292 environmental data (pollutants and environmental variables), the distance-based Linear Modeling
293 (DistLM) procedure was utilized with a backward selection of the variables, and each model
294 assessed by means of the Akaike's Information Criterion corrected for small samples (AICc;
295 Anderson *et al.*, 2008). Since the environmental and pollutant variables were too numerous (even
296 after multicollinearity assessment) respect to the meio- and macrofaunal ones, to further reduce
297 their number, both type of variables was analyzed (separately) by means of another (PCA) to
298 identify groups with similar variability. Only those principal components showing eigenvalues
299 >1 (Kaiser-Guttman criterion; Zwick & Velicer, 1986) were considered. To obtain a better
300 insight into the output loadings, the orthogonal varimax rotation of extracted PCA components
301 was performed. After a varimax rotation, each original variable tends to be associated with one
302 (or a small number) of PCA axis. By doing so, groups of variables were created each of which
303 represented by a single PCA score, and all the scores obtained were used as covariates in the
304 above DistLM analysis to assess the relations between faunal communities and the
305 environmental features. A distance-based Redundancy Analysis (dbRDA) was then applied to
306 visually investigate the relationship between the community assemblages and the environmental
307 data (Anderson *et al.*, 2008). All the above statistical tests were considered significant at α =
308 0.05. Moreover, the RELATE routine (a Mantel test like analysis) was used to search for cross-
309 taxon correlations between meio- and macrofaunal similarity matrices.
310 The PERMANOVA, PERMDISP, nMDS, SIMPER, PCA, RELATE and DistLM procedures
311 were performed with PRIMER™ and PERMANOVA+ ecological software (Clarke & Gorley,
312 2006; Anderson *et al.*, 2008); the ANOVA, the Tukey's test and the VARIMAX procedures
313 were performed using the free software R (R Core Team, 2018; v. 4.1.3).

314
315 **Results**

316

317 **Environmental features and contamination levels in the Ancona harbor**

318

319 Bottom water temperature reported a mean value of 10.3±0.2°C and salinity ranged from a
320 minimum of 31.3 (at LR station) to a maximum of 37.6 PSU (at API station). The sandy fraction
321 (>63 μm) characterized stations located outside the harbor (DS, LR, and API stations), while the
322 silty muddy fraction (<63 μm) was predominant in the inner stations MAN and PORT (Table
323 S2). Chl-*a* and organic matter contents into the sediments showed significantly ($F_{4, 10} = 12.97$, *p*
324 = 0.001) higher values of 'fresh' material (*i.e.,* Chl-*a*) at MAN and DS stations, while the BPC
325 significantly ($F_{4,10} = 22.21$, *p* = 0.001) decreased from inside to outside the harbor (Table S2).
326 The significant ($F_{4,10} = 12.61$, *p* = 0.001) highest value in the quality of organic matter (*i.e.*
327 PRT/CHO) was reported at PORT station (Table S2), due to a particularly low concentration of
328 CHO into the sediment of this station (Table S3).
329 Analyses aimed at determining HM concentrations in the sediment of the Ancona harbor have
330 highlighted a general decrease of contamination values moving from the innermost sampling
331 station (MAN) to those one more external (Table S4). MAN station turned out to be affected by
332 pollution levels 3-4 times higher than those detected at API station, reaching even Cu and Zn
333 concentrations 13 and 6 times higher, respectively. Just these two elements, Cu and Zn, were the
334 only exceeding the threshold limits imposed by the Ministerial Decree 173/2016 (46.31 μg g^{-1} of
335 Cu detected at MAN station; 297.2 and 114.9 μg g^{-1} of Zn detected at MAN and PORT stations,
336 respectively). In the remaining sampling stations, there were no overruns (Table S4).
337 The concentration levels of Σ_{19} PAHs ranged from 73.38 to 213.4 μg g^{-1} following, although not
338 linearly, the same pattern highlighted for HMs, with higher values in the innermost sampling
339 station (MAN), decreasing towards outer ones (Table S4). All the sediment samples showed a
340 distinct predominance of Low Molecular Weight PAHs, mainly driven by the Naphthalene
341 concentration (exceeding the legislative thresholds at MAN, DS, and LR stations), which
342 averagely accounted for 37% of the Σ_{19} PAHs, followed by its methylated isomers: 1- and 2-
343 Methylnaphtalene (24 and 22%, respectively). A prevalence of volatile with 2-3 aromatic rings
344 was reported at all stations (Fig. 2a) (for wind direction and speed on Ancona harbor area during
345 February 2015, please see Fig. S1). Excluding Naphthalene and its related compounds, the PAH
346 residual contamination was mainly ascribable to Phenanthrene and Fluoranthene (Table S4), and
347 the principal PAH diagnostic ratios (Tobiszewski & Namieśnik, 2012), commonly used as a tool
348 to discriminate the analyte origin and sources (Giuliani *et al.*, 2019; Pizzini *et al.*, 2021),
349 indicated a petrogenic origin (Fig. 2b).
350 The results from PCA plot considering the environmental variables, summarized the differences
351 among the sampling stations (Fig. 3). In details, MAN station clearly separated from the other
352 stations being characterized by higher values in pollutants, food sources and mud content into the
353 sediment. This explained the variability along the first axis (41.8% of variation). The variability
354 along the second axis (23% of variation) was mainly explained by the contrast between the DS
355 and LR stations and all the others, the latter characterized by the lowest values in mud content

356   and Fluoranthene into the sediments and by intermediate values between the innermost stations
357   and the outermost station for most of the pollutants and environmental variables considered.
358
Meiobenthic assemblages
360
361   A total of 12 taxa were identified with Nematoda representing the dominant taxon at all sampling
362   stations, with a percentage ranging from 75 to 95% (Fig. 4a). The second most represented taxon
363   was Copepoda with their *nauplii*; among less represented taxa (*i.e.,* others) Bivalvia, Ciliata,
364   Foraminifera, Kinorhyncha, Oligochaeta, Ostracoda, Platyhelminthes, Polychaeta, Sipuncula,
365   and Tardigrada constituted from 3 to 15% of the meiobenthic community (Fig. 4b). Meiofauna
366   abundance and values of its diversity indices are reported in Table S5 and represented in Figure
367   5. The ANOVA tests detected significant differences among all the sampling stations for taxa
368   richness (N; Table 1), with the highest value reported at DS station (Fig. 5a). The ANOVA tests
369   reported significant differences among the sampling stations also for all diversity and equitability
370   indices (Table 1 and Table S5). Regarding the number of taxa, PORT station showed a
371   significantly lower value on average compared to all the other sampling stations (Fig. 5b).
372   Shannon's diversity and Pielou's equitability indices showed both similar patterns, with MAN
373   station presenting the highest value (Fig. 5c and Fig. 5d).
374   PERMANOVA analysis with pairwise test reported significant differences (Table 2) in
375   meiofaunal community composition among all the sampling stations; PERMDISP test did not
376   show any significant dispersion around centroids, confirming that the differences among the
377   sampling stations were due to a real difference in meiobenthic composition (Pseudo-$F_{2,4}$ = 5.38,
378   P(perm) = 0.088).
379   The nMDS plot (Fig. 6a) shows the separation among the sampling stations, as well as a low
380   inter-replica variability. In particular, the innermost MAN and PORT stations were separated
381   from to the outermost ones. SIMPER analysis detected a dissimilarity percentage from 15 (LR
382   *vs.* DS stations) to 54% (PORT *vs.* LR stations). The highest values of dissimilarity percentage
383   were always associated with PORT station and mainly due to very low abundances (*i.e.,* ind.
384   $10cm^{-2}$ 50 ± 8) in some of the most represented taxa such as: Copepoda, Foraminifera, Nematoda
385   and Polychaeta (Table S6). For the other sampling stations, the dissimilarity was mainly due to
386   the presence/absence or differences in the abundances of the taxa others: Bivalvia, Ciliata,
387   Kinorhyncha, Oligochaeta, Ostracoda, Platyhelminthes and Sipuncula (Table S6). Several
388   positive correlations emerged between meiofauna descriptors and quantity of organic matter;
389   moreover, meiofauna abundance and its diversity were positively correlated to some HMs (*i.e.,*
390   Cd, Cu, Hg, Pb and Zn) and to silt/mud content into the sediment (Table S7a). The best model
391   selected by the DistLM analysis, reported in Table 3, comprised only the pollutant compounds.
392   The resulting dbRDA showed that the first two axes explained 83.4% of the variance of
393   meiofaunal community composition, corresponding to the combination of the following
394   environmental factors: V/Ni ratio (commonly used as a diagnostic marker of maritime traffic;

395   Viana et al., 2014), Naphthalene, and Benzo[a]pyrene (ARC1) and Zn, Fluorene, and percentage
396   of silt/mud (ARC4; Fig. S2a).
397
398   **Macrofauna assemblages**
399
400   A total of 93 taxa were identified, these included: 43 Annelida (42 Polychaeta and 1
401   Oligochaeta), 29 Mollusca (22 Bivalvia, 6 Gastropoda, and 1 Scaphopoda), 10 Crustacea (6
402   Amphipoda, 2 Cumacea, 1 Isopoda, and 1 Tanaidacea), 5 Nematoda, 2 Bryozoa, 2 Cnidaria (1
403   Anthozoa and 1 Hydrozoa), 1 Nemertea, and 1 Ophiuroidea (Table S8). Annelida was the most
404   represented group (from 68 to 90% at LR and MAN stations, respectively), followed by
405   Mollusca (from 4 to 24% at MAN and LR stations, respectively) at all the sampling stations.
406   Other less represented groups such as Cnidaria, Isopoda, Ophiuroidea, and Tanaidacea were
407   found only at one or two sampling stations (Fig. 7). Macrofauna abundance ranged from 610 ±
408   241 to 3455 ± 425 individuals m^{-2} at MAN and LR stations, respectively; values of its diversity
409   indices are reported in Table S5 are represented in Figure 8. The ANOVA tests reported
410   significant higher abundance value at LR station (Fig. 8a) compared to all the other stations
411   (Table 1). LR station was also characterized by the highest number of taxa (Fig. 8b) but also by
412   lowest values (Fig. 8c and Fig. 8d) of Shannon's diversity and Pielou's equitability indices
413   (Table 1 and Table S5). PERMANOVA analysis with pairwise test reported significant
414   differences (Table 4) in macrofaunal community composition among the majority of the
415   sampling stations; PERMDISP test did not show any significant dispersion around centroids,
416   confirming that the differences among the sampling stations were due to a real difference in
417   macrobenthic composition (Pseudo-$F_{2,4}$ = 0.99, P(perm) = 0.767).
418   The nMDS plot (Fig. 6b) shows the separation among the sampling stations. In detail, the
419   innermost MAN station and for a lesser extend the outermost API station were distinguished
420   from the others. SIMPER analysis reported high percentages of dissimilarity ranging from 63.4
421   (LR *vs.* DS stations) to 88.3% (MAN *vs.* API stations) between all pairs of sampling stations.
422   The great dissimilarity is mainly due to the presence/absence of species or to a particularly
423   abundant presence of them in one station compared to the others (Table S9). Species like
424   *Ampelisca diadema, Aponuphis bilineata, Jasmineira caudata, Spiophanes bombyx, Kurtiella*
425   *bidentata,* and *Euclymene oerstedii* characterized mainly the outermost API station, while some
426   other species such as *Tubificoides swirencoides, Streblospio* sp*., Heteromastus filiformis,* and
427   *Chaetozone caputesocis* were found inhabiting the innermost stations (Table S9). Four
428   significant correlations (three out of four were negative) were detected between macrofauna
429   descriptors and environmental variables; only macrofauna species richness was (negatively)
430   correlated to three TEs and to the percentage of finest sediment fraction (Table S7b).
431   The best model selected by the DistLM analysis, reported in Table 5, comprised only ARC2
432   (Benzo[ghi]perylene, and Anthracene/(Anthracene + Phenanthrene) and
433   Fluoranthene/(Fluoranthene + Pyrene) diagnostic ratios; Tobiszewski and Namieśnik, 2012) and
434   ARC4 (Zn, Fluorene, and percentage of silt/mud).The resulting dbRDA showed that the first two

435   axes explained the 42.9% of the variance in the macrofaunal community composition (Fig. S2b).
436   This latter low percentage indicates that the residual variance associated to the community was
437   not captured by the graph, and it is likely that an unobserved variable should have had improved
438   the general plot. In any case, along the first axis of the dbRDA there was a clear separation
439   between the innermost stations MAN and PORT, characterized by high values of Zn and
440   percentage of silt/mud compared to the outermost stations LR, DS, and API; while along the
441   second axis LR and DS stations were separated from API, MAN, and PORT stations.
442
443

444   **Discussion**

445

446   **Environmental features of Ancona harbor**

447

448   The presence of muddy sediments in the innermost stations and sand in the outermost ones was
449   clearly due to a reduced exposure to hydrological factors (wind, waves, and currents) which
450   create conditions of poor water renewal inside the Ancona harbor, favoring the presence of fine
451   sediments (Spagnolo *et al.*, 2011). The sediment particle size can influence sediment organic
452   matter load and pollutant content, with fine-grained components commonly showing a high
453   content in organic matter and pollutants (Papageorgiou *et al.*, 2010). This might have facilitated
454   an overall accumulation of HMs and PAHs inside the harbor, as reported in other harbor areas
455   (*e.g.,* McCready *et al.,* 2006; Losi *et al.,* 2021). In particular, the high values of Cu and Zn
456   detected at MAN and PORT stations were likely correlated to the shipyard activities present
457   within the harbor, especially with the use of new generation antifouling paints (Costa *et al.*,
458   2016; Pereira *et al.*, 2018). Furthermore, the pronounced prevalence of volatile, easily
459   transportable PAHs (*e.g.,* Naphthalene; Fig. 2a) pointed out that fuel combustion linked to
460   maritime traffic was the major source of these organic contaminants in the harbor basin. The
461   petrogenic origin of the PAH residual contamination (*e.g.,* Phenanthrene and Fluoranthene; Fig.
462   2b) was supported by the detection in the sediment samples of V, Ni, and Pb, commonly
463   considered as tracers of accidental oil spills and/or marine fuels (El Nemr *et al.*, 2006), as well as
464   by the values of the V/Ni ratio, marker of an intense maritime traffic (Viana *et al.*, 2014).
465   Considering the threshold values for chemicals specified in the Ministerial Decree 173/2016, few
466   values were reported exceeding the established alerting thresholds and always from the
467   innermost stations.
468   Not only contaminants, but also natural and/or anthropogenic changes in the benthic trophic
469   status (*i.e.,* organic matter quantity and sediment biochemical composition) may affect the
470   benthic communities (Pusceddu *et al.*, 2011; Foti *et al.*, 2014). The protein, carbohydrate, lipid,
471   and BPC content in the sediments have been proposed and utilized to assess the benthic trophic
472   status of marine coastal environments, including the Adriatic Sea (Vezzulli & Fabiano, 2006). In
473   Dell'Anno *et al.* (2002) PRT and CHO sedimentary contents were suggested as proxies for
474   organic matter quality and threshold values were established for ranking the trophic status and

475   the environmental quality of coastal marine ecosystems. Applying those thresholds to the
476   investigated sediments, the trophic status of Ancona harbor could be ranked as hyper-trophic
477   (PRT >4 mg g^{-1}) with the exception of the API station, ranked as eutrophic (PRT ≈ 4 mg g^{-1}).
478   However, in terms of CHO content, Ancona harbor should be ranked as meso-oligotrophic
479   system (CHO <5 mg g^{-1}). Pusceddu *et al.* (2009; 2011) identified as eutrophic systems those
480   characterized by BPC concentration >3 mgC g^{-1}, as found at MAN, PORT, LR, and DS stations,
481   and as mesotrophic systems those characterized by BPC concentration in the range 1-3 mgC g^{-1},
482   as in the case of the API station. In Ancona harbor PRT/CHO ratio resulted always >1, indicating
483   a great input of recent production's material and highlighting the good trophic quality of the
484   organic matter (Pusceddu *et al.*, 2009). Increasing of organic loads in the sediment have been
485   usually reported from other harbor areas, especially from the innermost zones (Danulat *et al.,*
486   2002; Covazzi-Harriague *et al.,* 2012; Xu *et al.*, 2014; Rebai *et al.,* 2022). Concentrations of Chl-
487   *a* here reported were extremely high if compared to those reported in February in a previous
488   study conducted along the Adriatic coasts (0.11-0.23 µg g^{-1}; Bianchelli *et al.*, 2016), indicating
489   the presence of 'fresh' primary organic matter. However, similar results in Chl-*a* concentrations
490   were reported from Tunisian harbors (Rebai *et al.,* 2022) along with high level of organic matter.
491   The PCA on measured environmental variables indicated the presence of a clear spatial
492   heterogeneity among the sampling stations and a separation between innermost stations and
493   outermost ones due to higher organic matter -and contaminant loads inside the harbor basin, as
494   previously reported in the same study area (Spagnolo *et al.*, 2011; Baldrighi *et al.*, 2019) and in
495   other enclosed systems (Guerra-Garcìa *et al.,* 2003; Vezzulli *et al.*, 2003; Losi *et al.*, 2013;
496   Dauvin *et al.,* 2017; Mehlhorn *et al.,* 2021). This marked environmental variability in harbors is
497   a common feature. Indeed, environmental disturbance within harbors may change rapidly over
498   spatial scales of a few meters, depending on various factors like the localization and magnitude
499   of pollution sources, allochthonous inputs of different nature, tidal regime, water circulation,
500   harbor position, shape, and size (McCready *et al.,* 2006; Vassallo *et al.*, 2006; Xu *et al.,* 2014).
501
502   **Meiofaunal response to harbor environmental conditions**
503
504   In Ancona harbor, the meiofaunal total abundance, community structure and, for a lesser extent,
505   univariate measures (*i.e.,* diversity and equitability indices) reflected the marked spatial
506   heterogeneity showed by the PCA and the clear separation both between inner and outer
507   sampling stations and among the sampling stations themselves (MAN *vs.* PORT *vs.* DS + LR *vs.*
508   API). Meiofaunal abundance was in the range of values reported by Baldrighi *et al.* (2019) for
509   Ancona harbor (*i.e.* from 912±404 to 2512±717 ind.10cm^{-2}) with lower values characterizing the
510   outermost station and for other harbors and coastal areas affected by pollution and/or high
511   organic matter loads (Vezzulli *et al.*, 2003; Veiga *et al.*, 2009; Pusceddu *et al.*, 2011; Dal Zotto
512   *et al.*, 2016; Semprucci *et al.*, 2016; Sedano *et al.,* 2014; Kulakova, 2022). The only exception
513   was represented by the paucity of meiofaunal organisms found at PORT station. Considering that
514   total meiofaunal abundance was positively linked to products derived from primary production

515 (Chl-*a*, Phaeo, and chloroplast pigment equivalents - CPE), its low abundance at PORT station
516 could be partially justified by the lowest detected value of 'fresh' material (Chl-*a*) and/or other
517 factors known to regulate the coexistence of different organisms such as competition for
518 resources and predation (Schratzberger *et al.*, 2003; Schratzberger *et al.*, 2008). Moreover, given
519 its small size, low mobility, and lack of dispersive life stages (Giere, 2009), meiofauna is more
520 susceptible to within-habitat physical variability and environmental disturbances than larger,
521 more mobile, and potentially more highly dispersed members of the macrofauna (Austen &
522 Widdicombe, 2006; Schratzberger *et al.*, 2008). This would explain the drop in meiofauna
523 abundance, not reported for the macrofauna, at that sampling station. A total of 12 meiofauna
524 taxa (Orders or Classes) were identified and in four sampling stations out of five the majority of
525 taxa were represented. The measures of diversity (*i.e.,* S, H', and J') were comparable to the
526 values reported in harbor areas (*e.g.*, Moreno *et al.*, 2008a; Moreno *et al.*, 2008b; Sedano et al.,
527 2014; Kulakova, 2022) and in enclosed/transitional systems in the Adriatic Sea (*e.g.*, Pusceddu *et
528 al.*, 2007; Pusceddu *et al.*, 2011; Frontalini *et al.*, 2014; Covazzi-Harriague *et al.,* 2012).
529 However, the strong dominance of Nematoda justified the low values reported for the Pielou's
530 equitability index, particularly at LR and DS stations. The dominance of the most resistant and
531 adaptable group is a peculiarity of more stressed, less stable environments and characterized by
532 fine sediment fraction, such as harbors (Semprucci *et al.*, 2015). Abundance and diversity indices
533 were correlated to different proxies of food sources (quantity and quality) into the sediment and
534 to its grain size, confirming the effect of these environmental variables on meiofaunal
535 populations (Balsamo *et al.*, 2010).
536 The dissimilarity among the sampling stations was mainly due to some less abundant and more
537 sensitive taxa, not present in the innermost stations. Usually, organisms that can cope with
538 unfavorable conditions take over (*e.g.,* Nematoda), whereas more sensitive taxa disappear or
539 become rare (Mirto *et al.*, 2014; Zeppilli *et al.*, 2015). In the case of Ancona harbor, Bivalvia,
540 Kinorhyncha, Platyhelminthes, and Tardigrada were found only at the outermost stations (LR,
541 DS, and API) being identified as less tolerant taxa (Baldrighi *et al.*, 2019 and literature therein).
542 Conversely, the more tolerant and widespread groups of Ciliata, Oligochaeta, and Polychaeta
543 (Pusceddu *et al.*, 2007; Moreno *et al.*, 2008a; Moreno *et al.*, 2008b; Semprucci *et al.*, 2015)
544 characterized the innermost stations, along with a non-negligible presence (from 34% to 80%
545 among *other* taxa, Fig.4b) of soft-shelled Foraminifera inhabiting the sediment at all the
546 investigated sampling stations. Soft-shelled monothalamous Foraminifera are an important
547 component living in the sediment and populating the Adriatic Sea (Sabbatini *et al.*, 2013), but
548 most of the time this component is overlooked in meiofauna studies. The high presence of this
549 group has been found to be associate to high values of Chl-*a,* eutrophic conditions, and high
550 variability of environmental parameters (*e.g.*, organic matter loads, salinity, temperature, oxygen
551 content; Sabbatini *et al.*, 2013). The strong tolerance and positive response of some
552 monothalamous species to environmental stress (Sabbatini *et al.*, 2010) may justify their
553 presence in the Ancona harbor. A further identification of the species characterizing these
554 sediments will be necessary, in any case, to confirm our hypothesis. Changes in the community

555 structure were supported by DistLM analysis, which revealed that pollutants and, secondly, the
556 grain size could explain the variability in the meiofaunal composition.
557 Food sources did not have any effect on the meiobenthic community. According to Dell'Anno *et*
558 *al.* (2002) and Pusceddu *et al.* (2009), the system of Ancona harbor can be ranked as eutrophic
559 (inside) - mesotrophic (outside) with high quality of organic matter. Thus, food sources did not
560 constitute a limiting factor for the meiofaunal community (Muniz *et al.,* 2005; Covazzi
561 Harriague *et al.*, 2012; Dal Zotto *et al.,* 2016), as reported instead for oligotrophic systems (*e.g.,*
562 Covazzi Harriague *et al.*, 2013). Same results were reported in Franzo *et al.* (2022) analyzing the
563 nematode communities inhabiting different Adriatic harbors, including that of Ancona. Authors
564 showed that the main environmental factor that shaped the nematode assemblages in all harbors
565 were the PAH concentration levels, while food sources and the grain size were much less
566 relevant. Interestingly, some positive correlations between HMs and meiofaunal abundance and
567 its related univariate measures were reported in the present study, as elsewhere (Schratzberger *et*
568 *al.,* 2003; Xu *et al.,* 2014). In the study conducted by Cibic *et al.* (2017), authors pinpointed as
569 heavy metal content may influence meiofaunal abundance and its composition. The positive
570 nature of the correlation could be the result of a meiobenthic community well adapted to
571 permanent stress conditions (Cibic *et al.*, 2017).
572
573 **Macrofaunal response to harbor environmental conditions**
574
575 In the present study, total macrofaunal abundance and its measures of diversity (*i.e.,* S, H', and
576 J'), fell within the range of values reported by Spagnolo *et al.* (2011) and Travizi *et al.* (2019) for
577 Ancona harbor and from other ports worldwide (*e.g.,* Gusmao *et al.,* 2016; Dauvin *et al.,* 2017;
578 Li *et al.,* 2017; Rebai *et al.,* 2022). Results here reported showed an overall increasing trend in
579 macrofaunal abundance and species richness from inside to outside the study area, however, LR
580 station significantly differed from the other sampling stations when univariate measures were
581 considered. The low values of the Shannon's diversity and of the Pielou's equitability indices
582 detected at LR station pointed to the presence of few dominant species such as *Mytilus*
583 *galloprovincialis* and *Capitella capitata*. Regarding the community structure, species
584 composition differed moving from inside to outside the harbor, overall reporting high
585 percentages of dissimilarity among stations (SIMPER analysis, Table S9).
586 The macrobenthic community was mostly composed by the dominant groups of Annelida,
587 Mollusca, and Crustacea, as usually reported from enclosed environments impacted by pollutants
588 and characterized by high organic matter loads (Guerra-García & García-Gómez, 2004b;
589 Spagnolo *et al.*, 2011; Travizi *et al.*, 2019). Among the group of Annelida, the Oligochaeta
590 species *T. swirencoides* was identified for the first time in Ancona harbor and it was found to be
591 particularly abundant in all the sampling stations and even dominant at LR station. Only at API
592 station the species was absent. Tubificid oligochaetes, also called sludge worms, are very
593 common in high polluted areas (Brusca & Brusca, 2003) and they are recognized as a pollution-
594 tolerant taxon (Pelletier *et al.*, 2010). Thus, *T. swirencoides* was the species most responsible for

595   the difference between the innermost sampling stations and the outermost one (SIMPER
596   analysis, Table S9). Species composition may be affected by pollutant concentrations and high
597   levels of organic matter, through a decrease in diversity and abundance of sensitive species
598   (Callier *et al.*, 2009). Most of the species found inhabiting the Ancona harbor sediments were
599   typically soft-bottom species and belonged to the ecological groups of disturbance-tolerant,
600   second- and first-order opportunistic species (Borja *et al.*, 2000). Polychaeta, along with the
601   tubificid oligochaeta *T. swirencoides,* were the taxa most represented and diversified. Many
602   Polychaeta species have a high level of tolerance to adverse effects such as pollution and natural
603   perturbations (Borja *et al.*, 2000), and for this reason they usually constitute the majority of
604   benthic organisms living in harbor systems (Guerra-García & García-Gómez, 2004b). Usually,
605   Polychaeta species richness and their diversity inside of harbor areas are low because of high
606   pollution levels and the lack of oxygen in the water column (Estacio *et al.*, 1997; Dhainaut-
607   Courtois *et al.*, 2000). The same trend of increasing Polychaeta diversity moving outside the
608   harbor area was also reported in the present study, with tolerant and opportunistic species such as
609   *Capitella capitata*, *C. caputesocis*, *H. filiformis*, *Sternaspis scutata* and *Streblospio* sp. (Borja *et
610   al.*, 2000) particularly abundant in the innermost sampling stations. The species *Prionospio
611   cirrifera*, mainly recorded outside the harbor (API station), is traditionally identified as an
612   opportunistic spionid living in silty-clay sediments with high organic content (Borja *et al.*, 2000;
613   Simonini *et al.*, 2004). Spagnolo *et al.* (2011) reported the same finding and authors explained
614   this as a result of a scarce tolerance of *P. cirrifera* to copper, detected at a concentration 9 times
615   higher at the innermost MAN station compared to the average concentration detected at all the
616   other sampling stations. A similar consideration could arise for the high abundance of the
617   opportunistic species *S. bombyx* at API station. Polychaeta species ranked as disturbance-
618   sensitive (Borja *et al.*, 2000), such as *Aricidea fragilis, Glycera capitata, Paradoneis armata*,
619   and *Paraonis fulgens* have also been found inhabiting the most impacted sampling stations inside
620   the harbor but in lower abundances compared to the opportunistic species. This leads us to think
621   about a certain kind of adaptation of these species to cope with less favorable conditions, but this
622   aspect needs further investigation. Due to their economic and ecological importance, as well as
623   their sedentary life, Mollusca has assumed a major role in monitoring contaminants worldwide
624   (Pizzini *et al.*, 2015; Pizzini *et al.*, 2017; Grotti *et al.*, 2016). *Kurtiella bidentata*, *M.
625   galloprovincialis*, and *Nucula nitidosa* are defined as disturbance-tolerant species and they
626   tended to dominate the innermost sampling stations in Ancona harbor, with the only exception
627   for *K. bidentata*, particularly abundant at API station. *Abra alba* characterized LR and DS
628   stations, confirming its preference for sandy sediments with medium-high levels of organic
629   matter quantity and quality (Guerra-García & García-Gómez, 2004b). This species has been
630   reported to abound in harbors affected by heavy metal pollution (Dhainaut-Courtois *et al.*, 2000).
631   LR station was characterized by a conspicuous presence of the Nonindigenous species (NIS)
632   bivalve *Anadara transversa*, bivalve of Indo-Pacific origin (Streftaris & Zenetos, 2006)
633   belonging to the family Arcidae. Members of this family have a red color blood due to a high

634 consistent level of hemoglobin in their bodies, allowing them to colonize habitats with low
635 oxygen concentrations (e.g., Zenetos, 1994; Morello *et al.,* 2004).
636 A large number of crustaceans (Amphipoda, Isopoda, Tanaidacea) have been categorized as
637 pollution-sensitive taxa, especially compared to Polychaeta (Pelletier *et al.*, 2010). Crustacean
638 communities have been considered to be among the most sensitive to changes in environmental
639 variables (Gómez-Gesteira & Dauvin, 2000), and for this reason crustacean species richness and
640 diversity inside harbors are generally considerably low (Estacio *et al.*, 1997; Dhainaut-Courtois
641 *et al.*, 2000). Three abundant species characterized the innermost sampling stations in Ancona
642 harbor: the Amphipoda *Leptocheirus pilosus,* the Caprellida *Phtisica marina*, and the Tanaidacea
643 *Apseudopsis latreillii. P. marina* and *A. latreillii* have been reported in high number in sediments
644 containing less sand and high concentrations of N, P, Cu, and organic matter (Guerra-García &
645 García-Gómez, 2004a). *A. latreillii* belongs to the group of species that may occur under normal
646 conditions, but whose populations are stimulated by organic enrichment, while *P. marina*
647 belongs to the group of species very sensitive to organic enrichment. According to the present
648 study and the results of other previous investigations (Conradi *et al.*, 2000; Guerra-García &
649 García-Gómez, 2001; Guerra-García & García-Gómez, 2004a) this species is able to live even in
650 impacted habitats with moderate-high levels of heavy metals and PAHs. Conversely, the more
651 sensitive Amphipoda *Ampelisca diadema* dominated the crustacean assemblages at API station.
652 As for the meiofauna, pollutant content and the different sediment texture inside and outside the
653 Ancona harbor affected the macrofaunal composition, as previously reported (Guerra-García and
654 García-Gómez, 2001; Guerra-García & García-Gómez, 2004a; Guerra-García & García-Gómez,
655 2004b; Spagnolo *et al.*, 2011; Travizi *et al.*, 2019). Univariate descriptors as well as the analysis
656 of species characterizing the benthic communities indicated the presence of modified, but quite
657 diverse and presumably well-established soft-bottom communities in all the investigated
658 sampling stations. This might reflect the successful adaptation of many pollution-tolerant species
659 to the long-term pollution and unstable environmental conditions of Ancona harbor (Travizi *et*
660 *al.*, 2019).

661 **Meiofauna and Macrofauna comparison**
662
663 Meio- and macrofauna can co-vary, even if the contrasting ecology of these two benthic
664 components can give us different information on the environment where they live in (Austen et
665 al., 1989; Schratzberger et al., 2003; Patrìcio et al., 2012). When compared together, both
666 taxonomical groups inhabiting Ancona harbor showed weak but significant relationship (Table
667 S10), as also evident from the nMDS (Figure 6). The weakness was likely due to the different
668 response of meiofauna to the PORT conditions, characterized by an extreme paucity in this
669 smaller benthic component.
670 However, with the only exception for PORT station, all the other stations followed the same
671 distributions for both benthic component, notwithstanding the different intra-replica variability.
672 This suggest that, even if the two faunal categories respond differently to the environmental and
673 pollutant variables (or at least with different intensity), both capture and highlight the

674 disturbances affecting the harbor area. This result, enforce the concept of cross-taxon congruence
675 (i.e., an interoperability among groups of organisms that respond as a unique community,
676 although both conserving their peculiarities) (Su *et al.*, 2004). Similar congruence between
677 meiofauna and macrofauna groups was described by, *e.g.,* Corte *et al.* (2017); Cronin-O'Reilly et
678 al. (2018). Notwithstanding, this result should be taken with caution as the relations between
679 different groups of organisms could be weak, as it is in the present case, to provide reliable
680 predictions of biodiversity in impacted areas (Heino, 2010), thus more data are needed to
681 confirm or definitely discard the usability of cross-taxon congruence.
682

683 ## Conclusions
684 In considering two benthic size components at the same time (meio- and macrofauna), we were
685 provided by a broader response to environmental conditions in the Ancona harbor. The following
686 considerations emerged:
687 - The meiofauna was affected by the quality and quantity of organic matter, suggesting that
688 meiobenthic assemblages were more receptive to within-habitat food variability than
689 macrofauna.
690 - Both invertebrate groups were characterized by distinctive assemblages across the harbor,
691 particularly evident for the meiofauna, consistent with changes detected for environmental
692 features.
693 - The present investigation confirmed the fundamental advantage of a multi-benthic size
694 approach, with the inclusion of different taxonomical groups considered to cover a broader
695 range of functions into the ecosystem. Optimally, both groups should be used in marine
696 pollution monitoring programs included in the EU Marine Strategy Framework Directive
697 (MSFD; Directive 2008/56/EC) in the context of its Descriptor 1 'maintenance of
698 biodiversity' and Descriptor 6 'sea floor integrity'.

699

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704

705

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Figure 1

Map of the sampling area (Ancona harbor) and location of the five sampling stations.

The pink rectangle indicates the geographical position of the Ancona harbor, Italy.

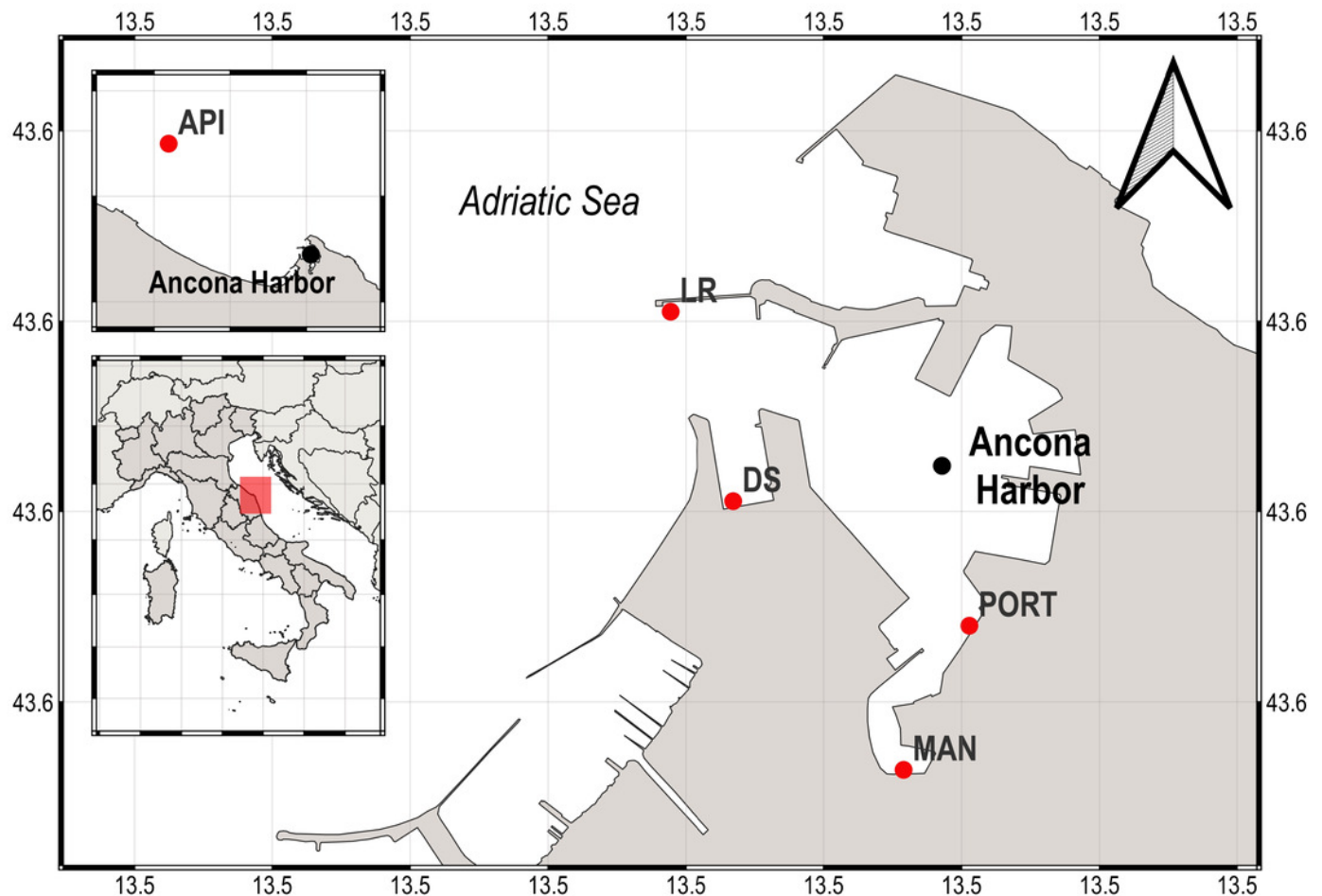



Figure 2

(a) Distribution pattern and (b) origin of Polycyclic aromatic hydrocarbons (PAHs) characterizing the sediment at the investigated sampling stations.

Σ LMW = sum of Low Molecular Weight compounds (Naphthalene, 1-Methylnaphtalene, 2-Methylnaphtalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene); Σ HMW = sum of High Molecular Weight compounds (Fluoranthene, Pyrene, Benz[*a*]anthracene, Chrysene, Benzo[*b*]fluoranthene, Benzo[*k*]fluoranthene, Benzo[*a*]pyrene, 7,12-Dimethylbenz[*a*]anthracene, Benzo[*ghi*]perylene, Indeno[1,2,3-*cd*]pyrene, Dibenz[*a*,*h*]anthracene); Σ COMB = sum of 9 non-alkylated PAHs (Fluoranthene, Pyrene, Benz[*a*]anthracene, Chrysene, Benzo[*b*]fluoranthene, Benzo[*k*]fluoranthene, Benzo[*a*]pyrene, Benzo[*ghi*]perylene, Indeno[1,2,3-*cd*]pyrene).

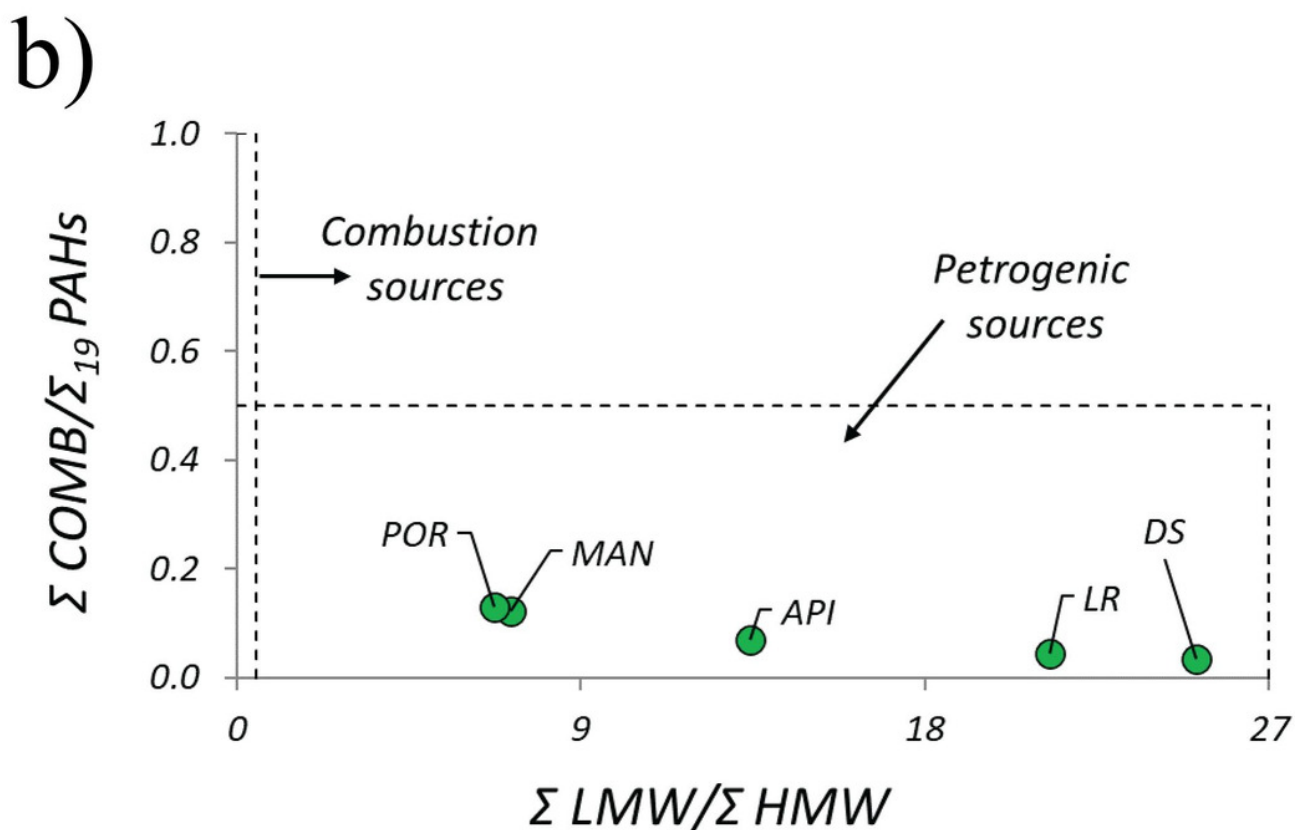
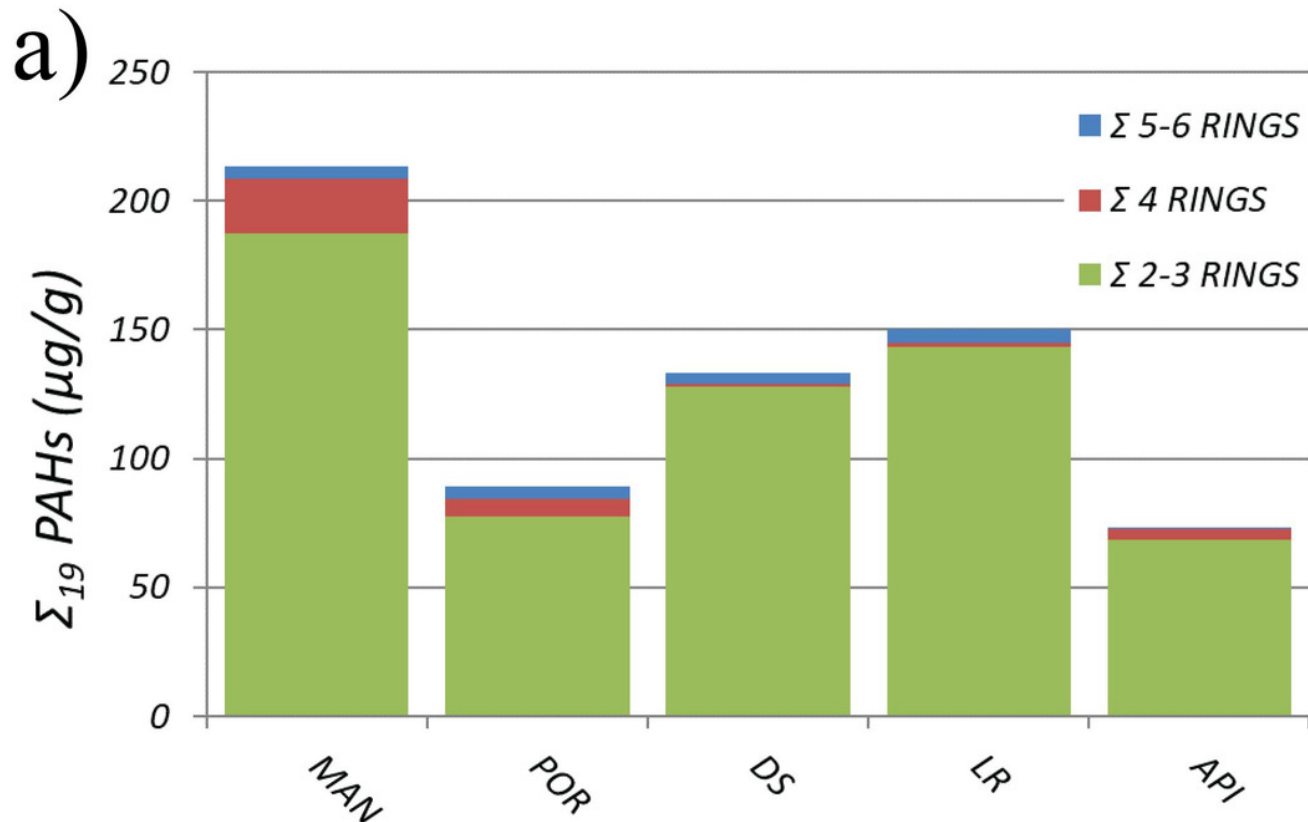



Figure 3

Principal Component Analysis (PCA) with environmental variables.

BPC = Biopolymeric organic carbon; CPE = Chloroplastic pigment equivalent; BPER = Benzo[*ghi*]perylene; BAP = Benzo[*a*]pyrene; FLU = Fluorene; ANT/PHE = Anthracene/ Phenanthrene; FLT/PYR = Fluoranthene/Pyrene.

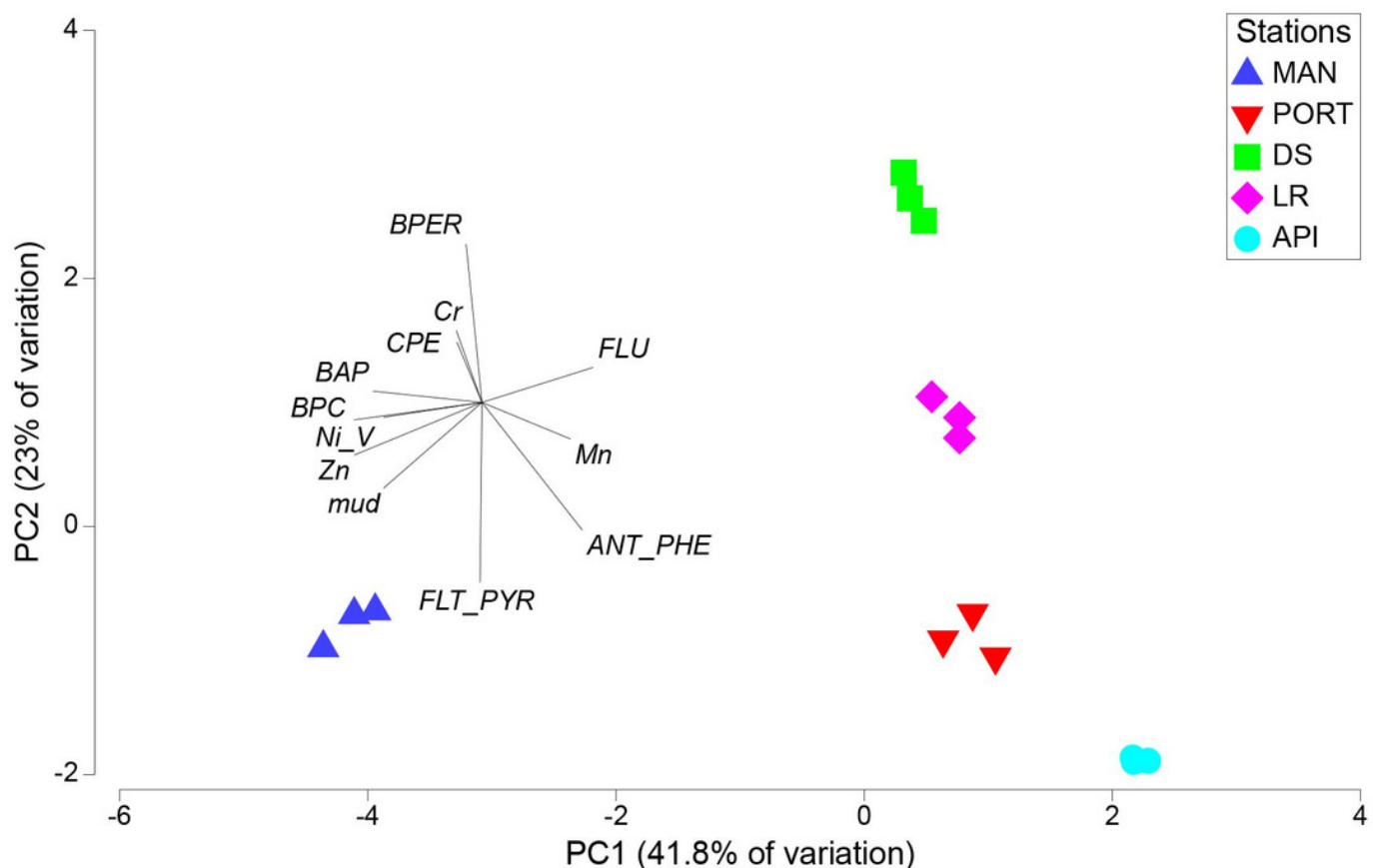



Figure 4

(a) Meiofaunal community structure and (b) contribution of taxa *others* at each sampling station.

Mean values of replicated samples (n =3) are shown. Bold values reported inside the bars are total abundances (ind.10cm^{-2}) of meiofauna (4a) and others (4b).

a)

b)

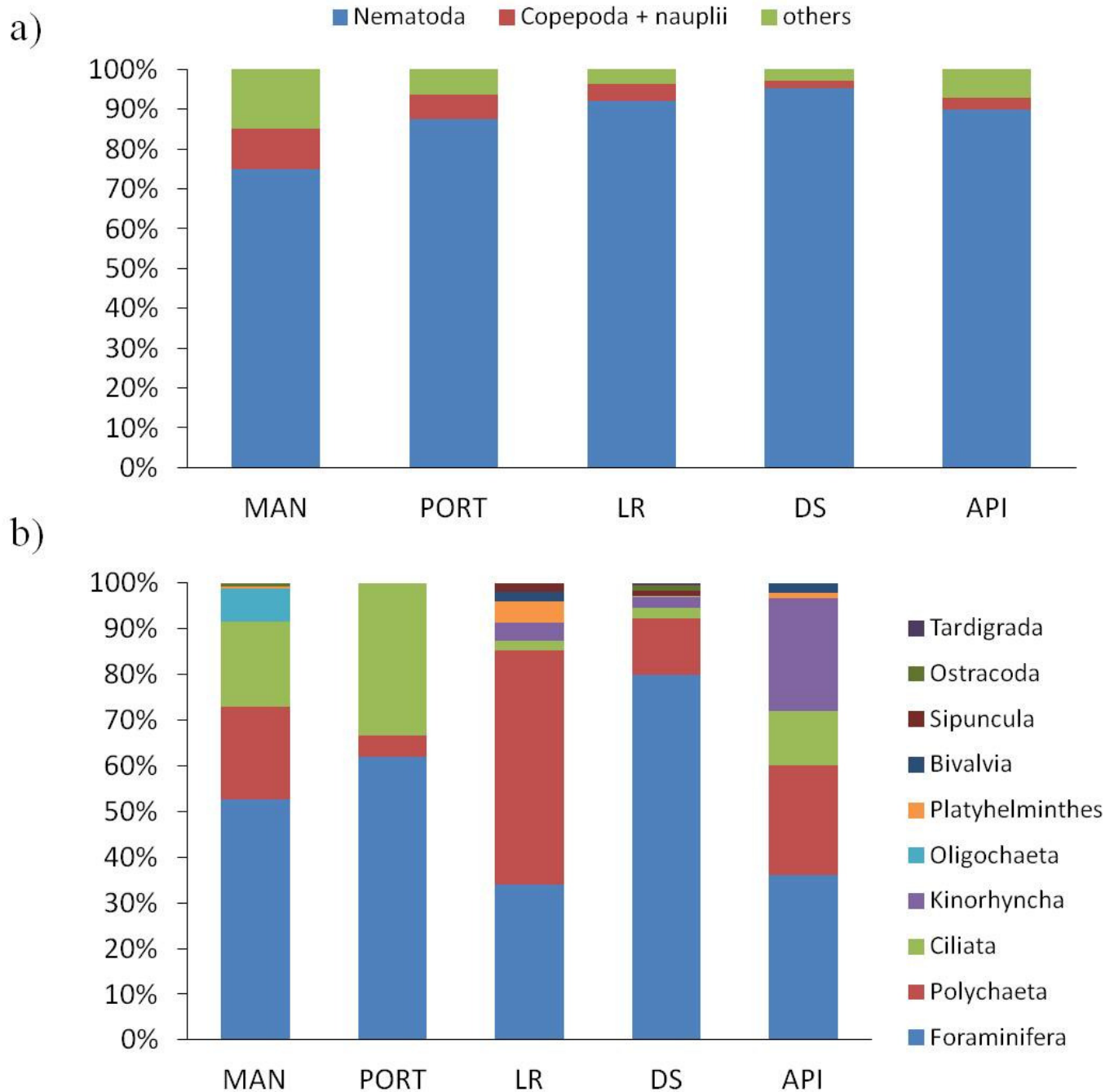



Figure 5

Meiofaunal univariate measures.

(a) Meiofauna abundance (N = no. of individuals 10 cm^{-2}) and its diversity indices: (b) meiofauna taxa richness (S), (c) Shannon's diversity index (H', based on log$_2$), and (d) Pielou's equitability index (J'). Bars represent 95% confidence intervals.

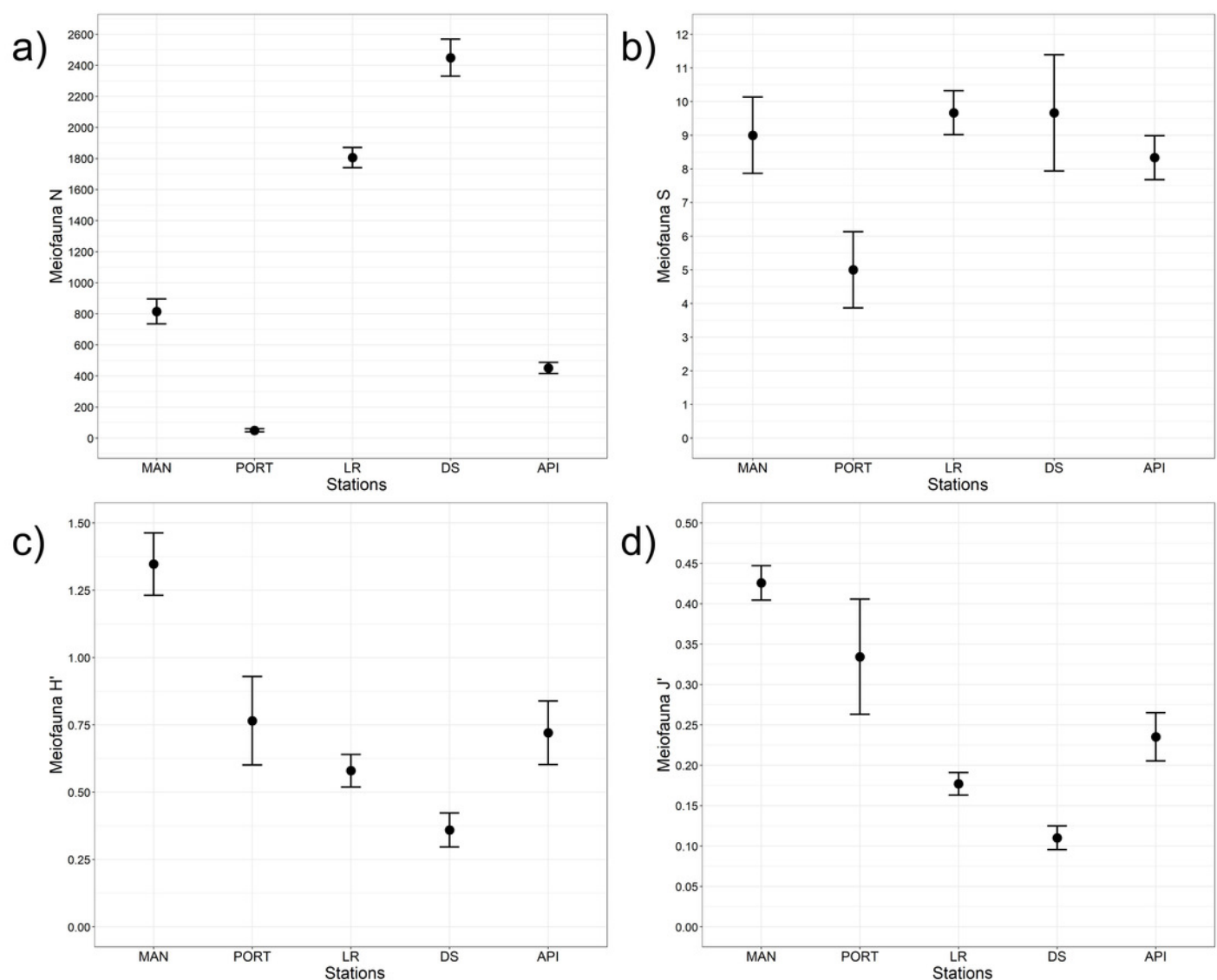



Figure 6

Non-metric Multidimensional Scaling (nMDS) of benthic communities.

Non-metric Multidimensional Scaling (nMDS) plots on (a) meiobenthic and (b) macrobenthic community structures characterizing the sediment at the investigated sampling stations. Data presented were fourth root scale transformed prior to analysis.

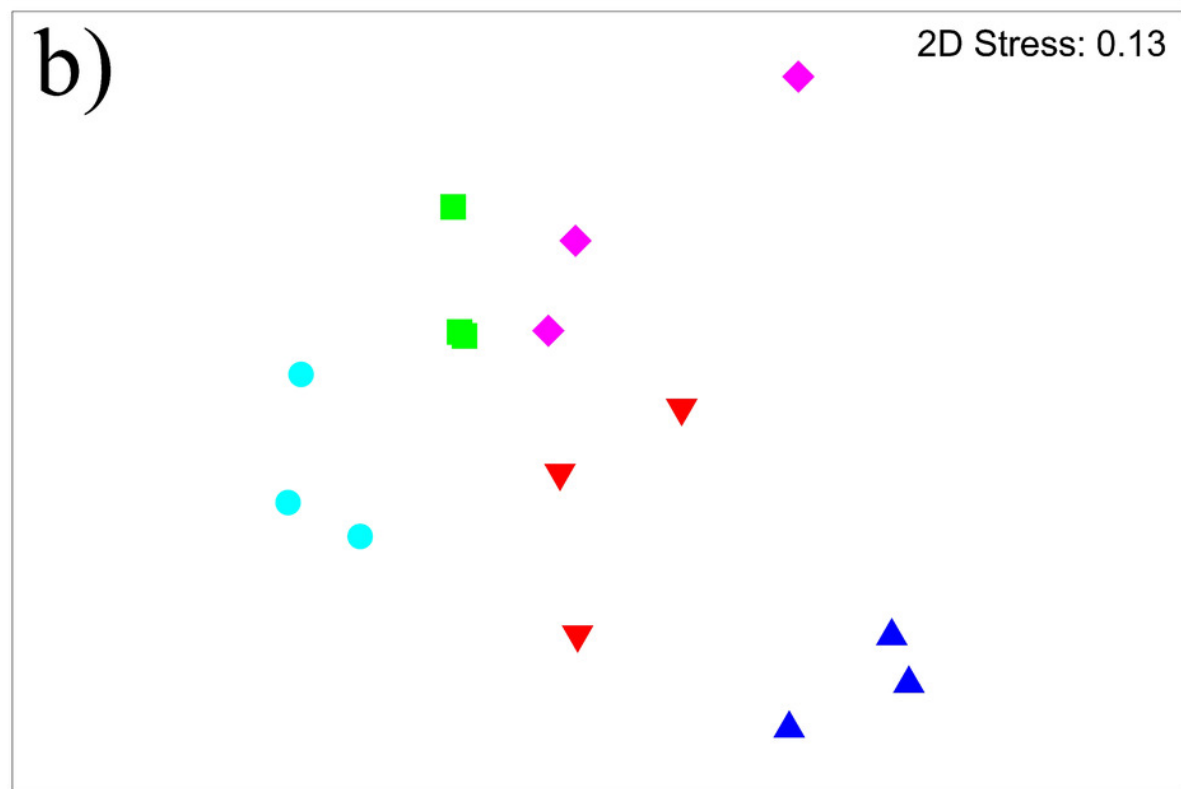



Figure 7

Macrofaunal community structure at each sampling station.

Mean values of replicated samples (n =3) are shown.

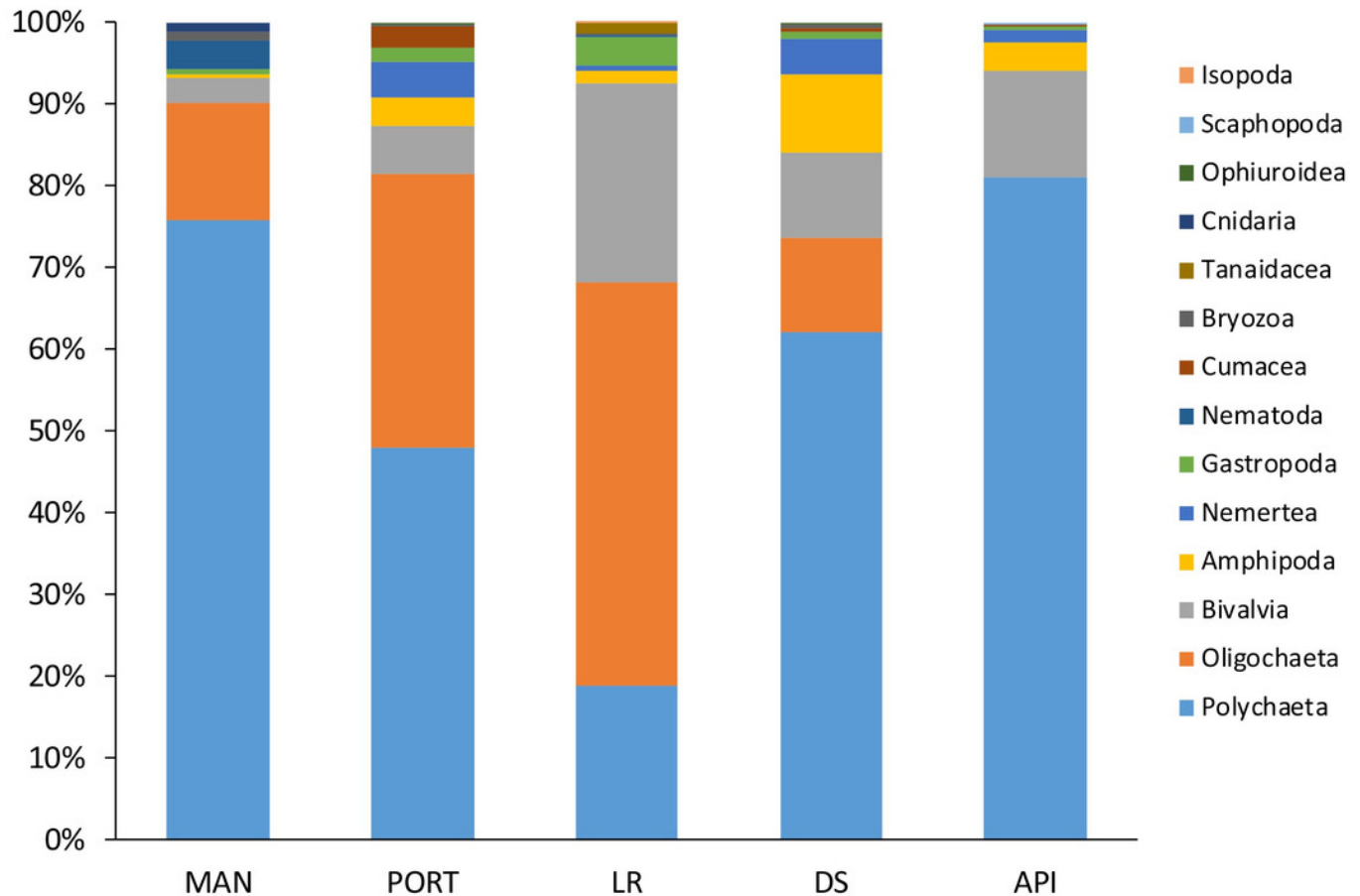
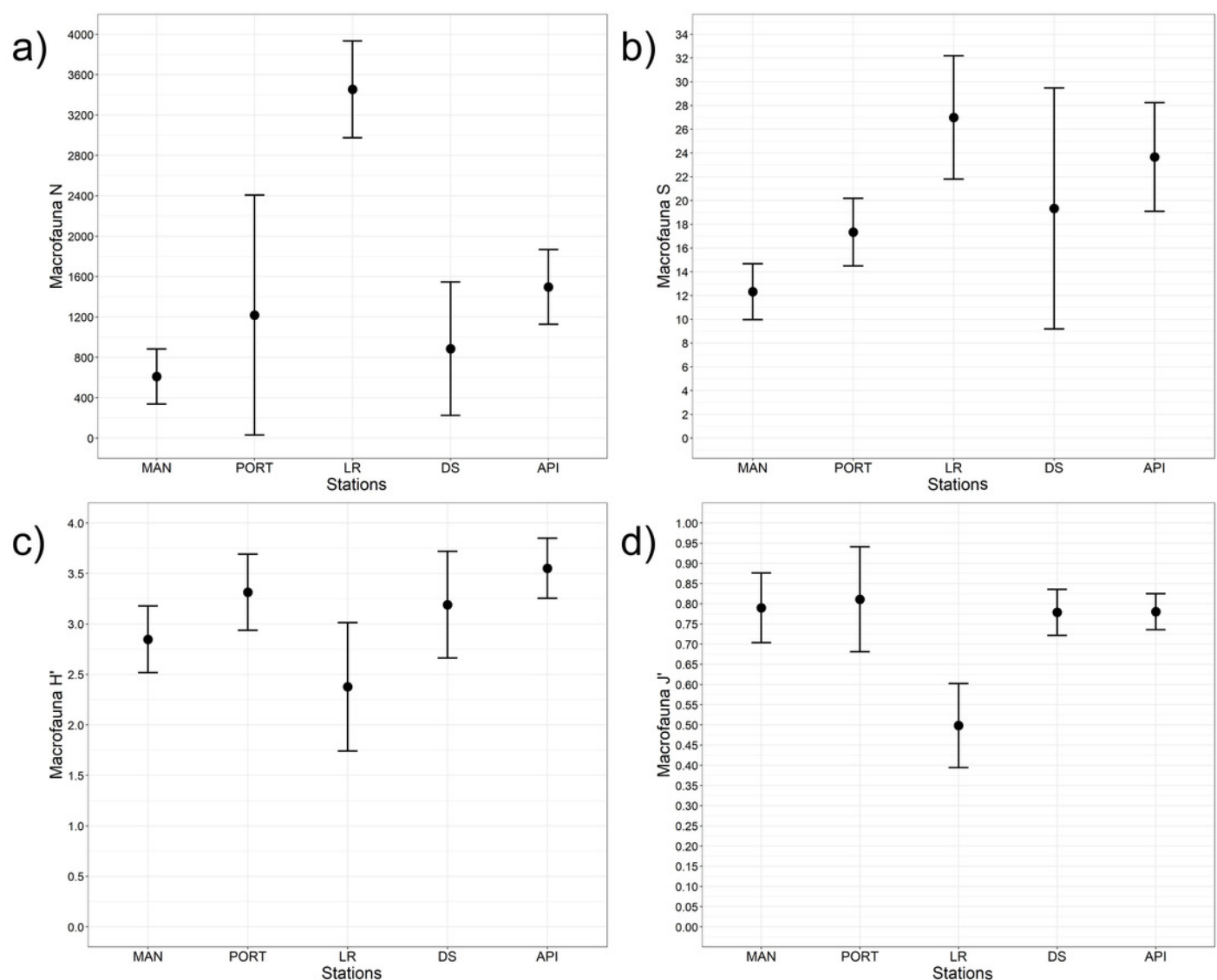



Figure 8

Macrofaunal univariate measures.

(a) Macrofauna abundance (N = no. of individuals m^{-2}) and its diversity indices: (b) macrofauna taxa richness (S), (c) Shannon's diversity index (H', based on log$_2$), and (d) Pielou's equitability index (J'). Bars represent 95% confidence intervals.

One-way Analysis of Variance (ANOVA) results on total meiofauna and macrofauna abundance (N) and their diversity indices.

Number of taxa (S), Shannon's diversity index (H', based on $\log_2$), and Pielou's equitability index (J'), characterizing the sediment at the investigated sampling stations. In bold significant p values.

1 Table 1. **One-way Analysis of Variance (ANOVA) results on total meiofauna and macrofauna**
2 **abundance (N) and their diversity indices**. Number of taxa (S), Shannon's diversity index (H', based on
3 $\log_2$), and Pielou's equitability index (J'), characterizing the sediment at the investigated sampling
4 stations. In bold significant *p* values.

Meiofauna					
Index		**d.f.**	**F value**	***p* value**	**Tukey's HSD *post hoc* test**
N	Stations	4	714.30	**<0.001**	DS>LR>MAN>API>PORT
	Residuals	10			
S	Stations	4	11.30	**<0.001**	PORT<API=DS=LR=MAN
	Residuals	10			
H'	Stations	4	44.10	**<0.001**	MAN>PORT>API=LR(=DS)>DS
	Residuals	10			
J'	Stations	4	41.60	**<0.001**	MAN>PORT=API=LR(=DS)>DS
	Residuals	10			
Macrofauna					
Index		**d.f.**	**F value**	***p* value**	**Tukey's HSD *post hoc* test**
N	Stations	4	10.60	**0.002**	LR>API=DS=MAN=PORT
	Residuals	10			
S	Stations	4	3.80	**0.041**	MAN<LR
	Residuals	10			
H'	Stations	4	8.20	**0.003**	LR<API=DS=MAN=PORT
	Residuals	10			
J'	Stations	4	3.90	**0.036**	LR<API
	Residuals	10			

5 d.f. = degrees of freedom; HSD = Honestly Significant Difference.

Table 2_(on next page)

Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise test and results on total meiofaunal community composition.

In bold significant values are reported.

1   **Table 2. Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise test and**
2   **results on total meiofaunal community composition**. In bold significant values are reported.

PERMANOVA						
Source	**d.f.**	**SS**	**MS**	**Pseudo-F value**	**P(perm)**	**Unique permutations**
Stations	4	7315.80	1829	38.0437	**0.001**	9915
Residuals	10	480.84	48.084			
Total	14	7796.70				
PAIRWISE TEST						
Groups	**t value**	**P(perm)**	**P(MC)**			
MAN vs. PORT	7.39	0.103	**<0.001**			
MAN vs. LR	8.21	0.099	**<0.001**			
MAN vs. DS	4.86	0.099	**0.004**			
MAN vs. API	6.12	0.099	**0.002**			
PORT vs. LR	8.23	0.095	**<0.001**			
PORT vs. DS	6.44	0.101	**<0.001**			
PORT vs. API	5.35	0.098	**0.002**			
LR vs. DS	2.95	0.101	**0.016**			
LR vs. API	5.20	0.101	**0.002**			
DS vs. API	3.98	0.098	**0.005**			

3   d.f. = degrees of freedom; SS = Sum of Squares; MS = Mean Square; P(perm) = Permutation p-value; P(MC) =
4   Monte Carlo p-value.

Distance-based Linear Modeling (DistLM) analysis on meiofaunal community composition characterizing the sediment at the investigated sampling stations.

Variables are coded after Varimax Rotated PCA Axis as follow: ARCx = Abiotic variables associated to the rotated axis (x indicates the number of the axis); BRCx = Biotic variables associated to the rotated axis. For a complete list of the variables refers to the text.

1   **Table 3. Distance-based Linear Modeling (DistLM) analysis on meiofaunal community composition**
2   **characterizing the sediment at the investigated sampling stations.** Variables are coded after Varimax
3   Rotated PCA Axis as follow: ARCx = Abiotic variables associated to the rotated axis (x indicates the
4   number of the axis); BRCx = Biotic variables associated to the rotated axis. For a complete list of the
5   variables refers to the text.

START SOLUTION							
AICc	**R^2**	**RSS**	**No. of variables**	**Selections**			
80.92	0.94	447.00	6	All			
SEQUENTIAL TESTS							
Variable	**AICc**	**SS**	**Pseudo-F value**	***p* value**	**Prop.**	**Cumul.**	**Res. d.f.**
BRC1	76.25	92.83	1.66	0.224	0.01	0.93	9
BRC2	74.60	173.62	2.89	0.53	0.02	0.91	10
BEST SOLUTION							
AICc	**R^2**	**RSS**	**No. of variables**	**Selections**			
74.59	0.91	713.44	4	ARC1-ARC4			

6   AICc = Akaike's Information Criterion corrected for small samples; R^2 = Coefficient of determination; RSS =
7   Residuals Sums of Squares; SS = Sums of Squares; Prop. = Proportion of variation explained by the variable;
8   Cumul. = Cumulative total of Prop; Res. d.f. = Residual degrees of freedom.

Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise test and results on total macrofaunal community composition.

In bold significant values are reported.

1 **Table 4. Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise test and**
2 **results on total macrofaunal community composition**. In bold significant values are reported.

PERMANOVA						
Source	**d.f.**	**SS**	**MS**	**Pseudo-F value**	**P(perm)**	**Unique permutations**
Stations	4	23534	5883.5	4.55	**0.001**	9915
Residuals	10	12936	1293.6			
Total	14	36470				
PAIRWISE TEST						
Groups	**t value**	**P(perm)**	**P(MC)**			
MAN vs. PORT	2.03	0.12	**0.028**			
MAN vs. LR	2.67	0.10	**0.011**			
MAN vs. DS	2.41	0.09	**0.015**			
MAN vs. API	3.09	0.10	**0.007**			
PORT vs. LR	1.57	0.10	0.100			
PORT vs. DS	1.71	0.10	0.069			
PORT vs. API	2.03	0.10	**0.031**			
LR vs. DS	1.57	0.10	0.106			
LR vs. API	2.23	0.10	**0.024**			
DS vs. API	2.03	0.10	**0.036**			

3 d.f. = degrees of freedom; SS = Sum of Squares; MS = Mean Square; P(perm) = Permutation *p*-value; P(MC) =
4 Monte Carlo *p*-value.

Distance-based Linear Modeling (DistLM) analysis on macrofaunal community composition characterizing the sediment at the investigated sampling stations.

Variables are coded after Varimax Rotated PCA Axis as follow: ARCx = Abiotic variables associated to the rotated axis (x indicates the number of the axis); BRCx = Biotic variables associated to the rotated axis. For a complete list of the variables refers to the text.

1 **Table 5. Distance-based Linear Modeling (DistLM) analysis on macrofaunal community**
2 **composition characterizing the sediment at the investigated sampling stations**. Variables are coded
3 after Varimax Rotated PCA Axis as follow: ARCx = Abiotic variables associated to the rotated axis (x
4 indicates the number of the axis); BRCx = Biotic variables associated to the rotated axis. For a complete
5 list of the variables refers to the text.

START SOLUTION							
AICc	**R²**	**RSS**	**No. of variables**	**Selections**			
28.74	0.70	11856	6	All			
SEQUENTIAL TESTS							
Variable	**AICc**	**SS**	**Pseudo-F value**	***p* value**	**Prop.**	**Cumul.**	**Res. d.f.**
BRC1	123.48	1746.70	1.23	0.251	0.048	0.65	9
ARC3	120.00	2136.70	1.53	0.131	0.059	0.59	10
BRC2	118.28	3193.80	2.17	0.022	0.000	0.51	11
ARC1	116.72	2906.30	1.78	0.049	0.080	0.43	12
BEST SOLUTION							
AICc	**R²**	**RSS**	**No. of variables**	**Selections**			
116.72	0.43	20821	2	ARC2; ARC4			

6 AICc = Akaike's Information Criterion corrected for small samples; R² = Coefficient of determination; RSS =
7 Residuals Sums of Squares; SS = Sums of Squares; Prop. = Proportion of variation explained by the variable;
8 Cumul. = Cumulative total of Prop; Res. d.f. = Residual degrees of freedom.