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Benthic ecosystem functioning in the severely contaminated Mar Piccolo of Taranto (Ionian Sea, Italy): focus on heterotrophic pathways

A. Franzo¹ · R. Auriemma¹ · F. Nasi¹ · J. Vojvoda^{1,2} · A. Pallavicini² · T. Cibic¹ · P. Del Negro¹

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Abstract The benthic ecosystem functioning is a rarely applied holistic approach that integrates the main chemical and biological features of the benthic domain with the key processes responsible for the flux of energy and C through the system. For the first time, such conceptual model, with an emphasis on the heterotrophic pathways, has been applied to the sediments at four stations within one of the most polluted coastal areas in Italy: the Mar Piccolo of Taranto. The functioning of the benthic ecosystem was different according to the investigated site. Nearby the military arsenal, i.e., the main source of organic contaminants and heavy metals, the system seemed inhibited at all the investigated structural and functional levels. Slow microbial processes of C reworking together with very limited densities of benthic fauna suggested a modest transfer of C both into a solid microbial loop and to the higher trophic levels. On the other hand, the ingression of marine water through the “Navigabile” channel seemed to stimulate the organic matter degradation and, consequently, the proliferation of meiofauna and macrofauna. In the innermost part of the basin, the system functioning, to some extent, is less impacted by contaminants and more influenced by mussel farms. The organic matter produced by these bivalves

fueled faster C reworking by benthic prokaryotes and enhanced the proliferation of filter feeders.

Keywords Sediments · Mar Piccolo of Taranto · Semi-enclosed basin · Organic enrichment · Contamination · Ecosystem functioning · Benthic communities · Heterotrophic pathways

Introduction

The United Nations Convention on the Law of the Sea defined pollution as “the introduction by man, directly or indirectly, of substances or energy into the marine environment, including estuaries, which results or is likely to result in such deleterious effects as harm to living resources and marine life, hazards to human health, hindrance to marine activities, including fishing and other legitimate uses of the sea, impairment of quality for use of the sea water and reduction of amenities”. Contaminants of major concern include persistent organic pollutants, nutrients, oil, heavy metals, pathogens, etc. Categorization of pollution only facilitated discussion: most contaminants are interrelated and jeopardize the environment and organisms regardless of the source of contamination (Islam and Tanaka 2004). Coastal areas deserve proper attention regarding diffuse and synergistic forms of pollution. Disposal into waterways is, in fact, a very ancient practice of dealing with wastes and the open waterways have been used by mankind for dumping all kinds of waste. Furthermore, the industrial areas are generally highly populated or the industries are usually established near highly populated areas. Therefore, the pollution load from industrial resources is generally coupled with domestic and sewage wastes. Among coastal areas, the effects of diffuse and synergistic forms of pollution are even more emphasized in enclosed basins, where

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✉ A. Franzo
afranzo@ogs.trieste.it

¹ OGS (Istituto Nazionale di Oceanografia e Geofisica Sperimentale), Sezione Oceanografia, v. A. Piccard 54, 34151 Trieste, Italy

² Department of Life Sciences, University of Trieste, via Licio Giorgieri 5, Trieste, Italy

the water circulation is restricted and, therefore, the dilution effect of contaminants is limited.

The Mar Piccolo of Taranto (Ionian Sea, southern Italy) appears to comprise all these features since this semi-enclosed basin collects the wastes from the densely populated urban center of Taranto and from the long-lasting industrial activities settled in the area. Since the 1960s, the city of Taranto and its coastline have been subjected, in fact, to the industrialization process that has caused profound environmental changes. The industrial zone is characterized mainly by the presence of the largest steelworks in Europe and navy arsenal in Italy, a major oil refinery, shipbuilding, and other industrial activities that are responsible for severe environmental contamination, mainly due to heavy metals, asbestos, polycyclic aromatic hydrocarbons (PAHs), organic solvents, polychlorinated biphenyls (PCBs), and dioxins. Previous surveys focused on the evaluation of organic and inorganic pollutants have shown so high levels of contamination as to consider the Mar Piccolo of Taranto one of the most polluted areas in Italy (Cardellicchio et al. 2007; Spada et al. 2012). This basin is included, in fact, in one of the largest Sites of National Interest (SIN) that has been declared as such by the Italian State according to the Law n. 426/ 1998. SIN are identified in relation to the characteristics of the site, the quantity and hazard of pollutants, the importance of the impact on the surrounding environments, in terms of health and ecology, as well as injury to the cultural and environmental heritage. The basin is even the most important area of mussel farming in Italy with an annual production of bivalves of about 40, 000 tons per year (Caroppo et al. 2012). This economic activity ensures noticeable incomes to the local population, but it could be endangered by the high contamination levels that affect the Mar Piccolo.

In a severely polluted environment such as the Mar Piccolo of Taranto, sediments deserve special attention. Due to the interactions with the water column, in fact, the benthic domain may be considered as the repository of the overlying water and consequently may act as a biogeochemical record of the past. Sediments represent a sink of contaminants that occur in concentrations up to orders of magnitude higher than in the overlying water (Cardellicchio et al. 2007). Benthic organisms, remaining in place, react to these high contamination levels without any escape possibility. The resulting community, in terms of species composition, replacements, eliminations, diversity, or abundance changes can give a proper idea of the recent history of the environmental events affecting the area (Solis-Weiss et al. 2001). In the Mar Piccolo, the chemical characterization of sediments, with an emphasis on contaminants, is well documented (Cardellicchio et al. 2007; Spada et al. 2012), and benthic communities have been studied focusing more on their macroscopic fraction (Matarrese et al. 2004; Prato and Biandolino 2005). On the contrary, the benthic ecosystem functioning, i.e., a holistic approach that

integrates the main chemical and biological features of the system together with the main processes responsible for the flux of energy and C, has not been investigated yet.

The benthic ecosystem is able to adapt to anthropogenic pressure, to some extent, by trying to minimize its impact through the development of stress-resistant communities that occupy new ecological niches. A highly specialized food web, which allows the benthic ecosystem to reach a state of equilibrium, can thus be established even in a heavily contaminated site (Cibic et al. 2012). The primary step of organic matter breakdown is mediated by extracellular enzymatic activities that render organic macromolecules available for bacterial uptake. Sediment organic matter can be channeled to higher trophic levels both directly (e.g., through deposit-feeding meiofauna and macrofauna) and/or indirectly, i.e., through bacterial uptake, Heterotrophic C Production (HCP), and subsequent consumption by bacterial grazers such as protozoa and bacterivorous meiofauna (Manini et al. 2003).

Due to its particular features, the Mar Piccolo of Taranto can be considered as a unique macrocosm where the application of a holistic approach could help to properly address future remediation actions that are urgently required in the area. Thus, we applied the benthic ecosystem functioning approach at four sites that should represent different forms/degrees of pollution. More precisely, we focused mainly on the key heterotrophic pathways and investigated in particular (1) how the main benthic heterotrophic communities (prokaryotes, meiofauna, and macrofauna) respond to severe forms of pollution in terms of abundance and composition, and (2) to what extent does the ability of the system in transferring organic detritus to the higher trophic levels change through microbial degradation activities and the subsequent incorporation of mobilized C into prokaryotic biomass.

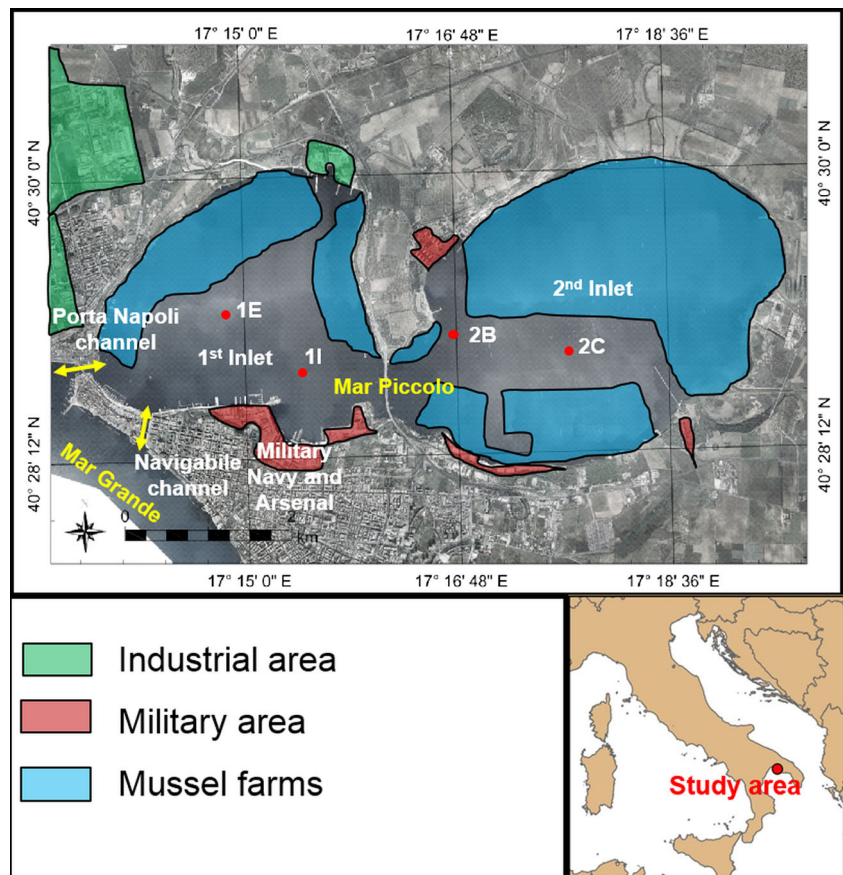
Materials and methods

Study site and sampling

The study area is an inner, semi-enclosed basin, with lagoonal features and divided into two inlets: the first is close to the navy arsenal and the steelworks plant, while the second is mainly influenced by mussel farms. The Mar Piccolo is characterized by scarce hydrodynamism and low water exchange since it is connected with the Mar Grande by just two narrow channels in the first inlet: the “Navigabile” and the “Porta Napoli” channels (Fig. 1). The main hydrological and chemical-physical features of the study area have been exhaustively described by Cardellicchio et al. (2015, this issue).

In June 2013 and April 2014, sampling was carried out at four sites selected as representatives of different environmental issues and anthropogenic impacts (Fig. 1). In the first inlet, St. 1E (40° 29' 01 N, 17° 14' 46 E) and St. 1I (40° 28' 46 N,

Fig. 1 Location of the four sampling stations in the Mar Piccolo of Taranto (red circles). The main anthropogenic pressures that exist along its coast are highlighted: the industrial area (green), the military area (red), and the mussel farms (blue). “Porta Napoli” and “Navigabile” channels are indicated by yellow arrows



17° 15' 38 E) should summarize the environmental features of the area in front of the “Navigabile” channel and the one nearby the military navy arsenal, respectively. In the second inlet, St. 2B (40° 28' 57 N, 17° 16' 42 E) and St. 2C (40° 28' 57 N, 17° 17' 41 E) were selected as representatives of the most enclosed part of the Mar Piccolo. Such stations were chosen following an increasing distance from the main sources of industrial pollution (located in the first inlet) and, at the same time, a more pronounced influence of mussel farms.

At each station, scuba divers collected five virtually undisturbed sediment cores using polycarbonate sample tubes (12.7 cm i.d. with a sample area of 127 cm²). Scuba divers avoided macroalgae when conspicuous as at St. 2B and 2C in order to maintain the same sampling conditions in all sites. One sediment core was partially extruded on board and the oxic sediment layer (0–1 cm ca) was subsampled and processed for the incubation in situ of the Heterotrophic C Production (HPC). The other cores were brought to the laboratory; one was dedicated to meiofauna subsampling while the others were partially extruded and the collected oxic sediment layer (0–1 cm ca) of each core was homogenized for the analyses of the main chemical (i.e., total and organic C, total N and biopolymeric C) and microbial parameters (i.e., prokaryotic

abundances and exoenzymatic activities). The same homogenized oxic sediment was also subsampled for the determination of heavy metals and PCBs as detailed in Cibic et al. (2015, this issue).

During each sampling, seawater temperature, dissolved oxygen, and salinity at the bottom were measured using a Seabird 19 Plus Seacat probe.

Total nitrogen (TN), total Carbon (TC), and total organic carbon (TOC)

For chemical element analyses, sediments were freeze-dried and ground using a mortar and pestle, and >250 μm fraction was separated from the rest of the sample. Triplicate of subsamples (~8–12 mg) were directly weighed in capsules (5 × 9 mm) on a microultrabalance Mettler Toledo model XP6 (accuracy of 0.1 μg). Tin and silver capsules were used for TC/TN and TOC analyses, respectively. The contents of TN, TC, and TOC were measured using an elemental analyzer CHNO-S Costech model ECS 4010. Before TOC determination, obtained following the methods of Pella and Colombo (1973) and Sharp (1974), subsamples were treated with increasing concentrations of HCl (0.1 and 1 N) to remove the carbonate (Nieuwenhuize et al. 1994).

Biopolymeric carbon (BPC)

Subsamples of homogenized sediment were freeze-dried and processed for the determination of carbohydrates, lipids, and proteins. Colloidal and EDTA extractable carbohydrates (CHO) were analyzed following the method described by Blasutto et al. (2005). Lipids were analyzed following the method proposed by Bligh and Dyer (1959) and modified for sediments. Proteins were extracted in NaOH (0.5 M) for 4 h and determined according to Hartree (1972). All analyses were carried out in four replicates. Carbohydrate, lipid, and protein concentrations were converted to carbon equivalents (Fichez 1991). The sum of carbohydrates, lipids, and proteins carbon was referred to as biopolymeric carbon (BPC).

Prokaryotic abundance

Three aliquots of sediment (0.5 g_{wet}) were withdrawn from each sediment sample, transferred into sterile test tubes, and fixed with 3 mL of pre-filtered (0.2- μ m pore size) and buffered formaldehyde solution (3 %v/v final concentration in autoclaved seawater). The sediment slurry was kept at +4 °C for 24 h, then washed twice with 3 mL of 1 \times phosphate-buffered saline (PBS) (pH=7.2) by centrifuging at 500 \times g to remove the supernatant, and stored in 1 \times PBS-ethanol (1:1) at -20 °C (Ravenschlag et al. 2000). For the subsequent sample processing, a modified protocol by Lunau et al. (2005) was followed: 100 μ L of sediment slurry was diluted with 900 μ L sterilized Milli-Q water and 100 μ L methanol. The diluted samples were placed in a water bath at 35 °C for 15 min, sonicated in ice (three times for 1 min with two 30-s in-between breaks), diluted again with sterile Milli-Q water to a final concentration of 1:110, and filtered on black Nuclepore polycarbonate 0.2- μ m-pore-size filters. Filters were mounted on microscope slides, stained with a SYBR Green I-mounting medium Mowiol solution (1:15), and counted by epifluorescence microscopy at \times 1000 magnification (LEICA DM2500). A minimum of 300 cells were counted for each filter in at least 20 randomly selected fields under a blue filter set (BP 450–490 nm, BA 515 nm).

Benthic bacterial community structure

Bacterial community structure was assessed in the homogenized oxic sediment only in April 2014. Bacterial community DNA was extracted from 0.5 g_{wet} of sediment using PowerSoil DNA extraction kit (MoBIO) with a slightly modified protocol. To facilitate the cell lysis, 0.5 g of muffled glass beads (0.1 mm) was added before the bead-beating step, which was then repeated four times for 2.5 min followed by 10 min of incubation at 70 °C. DNA was eluted in 50 μ L of preheated (70 °C) elution buffer. Bacterial 16S rRNA amplicon library was generated by

two PCR amplifications. The first PCR was performed using universal bacterial primers F8 (5'-AGA GTT TGA TCC TGG CTC AG-3') and U534R (5'-ATT ACC GCG GCT GCT GGC-3') in a reaction containing 1 \times KAPA HiFi HotStart ReadyMix, BSA (3 mM), 0.5 μ M of each primer, 1 \times EvaGreen, up to 10 μ L of molecular-grade water, and 1 μ L of extracted DNA. Amplification was carried out in a qPCR cycler with an initial denaturation step at 95 °C (3 min), followed by 23 cycles of denaturation at 98 °C (20 s), primer annealing at 59 °C (15 s), and extension at 72 °C (15 s). Cycling was completed by a final extension at 72 °C for 1 min, followed by melting curve analysis obtained by increasing the temperature from 65 to 95 °C. PCR products were diluted based on Ct values to obtain the equimolar concentrations for all of the samples and used as template to amplify the 16S rRNA hypervariable region V3. Amplification was performed in 25 μ L reaction containing 1 \times KAPA HiFi HotStart ReadyMix, 3 mM BSA, additional 0.5 mM MgCl₂, 1 \times EvaGreen fluorescent dye, and 0.4 μ M of both forward primer (B338F-5'-ACT CCT ACG GGA GGC AGC AG-3') fused with P1-adaptor sequence, and reverse primer (U534R-5'-ATT ACC GCG GCT GCT GGC-3') fused with A-Adaptor plus key sequence. Nested PCR was performed under the same cycling conditions as the first PCR with an exception of annealing temperature of 57 °C and 14 amplification cycles. Amplicons were gel-purified using HiYield™ Gel/PCR DNA Fragments Extraction Kit (RBC Bioscience). Amplicon library concentration was estimated with Qubit® dsDNA BR Assay Kit (Life Technologies), while the size and purity were examined by on-chip electrophoresis in 2100 BioAnalyzer instrument using DNA 1000 kit (Agilent). Using the concentration data and the average amplicon length (261 bp), the molarity of amplicon library was calculated, assuming that an average base pair has a molecular weight of 650 g mol⁻¹. All the libraries were pooled in equimolar amount and the pool diluted to the concentration of 25 pM just before sequencing. Sequencing of the amplicon libraries was carried out on the Ion Torrent Personal Genome Machine (Life Technologies) system using the Ion Sequencing 200 kit (Life Technologies) on a Ion 316 chip v2 (Life Technologies).

For sequence analysis, reads were processed for demultiplexing, adapters, and quality trimming using the CLC genomics workbench 7.5 (CLCbio). Reads from each sample were analyzed using several software packages. Richness and diversity metrics, including the number of observed OTUs, Chao1 estimator, Shannon index, and inverse Simpson's index, were calculated using the Mothur-based analysis embedded in the Clove software pipeline (<http://www.ncbi.nlm.nih.gov/pubmed/21878105>). Taxonomic assignments were performed with MG-RAST (<http://www.biomedcentral.com/1471-2105/9/386>) versus the

non-redundant database M5RNA. The on-line software METAGENassist was used to perform statistical differential OTUs representation and to link taxonomic assignments to metabolic features (<http://nar.oxfordjournals.org/content/40/W1/W88>).

Meiofauna

From a virtually undisturbed sediment core, three replicates were gently taken using cut-off plastic syringes (2.7 cm i.d., length 11.4 cm) and immediately frozen at -20°C (Higgins and Thiel 1988). The top 10 cm of the sediment core was considered; subsamples were preserved in buffered 4 % formaldehyde solution using prefiltered seawater and stained with Rose Bengal (0.5 g L^{-1}). Sediment samples were sieved through 1000 and $38\text{ }\mu\text{m}$ mesh net and the extraction of organisms (from the sediment retained on the $38\text{ }\mu\text{m}$ sieve) was performed by three times centrifugation ($1932\times g$, 10 min) with Ludox HS-40 (density $1.15\text{--}1.18\text{ g cm}^{-3}$) as described by Danovaro et al. (2004). All meiobenthic organisms were counted and taxonomically classified to the main groups according to Higgins and Thiel (1988) under a stereomicroscope (Olympus SZX12; final magnification of $\times 40$ or $\times 80$). The abundance was expressed as individuals per 10 cm^2 .

Macrofauna

Macrobenthos were sampled with a van Veen grab sampling area of 0.06 m^2 ; three replicates per station were taken, sieved through a $1000\text{ }\mu\text{m}$ sieve, and immediately fixed with formaldehyde solution (4 % v/v final concentration in seawater). After sorting, all animals were counted and identified to the lowest possible taxonomical level using a stereomicroscope (Zeiss Discovery V.12) at $\times 8$ to $\times 100$ magnification (Rees et al. 1990). For the identification of organisms, the taxonomical keys listed in Morri et al. (2004) were used. The abundance was expressed as individuals per square meter. Individuals were grouped in five trophic guilds according to several criteria as the feeding apparatus morphology, the feeding mode, and the food source (Desrosiers et al. 2000). Such groups were surface deposit feeders (SDF), subsurface deposit feeders (SSDF), filter feeders (FF), carnivores (CRN), and grazers (GRZ).

Degradative activities

Extracellular enzymatic activities were assayed using fluorogenic substrate analogues (Hoppe 1993) derived from 7-amino-4-methyl-coumarin (AMC) and 4-methylumbelliferone (MUF). Protease activity (leucine aminopeptidase activity-AMA) was assayed as the hydrolysis rate of leucine-AMC while β -glucosidase (β -GLU), lipase (LIP), and chitinase (CHIT) were assayed using MUF- β -D-glucoside and MUF-oleate and MUF- β -D-glucosamide

(Sigma-Aldrich), respectively. Enzyme activities were expressed in terms of the rate of MUF or AMC production.

Sediment slurries were prepared by adding 6 mL of $0.2\text{-}\mu\text{m}$ -filtered bottom water to 0.5 g of wet sediment. After evaluation of the saturating concentrations, hydrolysis rates were measured by incubating slurries with (final concentrations) $800\text{ }\mu\text{M}$ leucine-AMC, $400\text{ }\mu\text{M}$ MUF- β -D-glucoside, MUF-oleate, and $200\text{ }\mu\text{M}$ MUF- β -D-glucosamide for 1 h in the dark at in situ temperature. Before spectrofluorometric measurement, each sample was centrifuged for 2 min at 3000 rpm. Fluorescence increase due to MUF and AMC hydrolyzed from the model substrates was measured using a Jasco FP 6500 spectrofluorometer (MUF= 365-nm excitation and 455-nm emission; AMC= 380-nm excitation and 440-nm emission). Standard solutions of MUF and AMC were used to produce calibration curves with $0.2\text{-}\mu\text{m}$ -filtered bottom water. Triplicate blanks without fluorogenic substrate were used to determine the natural fluorescence increase in the samples not attributable to the tested enzymes. Hydrolytic activities were converted into C mobilization using the conversion factor 72 for glucose and leucine and 216 for oleic acid.

Heterotrophic Carbon Production (HCP)

HCP was measured following the method of ^3H -leucine uptake for sediment samples (van Duyl and Kop 1994, as detailed by Manini et al. 2004). Each sediment sample (0.2 mL of 1:1 v/v slurry) was added to $6\text{ }\mu\text{Ci}$ of ^3H -leucine (Perkin Elmer, specific activity= 69) and incubated in the dark for 1 h at experimental temperature. After incubation, radiotracer incorporation was stopped by adding ethanol 80 % (1.7 mL). After two washes of the samples with ethanol (80 %) by mixing, centrifuging, and supernatant removal, the sediment was transferred with ethanol (80 %) onto a polycarbonate filter ($0.2\text{ }\mu\text{m}$ mesh size). Subsequently, the filters were washed twice with 5 % trichloroacetic acid. Samples were heated in $\text{NaOH } 2\text{ M}$ for 2 h in a water bath at 100°C , cooled on ice, and centrifuged at $425\times g$ for 3 min. One milliliter of supernatant was transferred to scintillation vials and 10 mL of Hionic Fluor[®] (Perkin Elmer) scintillation fluid was added. Activity in the samples was determined by a β -counter (Packard Tri-Carb 2900TR). For each sample, three replicates and two ethanol-treated blanks were analyzed.

Statistical analyses

Descriptive statistics was performed on meiofauna and macrofauna in order to calculate the relative abundance (RA) of the main taxa.

Univariate diversity analysis, cluster analysis, and similarity percentage analysis (SIMPER) were performed on macrofauna using PRIMER software v.5 (PRIMER-E Ltd., Plymouth, UK). The applied univariate diversity indices were

number of taxa (S), richness (d; Margalef 1986), equitability (J', Pielou 1966), and diversity (H'log2; Shannon and Weaver 1949).

To test for the variability of the main variables as a whole among stations, the analysis of similarity (ANOSIM) was applied using PRIMER software v.5 (PRIMER-E Ltd., Plymouth, UK). ANOSIM tests a priori-defined groups (corresponding to the stations) against random groups in ordinate space. R_{ANOSIM} statistic values are a relative measure of separation of the a priori-defined groups. A zero (0) indicates that there is no difference among groups, while a one (1) indicates that all the samples within groups are more similar to one another than any samples from different groups.

Principal component analysis (PCA) was carried out on environmental data in order to visualize the trends of the main abiotic variables. TOC, proteins, lipids, and total carbohydrates were included in the analysis together with total PCBs and total Hg (data presented in Cibic et al. 2015, this issue). The biotic components (benthic prokaryotes, meiofauna and macrofauna abundances, extracellular enzymatic activities, HCP) were projected on the factor plane as additional variables without contributing to the results of the analysis. This can provide an insight into the possible influence of the environmental variables upon each benthic group (STATISTICA 7).

Multidimensional scaling analysis (MDS) was performed on the abundances of macrofaunal trophic guilds. Square root transformation of the data matrix and Bray-Curtis similarity were applied using PRIMER software v.5 (PRIMER-E Ltd., Plymouth, UK).

Results

Chemical characterization of sediments

The results of the main chemical parameters (TN, TC, TOC, and BPC) are reported in Table 1. During both campaigns, the highest values of TN were measured at St. 2B ($\sim 4 \text{ mg C g}^{-1}$) while minima at St. 1E ($< 2.5 \text{ mg C g}^{-1}$). Regarding TC, higher contents characterized the first inlet ($\sim 80 \text{ mg C g}^{-1}$) compared to the second one, especially St. 2C ($\leq 60 \text{ mg C g}^{-1}$). Almost half of the TC load was constituted by TOC, with maxima at St. 1I and minima at St. 2C during both campaigns. In June 2013 and April 2014, protein contents of St. 2C were slightly higher than lipids while the latter represented the dominant BPC fraction in the first inlet. The site in front of the navy arsenal (St. 1I) showed, in fact, elevated lipid contents with values equal to $5256.3 \pm 189.1 \text{ } \mu\text{g C g}_{\text{dry}}^{-1}$ and $3157.3 \pm 74.3 \text{ } \mu\text{g C g}_{\text{dry}}^{-1}$ in June 2013 and April 2014, respectively. Carbohydrates constituted the minor fraction of the BPC pool, with values at least one order of magnitude lower than proteins and lipids. A clear difference between the two inlets was not observed for the EDTA-extractable carbohydrates whereas the

colloidal carbohydrates were very low in the first inlet during June 2013.

Benthic prokaryotes and bacterial community structure

Although benthic prokaryotic numbers did not vary either among stations or during the two campaigns (Table 2), some differences were observed in the prokaryotic community structure in April 2014.

Out of 430,976 obtained reads, 396,591 were left after the removal of the low quality sequences (limit $p_{\text{err}}=0.5$) and/or with a length $< 150 \text{ bp}$. A total of 355,513 reads were recognized as bacterial 16S rRNA gene sequences, and out of this, approximately 56 % (197,692 reads) could be affiliated to a specific bacterial class. Based on 97 % similarity, 6537, 9761, 11,283, and 8963 OTUs were observed in samples from St. 1E, St. 1I, St. 2B, and St. 2C, respectively. According to the Shannon diversity index (H'), the most diverse communities were those from St. 1I and St. 2B ($H'=7.3$ and 7.2 , respectively), while at St. 1E and St. 2C diversity was slightly lower ($H'=6.5$ and 6.6 , respectively). Taxonomic classification of the reads revealed that the most abundant bacterial groups were *Gammaproteobacteria*, *Deltaproteobacteria*, *Bacteroidetes*, and *Alphaproteobacteria* (Fig. 2). On a family level, *Flavobacteriaceae* (*Bacteroidetes*), *Rhodobacteraceae* (*Alphaproteobacteria*), and *Desulfobulbaceae* (*Deltaproteobacteria*) were most frequently detected at St. 1E. *Desulfobacteraceae* (*Deltaproteobacteria*) were more abundant than *Flavobacteriaceae* at St. 1I and St. 2B, while all four families were equally abundant in the middle of the second inlet (St. 2C).

The taxon-to-phenotype mapping (done by METAGENassist) did not show clear differences among the four stations in the metabolic potential (Fig. 3). The most common trait identified at all four stations (around 20 % of population) was the oxidation of ammonia, followed by the dehalogenation ability, sulfate reduction and oxidation, nitrite reduction, and N_2 fixation.

Benthic communities at higher trophic levels

The absolute abundances of meiofaunal and macrofaunal organisms are summarized in Table 2.

In June 2013, meiofaunal numbers ranged from $321.5 \pm 223.3 \text{ ind. } 10 \text{ cm}^{-2}$ to $1303.9 \pm 721.9 \text{ ind. } 10 \text{ cm}^{-2}$ at St. 1I and St. 1E, respectively. During the second campaign, St. 1I showed very low densities ($91.3 \pm 58.7 \text{ ind. } 10 \text{ cm}^{-2}$) while the highest value was reported at St. 1E ($695.0 \pm 393.6 \text{ ind. } 10 \text{ cm}^{-2}$), comparable to the abundance observed at St. 2C ($636.9 \pm 218.9 \text{ ind. } 10 \text{ cm}^{-2}$) (Table 2).

Meiofauna were dominated by Nematoda during both campaigns and at all stations, with relative abundances (RA) $> 75 \%$. Copepoda and their naupliar stages were the second

Table 1 The main structural chemical data measured in the four sites

	St. 1E	St. 1I	St. 2B	St. 2C
TN (mg N g ⁻¹)	2.2 ± 0.0	3.0 ± 0.1	3.9 ± 0.1	3.7 ± 0.1
	2.5 ± 0.0	2.7 ± 0.0	4.0 ± 0.0	2.8 ± 0.0
TC (mg C g ⁻¹)	79.8 ± 0.9	81.1 ± 0.5	69.6 ± 0.6	60.4 ± 0.3
	76.9 ± 0.4	83.7 ± 1.3	64.4 ± 0.1	55.3 ± 0.5
TOC (mg C g ⁻¹)	35.6 ± 0.6	42.9 ± 1.5	38.1 ± 0.2	30.2 ± 0.5
	33.2 ± 2.1	38.1 ± 1.5	32.2 ± 1.6	22.3 ± 0.3
C-PRT (μgC g ⁻¹)	693.7 ± 12.0	1943.6 ± 30.0	1522.9 ± 8.6	2145.4 ± 69.3
	1200.3 ± 29.9	1963.8 ± 45.6	2420.7 ± 69.7	1591.5 ± 22.0
C-LIP (μgC g ⁻¹)	2497.0 ± 58.7	5256.3 ± 189.1	2580.3 ± 68.8	1896.1 ± 86.5
	2498.4 ± 97.4	3157.3 ± 74.3	1685.9 ± 90.5	1068.0 ± 75.3
C-CHO _{EDTA} (μgC g ⁻¹)	197.3 ± 10.0	292.8 ± 12.8	321.3 ± 16.5	269.7 ± 12.7
	132.8 ± 6.1	187.5 ± 6.4	154.2 ± 5.4	117.6 ± 4.0
C-CHO _{H2O} (μgC g ⁻¹)	13.1 ± 1.2	16.7 ± 0.5	109.8 ± 7.0	154.6 ± 1.3
	96.5 ± 2.6	90.8 ± 1.8	136.5 ± 3.5	93.2 ± 0.9
BPC (μgCg ⁻¹)	3401.1	7509.4	4534.3	4465.7
	3928.0	5399.4	4397.2	2870.4

Values are averages of three replicates (±standard deviations). White and gray backgrounds outline data of June 2013 and April 2014, respectively. TN total nitrogen, TC total carbon, TOC total organic carbon, C-PRT proteins, C-LIP lipids, C-CHO_{EDTA} EDTA-extractable carbohydrates, C-CHO_{H2O} colloidal carbohydrates extracted in water, BPC biopolymeric C

most abundant taxon with higher RA at St. 2C both in June 2013 and in April 2014. Kinorhyncha were observed only in sediments of St. 1E and in very low percentages (Table 3). Overall, in April 2014, a higher number of taxa was observed than during the first campaign.

In June 2013, total macrofaunal abundances varied from 605.0±543.1 ind. m⁻² to 1916.0±341.9 ind. m⁻² at St. 1I and St. 1E, respectively. In April 2014, values were lower than those observed during the previous campaign and the

minimum, equal to 145.0±7.1 ind. m⁻², was recorded at St. 1I, while the highest density was at St. 2C (1050.0±14.1 ind. m⁻²) (Table 2). Overall, in June 2013, the community was dominated by polychaetes (41 %) and molluscs (40 %), followed by crustaceans, which represented ~13 % of the total abundance. The remaining 6 % included taxa with a sporadic occurrence as anthozoans, sipunculids, and nemertines. The dominance of polychaetes was due to the high abundance of this taxon at St. 1E (RA=56.2 %), while at

Table 2 Mean abundance of the three benthic communities analyzed at the four stations. Values are averages of three replicates (± standard deviations)

	Benthic prokaryotes	Meiofauna	Macrofauna
	*10 ⁹ cells g _{dry} ⁻¹	ind. 10 cm ⁻²	ind. m ⁻²
St. 1E	2.3 ± 0.0	1303.9 ± 721.9	1916.0 ± 341.9
	2.5 ± 0.2	695.0 ± 393.6	755.0 ± 106.1
St. 1I	1.9 ± 0.0	321.5 ± 223.3	605.0 ± 543.1
	2.0 ± 0.1	91.3 ± 58.7	145.0 ± 7.1
St. 2B	2.5 ± 0.1	713.8 ± 154.0	683.3 ± 294.9
	1.8 ± 0.0	437.7 ± 185.9	255.0 ± 21.2
St. 2C	1.8 ± 0.3	589.3 ± 108.0	977.0 ± 209.2
	1.9 ± 0.1	636.9 ± 218.9	1050.0 ± 14.1

White and gray backgrounds outline data of June 2013 and April 2014, respectively

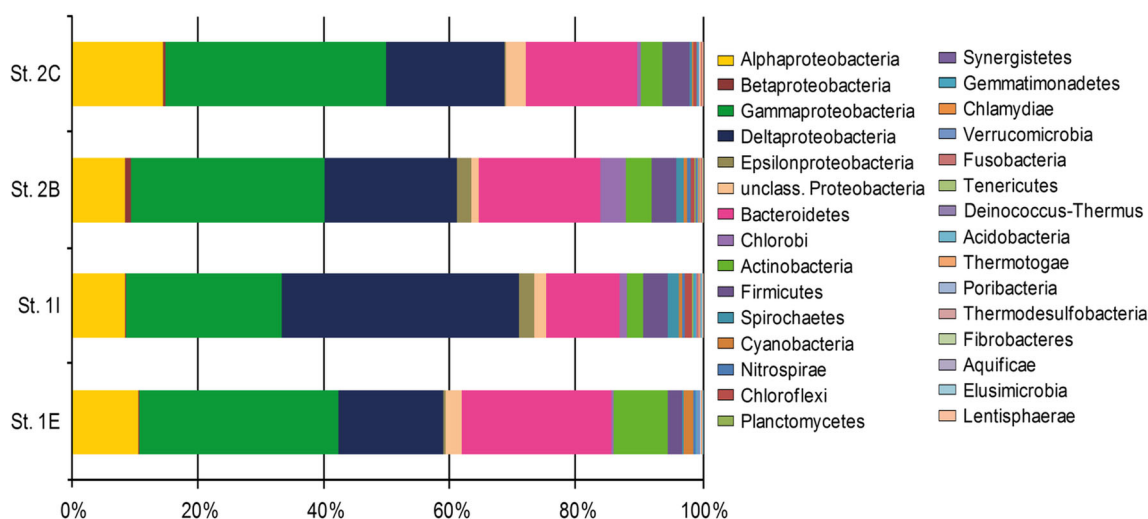


Fig. 2 Relative abundance and affiliation (class level) of the reads from each sampling station in April 2014

the other stations, molluscs represented the most abundant group of organisms (Table 3). Crustaceans accounted for only 4.3 % of the whole community at St. 1E and their RA increased considerably at the other three sites. Echinoderms were observed only in the first inlet and with low percentages. Similarly, in April 2014, macrofauna were constituted mainly by molluscs (41 %), polychaetes (38 %), and crustaceans (13 %), while the remaining 8 % comprised anthozoans, sipunculids, and nemertines. The dominance of molluscs was ascribable to their high numbers at St. 2C, where these organisms, mainly represented by bivalves, accounted for >50 % of the whole community. RA of polychaetes decreased from St. 1E to St. 2C while higher RA of crustaceans were reported at St. 1I and 2B. Similarly to the first campaign, echinoderms were observed in low RA and mainly at the station close to the channel, i.e., St. 1E (RA=10.6 %).

Focusing on the qualitative composition of macrofauna, sediments of St. 1E harbored typically marine species during both campaigns, i.e., *Papillicardium papillosum* and *Pitar rudis* among bivalves and *Amphiura chiajei* and *Ophiotrix quinquemaculata* among echinoderms. The other stations were characterized by organisms that inhabit paralic environments as *Abra segmentum*, *Cerastoderma glaucum* (bivalves), *Gammarus aequicauda*, and *Gammarus insensibilis* (amphipods). At St. 1I and 2B, taxa typically associated to the presence of macroalgae were observed: *Gammarus* sp. and *Leuchotoe* sp. among amphipods and *Musculus subpictus* and *Modiolula phaseolina* among bivalves.

Applying the diversity indices to macrofauna, H' values were higher in June 2013 than in April 2014 (Table 4). During the first campaign, St. 2B resulted the most diverse station with H' equal to 4.41 whereas the lowest diversity

Fig. 3 Relative abundances of specific metabolic traits obtained with taxon-to-phenotype mapping (METAGENassist)

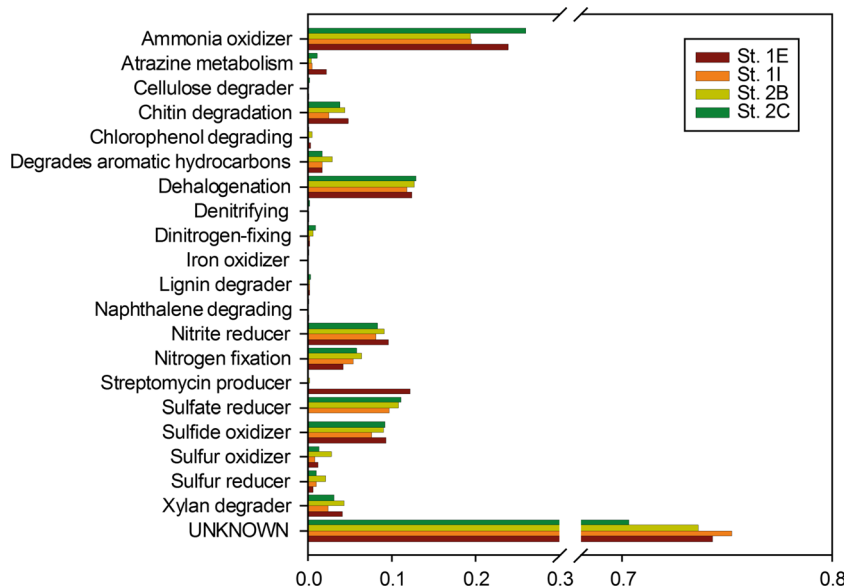


Table 3 Relative abundance (%) of major groups constituting meiofauna and macrofauna at the four stations

Meiofauna	St. 1E	St. 1I	St. 2B	St. 2C
Nematoda	94.0	93.1	93.4	90.3
	92.0	86.7	79.9	75.6
Copepoda	2.1	4.1	5.0	3.0
	3.1	4.2	5.6	6.6
Nauplii	1.7	1.9	1.5	6.8
	3.3	6.7	11.4	17.2
Kinorhyncha	1.8	0.0	0.0	0.0
	1.1	0.0	0.0	0.0
Others	0.3	0.9	0.1	0.0
	0.5	2.4	3.2	0.6
Macrofauna				
Polychaeta	56.2	27.5	31.7	24.4
	72.8	51.7	35.3	13.3
Mollusca	29.3	43.1	48.8	54.5
	7.3	10.3	23.5	73.8
Crustacea	4.3	26.6	16.3	18.8
	1.3	37.9	39.2	12.4
Echinodermata	3.8	0.9	0.0	0.0
	10.6	0.0	0.0	0.0
Others	6.4	1.8	3.3	2.3
	7.9	0.0	2.0	0.5

Meiofauna were analyzed in the top 10 cm. White and gray backgrounds outline data of June 2013 and April 2014, respectively

characterized St. 2C ($H'=3.72$). The highest number of taxa (S) and richness (d) were calculated at St. 1E. Although this site was characterized also by the second highest H' value, the minimum of equitability (J') was due to the dominance of

Notomastus formianus (polychaete) and *Abra alba* (mollusc). In April 2014, the maxima of both d and H' were calculated at St. 1E, whereas the lowest values characterized St. 2C. The dominance of *Arcuatula senhousia* at this site contributed also to the minimum of equitability ($J'=0.43$).

Table 4 Univariate diversity indices applied to macrofauna at the four stations

	S	d	J'	$H'(\log_2)$
St. 1E	57	9.66	0.75	4.39
	24	4.61	0.87	3.98
St. 1I	26	5.33	0.88	4.12
	12	3.30	0.89	3.20
St. 2B	35	7.10	0.86	4.41
	12	2.83	0.89	3.20
St. 2C	24	4.47	0.81	3.72
	16	2.82	0.43	1.71

White and gray backgrounds outline data of June 2013 and April 2014, respectively

S number of taxa, d richness, J' equitability, $H'(\log_2)$ diversity

While grazers were absent at St. 1E, macrofauna were dominated by deposit feeders that represented >69 % of the whole community during both periods. The highest contribution was attributable to surface deposit feeders (738.8 ind. m^{-2}) and subsurface deposit feeders (305.0 ind. m^{-2}) in June 2013 and April 2014, respectively. The other sampling stations were characterized by the presence of all trophic guilds during both campaigns, and some differences were observed. At St. 1I, macrofauna were dominated by grazers (the isopod *Paracerceis sculpta* and the bivalve *Haminoea navicula*) and carnivores (the polychaetes *Lumbrineris latreilli* and *Eunice vittata*), which together accounted for 55.2 % (June 2013) and 62.1 % (April 2014) of the whole community. At St. 2C, filter feeders were well represented, especially during April 2014, when this group accounted for 79.4 % of the total abundance due to the high density of

A. senhousia (bivalve). St. 2B showed an intermediate situation compared with the other sites and a clear dominance of a trophic association over the others was not highlighted.

Functional parameters

The rates of β -glucosidase, lipase, and chitinase were low ($<4 \text{ nmol cm}^{-3} \text{ h}^{-1}$) both in June 2013 and April 2014, and the variability among stations did not allow to highlight any pattern within the study area. The highest enzymatic activities were directed to the degradation of proteins. The minima were observed at St. 1I during both campaigns ($18.63 \pm 1.75 \text{ nmol cm}^{-3} \text{ h}^{-1}$ and $11.71 \pm 0.82 \text{ nmol cm}^{-3} \text{ h}^{-1}$) while the maxima were reached at St. 2C ($56.50 \pm 1.42 \text{ nmol cm}^{-3} \text{ h}^{-1}$) and St. 1E ($126.19 \pm 1.62 \text{ nmol cm}^{-3} \text{ h}^{-1}$) in June 2013 and April 2014, respectively. Alkaline phosphatase showed intermediate rates, with lower values in the first inlet and slightly higher values in the second one, both in June 2013 and April 2014 (Fig. 4).

In June 2013, HCP rates, ranging from $0.99 \pm 0.05 \mu\text{g C g}_{\text{dry}}^{-1}$ to $2.53 \pm 0.38 \mu\text{g C g}_{\text{dry}}^{-1}$, were higher than during April 2014, when data varied between $0.17 \pm 0.01 \mu\text{g C g}_{\text{dry}}^{-1}$ and $0.59 \pm 0.05 \mu\text{g C g}_{\text{dry}}^{-1}$. During both campaigns, sediments of St. 1I were characterized by the HCP minima, while the maxima were observed at St. 2B (June 2013) and St. 2C (April 2014), although the latter value was almost equal to the rate estimated at St. 2B (Fig. 4f).

PCA

The ordination plot of the considered functional and structural parameters accounted for 78.36 % of the total variance. Two principal components (factors) were identified (eigenvalue >1) which together explained 78.36 % of the total variance, whereas the first and the second factors explained 45.22 and 33.14 % of the total variance, respectively (Fig. 5). Total lipids, total PCBs, and TOC were the predominant elements of the first factor, while the major contributors of the second one were total proteins, total carbohydrates, and TN (Fig. 5a). All the sampling stations were plotted on the PCA factor-plane 1×2 , which grouped the stations according to their environmental conditions (Fig. 5b). During both periods, the two inlets of the Mar Piccolo of Taranto were well separated with St. 1E and St. 1I in the left half of the plot, and St. 2B and St. 2C in the right one. Focusing on the first inlet, St. 1E and St. 1I were clearly separated. The position of the latter was influenced mainly by lipid load, total Hg, and total PCBs.

Discussion

In the present study, the benthic ecosystem functioning of the Mar Piccolo of Taranto was investigated at four stations that could be considered, to some extent, as representatives of different environmental issues and anthropogenic impact. Furthermore, the survey was carried out in early summer and spring conditions, i.e., during June 2013 and April

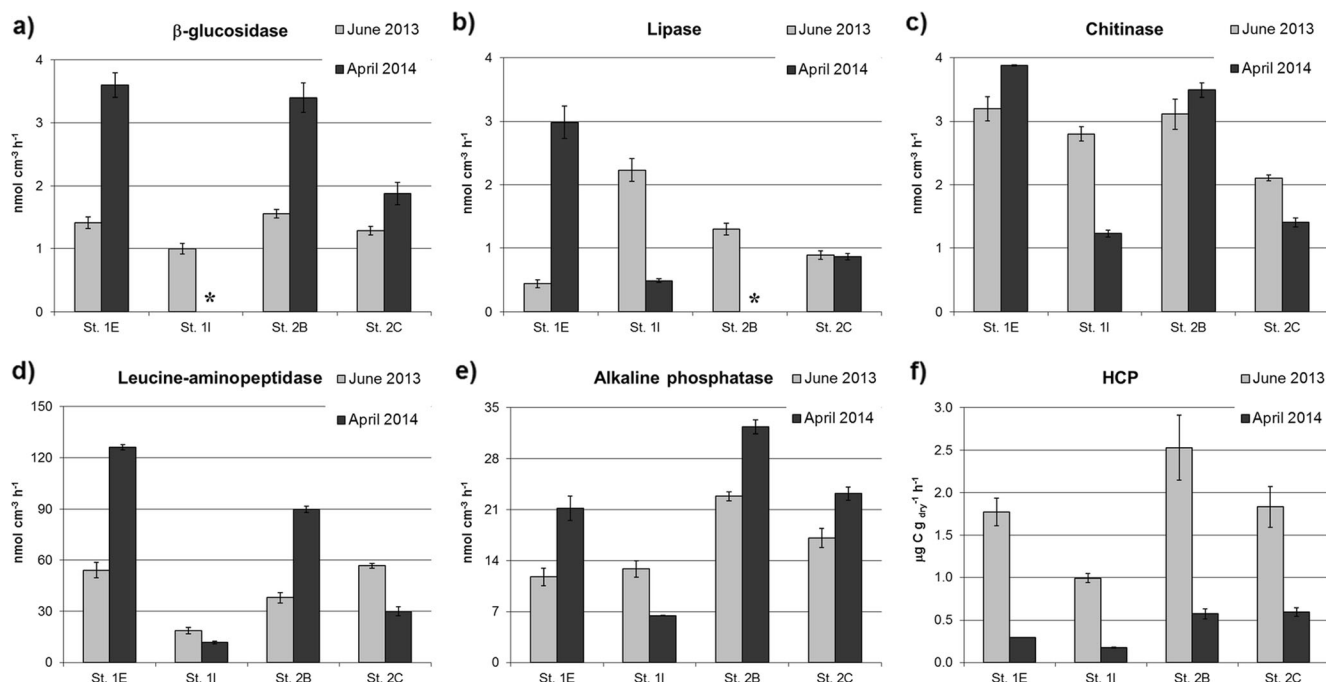


Fig. 4 Extracellular enzymatic activities and Heterotrophic C Production (HCP) at the four stations in June 2013 and in April 2014. Asterisks indicate that data are not available because below the detection limit of the instrument

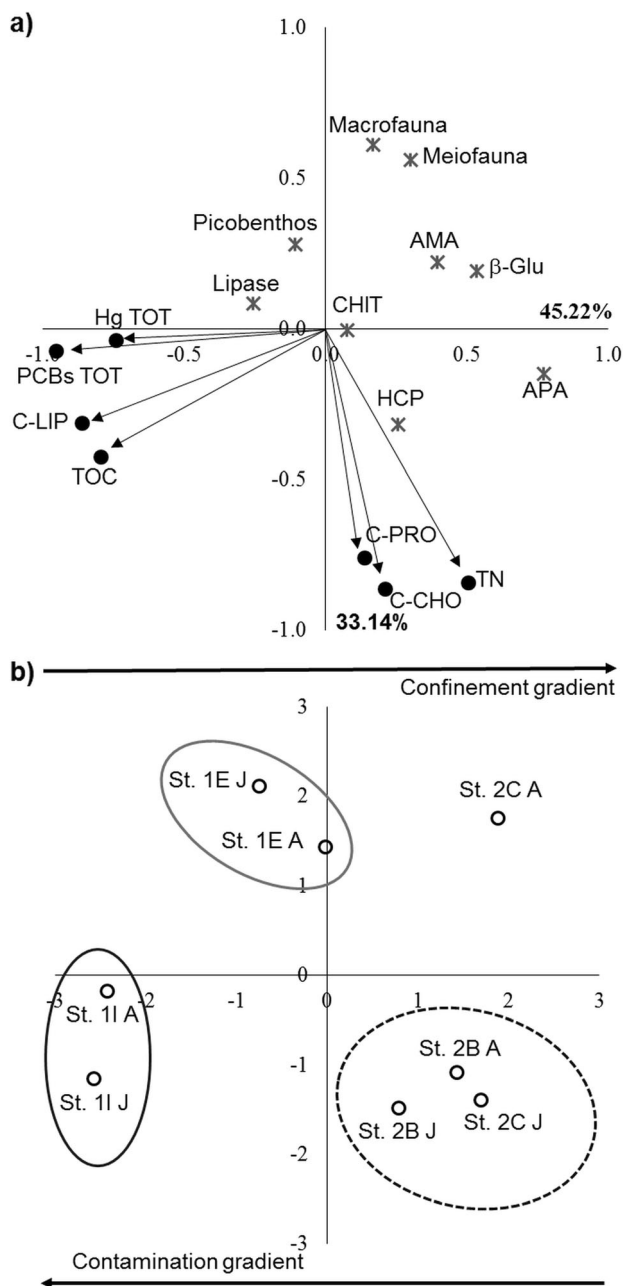


Fig. 5 PCA ordination diagram of the selected variables (a). Scatter diagram plotting factors 1 and 2 of sampling stations (b). *C-LIP* total lipids; *C-PROT* total proteins; *C-CHO* total carbohydrates; *TN* total nitrogen; *TOC* total organic carbon; *Hg TOT* total Hg; *PCBs TOT* total PCBs; *AMA* protease; *APA* alkaline phosphatase; *CHIT* chitinase; *β-Glu* β-glucosidase; *HCP* Heterotrophic C Production; *J* June 2013; *A* April 2014

2014, respectively. Focusing on the temporal variability, the ANOSIM test was performed using the two periods as discriminating factors in order to investigate the role exerted by seasonality in amplifying or reducing the differences among stations. The output highlighted that, for the selected variables (chemical structural parameters, total Hg, total PCBs, abundances of the main benthic communities, and the investigated

functional parameters), the stations of June 2013 were not statistically different from those of April 2014. Thus, we infer that for the main benthic parameters, the seasonal variation was not strong enough to hide the intrinsic and more profound differences among stations. On the other hand, performing the ANOSIM test on the same parameters using the stations as the discriminating factor, the output showed that the four sampling sites were statistically different among each other, corresponding, therefore, to different environmental situations at the bottom ($R_{ANOSIM}=0.521$, $p<0.05$). These findings suggest that the spatial variability among stations exceeded the temporal one, revealing the presence of different environmental contexts that were maintained over time.

Chemical characterization of sediments and synergistic effects of contaminants

Our results pointed out that the sediments of all the studied sites contained an elevated load of both TC and TOC. However, some differences among stations were detectable. The sampled site in front of the navy arsenal showed the maxima of TC and TOC during both periods while the station located in the middle of the second inlet displayed the minima. The high TC content observed in the first inlet could be ascribable to the industrial activities that exist along its coast, e.g., the introduction of coal, which could be associated to the steelworks plant. On the contrary, the increasing distance from these sources of C could explain its lower values in the second inlet.

Focusing on the organic fraction, a pattern similar to that observed for TC was highlighted, i.e., higher TOC content at St. 1I and a gradual decrease towards the second inlet. Our findings revealed a considerable organic enrichment that tends to remain in sediments over time. During both campaigns, TOC data were, in fact, just slightly higher compared to those previously reported in the same basin (Calace et al. 2005) and fell within the range observed in another severely polluted area characterized by brackish water and lagoonal features, i.e., the Banduzzi-Aussa river system (Cibic et al. 2012). Although the peculiarities of these two environments (different depth, salinity, etc.) limit any further comparison, data confirm the high organic enrichment as a common feature for environments subjected to a diffuse and multiple degree of pollution. In the Mar Piccolo of Taranto, besides the impact of several anthropogenic activities that exist in the area (e.g., mussel farming, industrial plants, etc.), the low hydrodynamism in this semi-enclosed basin likely contributes to the accumulation of organic matter.

The discharge of municipal and industrial wastes is generally associated with the introduction of a wide array of contaminants that are tightly linked to the sedimentary organic matrix. Among them, PAHs and PCBs are widely recognized as the major environmental issue in sediments of the Mar

Piccolo of Taranto (Storelli and Marcotrigiano 2000; Cardellicchio et al. 2007). Such contaminants show a great resistance to degradation and, then, they tend to accumulate in sediments for decades. Their toxicity affects benthic organisms at several trophic levels by reducing their abundances or altering the communities' structures. Focusing on PCBs, they occur in high or warning concentrations as reported in previous surveys (Cardellicchio et al. 2007) and confirmed by Cibic et al. (2015, this issue) in the uppermost sediment layer (0–1 cm). These hydrophobic contaminants enter the coastal marine areas through primary and secondary sources (e.g., effluent outfalls, river discharges, urban runoff, etc.) and tend to settle on the seabed where they are absorbed by fine mineral particles or organic matter forming sedimentary contaminant sinks (Kapsimalis et al. 2014). In the present study, the highest values of PCBs (1159.7 and 1067.6 ng g_{dry}⁻¹, in June 2013 and April 2014, respectively, from Cibic et al. 2015, this issue) co-occurred with the maxima of TOC, i.e., at St. 1I, indicating a high level of pollution nearby the navy arsenal. At this station, the maxima of lipids were also observed, confirming the strong association of PCBs with this biopolymeric C fraction. Sediments of the first inlet, in fact, were characterized by a lipid content of >70 and >58 % over the total BPC in June 2013 in April 2014, respectively, and at St. 1I this fraction reached extremely high values during June 2013 (5256.3 ± 189.1 µg C g⁻¹). On the other hand, sediments of the second inlet showed PCB concentrations about one order of magnitude lower than those reported at St. 1I, indicating that this area is less contaminated by such contaminants.

Both BPC and TN contents measured at St. 2B and St. 2C revealed a situation of substantial organic enrichment likely ascribable to mussel farming that is widely spread over the second inlet. The biodeposits (feces and pseudofeces) continuously produced by suspended bivalves settle to the bottom increasing the organic load as reported by Franzo et al. (2014) in a marine coastal site. In the Mar Piccolo of Taranto, the long line mussel farms, although present in both inlets, are more widespread in the second one (i.e., ~66 % of the whole second inlet's coverage against ~26 % of the first one; Caroppo et al. 2012), where the low hydrodynamism facilitates the accumulation of such biodeposits in the seabed.

Benthic communities

The ecotoxicological evaluation of sediments of the Mar Piccolo of Taranto, based on the international Sediment Quality Guidelines (SQGs) and mSQGq indices, was applied to organic contaminants (PAHs and PCBs) and heavy metals by Cardellicchio et al. (2007) and Cardellicchio et al. (2009), respectively. Overall, their findings demonstrated that sediments of the Mar Piccolo of Taranto, particularly those in the first inlet, show medium-high or high ecological risk for marine organisms, especially for benthic species. Organic

contaminants, and in particular PCBs that are strictly associated to the sedimentary organic matrix, could directly reduce deposit feeders' numbers by killing them. An indirect effect could be represented by the alteration of the inter-species relationships with the disappearance of sensitive taxa and the dominance of the most tolerant ones. We infer that the high PCB toxicity could be responsible for the low densities of meiofauna and macrofauna observed at St. 1I, where the concentration of such contaminants was extremely high. These low abundances could be reflected in a lower top-down pressure leading to the accumulation of OM, and in particular of lipids. This could partially explain the extremely high lipid contents in sediments nearby the navy arsenal.

Focusing on heavy metals, a clear distinction between the two inlets was observed, with high levels at St. 1I in accordance with previous studies in which the navy arsenal has been identified as one of the most important sources of these contaminants (Cardellicchio et al. 2009; Spada et al. 2012). Spada et al. (2012) reported that total Hg (THg) could be even 110 times higher than the background levels. However, THg in sediments is not the most important chemical form to consider, as the different Hg chemical species have also different bioavailabilities, thereby affecting their biogeochemical behavior and toxicity to organisms (Covelli et al. 2009). Oxygen concentrations in sediments modify the Hg speciation and consequently its bioavailability. In the Mar Piccolo of Taranto, although Hg is present mainly in its elemental form, a noticeable fraction of it may be "mercuric sulfide" (HgS), due to the co-occurrence of strong anoxic conditions and the high availability of sulfides (Caroppo and Cardellicchio 1995). Both these forms are insoluble and potentially less available for methylation (Covelli et al. 2009). However, bioturbation and particularly resuspension events, which often occur in the Mar Piccolo due to its shallow depth, could be responsible for the oxidation of HgS, rendering a fraction of this form eventually available for methylation. In the present study, the low abundances of meiofauna and macrofauna observed at St. 1I could be ascribable not only to PCBs but partially also to Hg contamination. Although data on Hg speciation are not available, oxygen microprofiles did not indicate a severe oxygen depletion (Rubino et al. 2015, this issue), suggesting that a fraction of HgS could be oxidized posing the risk for its consequent methylation. Bioaccumulation and biomagnification phenomena already documented in the whole area of the Mar Piccolo (Spada et al. 2012) and particularly in front of the navy arsenal corroborate the hypothesis that the benthic trophic chain is affected by Hg contamination. However, the evaluation of this aspect and its ecological implications need great caution and deserve further investigations.

To the best of our knowledge, very few data are available on meiofauna inhabiting sediments of the Mar Piccolo of Taranto. However, our findings are in accordance with

Sandulli et al. (2004) who reported the presence of a not well structured meiofaunal community characterized by low abundances. In our study, the higher densities observed at St. 1E could be ascribable to the ingression of marine water through the channel and to several associated effects as inputs of fresh organic matter and enhanced oxygenation of sediments. In the second inlet, gradually increasing meiofaunal numbers were observed both in June 2013 and April 2014, along a decreasing gradient of contamination. Although the determination at the level of the main meiofaunal groups limits further considerations about the structure and biodiversity of this community, our findings suggest that meiofauna were more structured at St. 1E due to the occurrence of several groups even if with very low RA. In accordance with our results, Sandulli et al. (2004) reported a community strongly dominated by Nematoda. Although this feature is common for both polluted and unpolluted environments, the authors observed the presence of nematode genera well adapted to strongly contaminated sediments as *Theristus*, *Terschellingia*, and *Desmodora*. Being the most diverse taxon of meiofauna, this well-represented group of organisms has a high ecological valence and therefore likely includes opportunistic taxa, i.e., able to tolerate such inhospitable environments. Moreover, Sandulli et al. (2004) observed higher meiofaunal numbers in sediments colonized by *Caulerpa racemosa*, and related the presence of such macroalgae to an increased microenvironmental complexity, which could offer new exploitable surfaces for meiofauna colonization. In our study, the presence of *Caulerpa* spp. and *Chaetomorpha linum* was observed in the second inlet during both campaigns (Cibic et al. 2015, this issue). These algae could alter the sedimentary environment by increasing the habitat complexity not only for meiofauna but also for macrofauna. The occurrence of new ecological niches could contribute, therefore, to the settlement of benthic communities different from those observed in the first inlet.

The geomorphological features of the Mar Piccolo of Taranto seem to play a pivotal role on macrofaunal abundance and community structure within the area. This semi-enclosed basin shows the peculiarities of coastal transitional environments, with decreasing salinity, limited depth, and slow water renewal rates going from the “Navigabile” channel towards the second inlet. The presence of a confinement gradient, in fact, was already reported by Alabiso et al. (2006). In similar environmental contexts, the increased distance from seawater inputs is associated to the gradual reduction of marine species, which are replaced by taxa adapted to brackish conditions (Perthuisot and Guelorget 1983). Along a confinement gradient, macrofauna tend to show large seasonal variations, low benthic diversity, and strong dominance of few paralic species (Arias and Drake 1994). In the present study, macrofauna seem to follow such general patterns. The station nearby the “Navigabile” channel (St. 1E) showed a well-developed

macrobenthic community as supported by the high values of richness and biodiversity. During both sampling campaigns, marine species were observed mainly at this site, such as the echinoderms *Amphiura chiajei* and *Ophiothrix fragilis*, the bivalves *Pitar rudis* and *Papillicardium papillosum*, and the polychaete *Notomastus formianus* (Pères 1967). These findings suggest that the proximity to the channel mouth, and the ingression of marine water through it, may influence the environmental characteristics of the sediments favoring marine environmental features. On the other hand, the inner part of the study area, i.e., the second inlet, displayed species typical of the paralic environment (the bivalves *Abra segmentum* and *Cerastoderma glaucum*; the amphipods *Gammarus aequicauda*, *G. insensibilis*, and *Microdeutopus anomalus*, Perthuisot and Guelorget 1983), reflecting the increased degree of confinement. Moreover, the paralic species *Arcuatula senhousia* and *Paracerceis sculpta* were only found at these stations.

Even considering the natural variability of the community due to the confinement gradient, different responses of macrofauna to anthropogenic stressors were detected. Focusing on the trophic guilds, the dominance of infaunal organisms and deposit feeders at St. 1E suggests, to some extent, the co-occurrence of oxygenated sediments and less severe levels of contamination. This hypothesis is corroborated by the lower load of contaminants reported at this site when compared to St. 1I and also by the oxygen microprofiles which revealed a slightly deeper oxygen penetration at that station, up to 3 mm in June 2013 (Rubino et al. 2015, this issue). On the other hand, the community nearby the navy arsenal was dominated by more mobile invertebrates as carnivores and grazers. This finding suggests that deposit feeders could be killed by the ingestion of toxic sediments due to high contaminants contents as those observed at St. 1I. Even if Hg is likely present in its insoluble forms, the digestive acids of such organisms could transform less bioavailable Hg compounds in more active ionic counterparts. Furthermore, in such inhospitable environment, mobile organisms could have more probability to survive because they are able to avoid hotspots of contaminants through their active movements (Ward et al. 2013). In the second inlet, the presence of mussel farms could enhance the proliferation of filter feeders, which obtain food via filtration of the organic matter settled from the suspended ropes. Since this pattern was more accentuated at St. 2C than at St. 2B, and the latter showed intermediate characteristics between St. 1I and St. 2C, we infer that the three stations are located along a decreasing gradient of chemical (heavy metals, PCBs, etc.) contamination. Increasing the distance from the main contamination source (that, overall, is represented by the first inlet), the community seems to adapt to an impacted environmental context in which the main stressor is the mussel farm activity. Moreover, as already mentioned for meiofauna, the presence of macroalgae could exert an influence on

macrofaunal composition and structure. During April 2014, the elevated abundances of *A. senhousia* (760 ind. m⁻²) at St. 2C could derive from the presence of the nitrophilic algae observed in our samples. The association between this bivalve and nitrophilic macroalgae has been already reported by Matarrese et al. (2004). Vegetation represents, in fact, a suitable substratum for the attachment of this species by byssal threads. The variability observed in its abundance between June 2013 and April 2014 could be explained by the intrinsic characteristics of this bivalve. It is well known, in fact, that *A. senhousia* tends to reach very high densities (150,000 ind. m⁻²; Crooks 2001) under favorable environmental conditions.

The MDS plot performed on trophic guilds shows a clear separation of the marine St. 1E from all the other sites, and the latter seem to follow a confinement gradient. The location of the most polluted stations (St. 1I) at the bottom right of the plot suggests that the sediments in front of the navy arsenal harbor a different macrofaunal assemblage. The reciprocal positions of the other sites (especially those of the second inlet) seem to follow a decreasing contamination gradient (Fig. 6).

Benthic ecosystem functioning in the Mar Piccolo of Taranto

Ecosystem functioning involves several processes which can be summarized as production, consumption, and transfer to higher trophic levels, organic matter decomposition, and nutrient regeneration (Danovaro and Pusceddu 2007). In this study, we investigated two aspects of the heterotrophic pathways that are crucial for the transfer of detritus to the higher trophic levels and, therefore, for the ecosystem functioning performance: the organic matter decomposition rates (i.e., extracellular enzymatic activities) and the incorporation of such mobilized C into new prokaryotic biomass (i.e., HPC).

In the four studied stations, all the tested hydrolytic rates were extremely low even compared with other polluted environments characterized by high sedimentary organic matter

(Manini et al. 2003; Cibic et al. 2012). Since both TOC and BPC data indicated an elevated organic content at all sites, the limited degradation rates suggest that just a small fraction of the organic pool was enzymatically degradable and, therefore, available for heterotrophic metabolism. We calculated the turnover rates of the main macromolecules as the ratio of enzymatically mobilized carbohydrates, lipids, and proteins to the corresponding labile organic fraction. Values are expressed as days required for completing the turnover of each molecules' group. St. 1I was characterized by the slowest turnover rates of both proteins (24.1 and 32.9 days in June 2013 and April 2014, respectively) and carbohydrates (28.3 and 33.6 days in June 2013 and April 2014, respectively) whereas the other stations showed values comparable with those reported for Lesina and Goro lagoons (Manini et al. 2003) and slightly lower than those calculated in the polluted Banduzzi-Aussa river system (Cibic et al. 2012). Lipid turnover was slow at all stations and particularly in the first inlet, where more than 400 days were potentially required for the degradation of the whole lipid pool (St. 1E in June 2013 and St. 1I in April 2014).

The refractory nature of sedimentary OM could be attributable to processes such as sorption or complexation, which may partially sequester potentially degradable substrates and, then, impede their mineralization. Mayer et al. (2004) demonstrated that the network of pores that allow the access of enzymes to organic matter within the aggregates might include "throats" of small mesopore size. Thus, some pores could be too small to allow enzyme diffusion to parcels of organic matter held in larger pore spaces. Furthermore, the diffusional hindrance caused by some combination of pore size and tortuosity will slow access of digestive agents to organic substrate, and return of solubilized food, that makes it physiologically unprofitable for an organism to make the attempt. In addition, Zimmerman et al. (2004) confirmed such "mesopore protection" mechanism for sequestration and preservation of sedimentary organic matter, extending the concept also to organic contaminants. Sediments of the Mar Piccolo of Taranto are an extremely complex environment where an elevated organic load co-occurs with several other compounds, such as organic contaminants and heavy metals. Unfortunately, we are not able to investigate the fraction of mesopores over the total range of pores in our sediment aggregates. However, the high complexity of the sedimentary matrix should not exclude the occurrence of adsorption/complexation processes, which alter the function of enzymes and/or their access to the substrates, resulting, therefore, in partially impeded carbon mineralization.

From an "ecosystem" point of view, the benthic system of the Mar Piccolo of Taranto showed different functioning scenarios. Nearby the navy arsenal, the high amounts of several pollutants strictly associated to the OM pool (and in particular to lipids) could increase its refractory nature, limiting

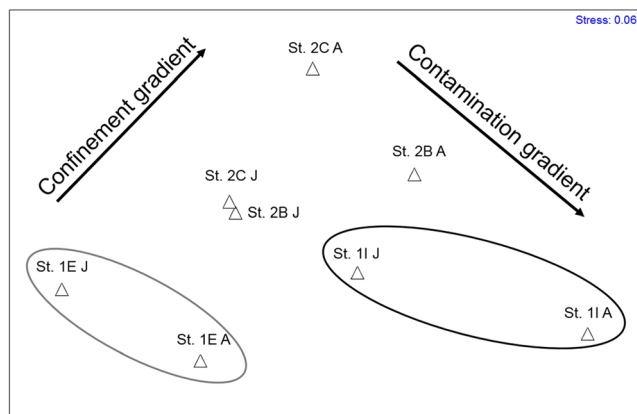


Fig. 6 MDS output performed on the abundances of main macrofauna trophic guilds. J June 2013; A April 2014

therefore the degradation of the organic detritus. Furthermore, the lowest HCP rates observed at St. 1I during both campaigns suggest a limited transfer of mobilized C to the higher trophic levels. From a top-down perspective, the scarce presence of meiofaunal and macrofaunal organisms at this station could reflect limited inputs of “fresh” organic matter (e.g., exudates, feces, etc.), which could be readily available to fuel the degradation activities. Moreover, it is important to take into account that a fraction of the observed enzymatic activities should be attributable to benthic microalgae (Misic and Covazzi Harriague 2009; Cibic et al. 2012). At St. 1I, Rubino et al. (2015, this issue) reported surprisingly modest abundances and activities associated to such organisms. These findings could contribute to explain the low degradation rates observed nearby the navy arsenal.

Although the degradation rates remained rather slow at all stations, the heterotrophic microbial community seems more active at the other investigated sites if compared to St. 1I. The ingression of marine water through the “Navigabile” channel (St. 1E) and the higher abundances of both benthic microalgae (Rubino et al. 2015, this issue) and meiofauna/macrofauna could in fact contribute to the faster prokaryotic processes. This hypothesis is corroborated by the higher HCP rates observed, especially during June 2013. On the other hand, the low HCP rates measured during the second campaign may reflect the winter conditions in which slower microbial metabolisms associated to the lower bottom temperature lead to a less active microbial community. Our findings are consistent with Cibic et al. (2012) who reported comparable HCP rates in a severely polluted environment during winter.

Focusing on the main actors responsible for the investigated mineralization processes, i.e., benthic bacteria, our data on their potential metabolic traits (Fig. 4) did not clearly reveal that specific pathways prevailed over the others according to the different stations. However, the lower microbial activities observed at St. 1I suggest that the more unfavorable environmental conditions nearby the navy arsenal could exert an inhibition effect on bacteria. Heavy metals, observed in higher amounts at this station, have documented toxic effects on bacterial activity and were already reported by Sun et al. (2013) as the main factor able to shape bacterial community composition, structure, and diversity in sediments of estuaries subjected to multiple contamination. Moreover, the large percentage of “unknown” metabolic traits makes the interpretation of results particularly arduous since the real metabolic potential of the community is likely hidden. Much of our knowledge on pollutant tolerant strains in literature comes from studies that involve laboratory isolation from environmental samples. However, moving from environment into controlled laboratory settings will favor certain bacterial types. The high abundance of unclassified groups in this and other studies (Sun et al. 2013) suggests that current knowledge on contaminant tolerant species has been biased by the difficulties faced in

mimicking environmental settings in laboratory contaminant exposures. Our findings reiterate how little is known about bacterial communities inhabiting coastal sediments, their diversity, and associated functions.

Finally, we cannot exclude that some methodological limitations could be partly responsible for the observed low degradative activities. In the present study, the hydrolytic rates were estimated using small substrate proxies, typically a monomer (an amino acid or a monosaccharide analogue) linked to a fluorophore. Such method measures only the activities of exo-acting enzymes, overlooking completely the action of endo-acting enzymes that cleave a polymer midchain. In addition to such considerations, the MUF and AMC fluorophores have excitation and emission maxima, which overlap with natural DOC in marine environments, so measurements can be problematic, particularly in high-DOC-content pore waters (Arnosti 2011). In the Mar Piccolo of Taranto, De Vittor et al. (2015, this issue) reported elevated pore water DOC concentrations especially at St. 1I, in correspondence to our lowest degradative activities. These findings corroborate the hypothesis of an antagonistic effect exerted by DOC fluorescence on that of the fluorophores used for measuring the exoenzymatic rates. Keeping in mind the drawbacks described above, we cannot exclude, therefore, that the measured degradative rates could represent an underestimation of the real mineralization processes ongoing in the area.

Conclusions

Our main findings suggest that the benthic ecosystem functioning could be quite different between the two inlets. Although a noticeable organic enrichment existed at all stations, the two inlets differed in the intrinsic characteristics of such sedimentary organic matter. The sites located in the first inlet showed an extremely elevated organic content strongly associated with high amounts of contaminants, rendering the sediments more toxic and limiting the role of OM as a food source for the analyzed benthic communities. The stations of the second inlet were characterized by less contaminated sediments with organic matter that derived mainly from mussel farming and that tended to accumulate due to the low renewal of bottom waters. St. 1I displayed the highest concentrations of both organic matter and contaminants (PCBs and heavy metals) due to the presence of one of the main sources of pollution, i.e., the military arsenal. The benthic system functioning seemed affected at all the investigated structural and functional levels. Both meiofauna and macrofauna showed low abundances, while likely inhibited microbial processes led to limited transfer of C either into a solid microbial loop or to the higher trophic levels. On the other hand, at St. 1E, the ingression of seawater through the “Navigabile” channel and

the increased distance from the navy arsenal likely enhanced the overall benthic ecosystem functioning as indicated by the faster microbial reworking of the organic matter and by the higher densities of meiofauna and macrofauna. St. 2B and 2C were located along a decreasing gradient of contamination (from St. 1I) and at the same time along an increasing gradient of confinement (from St. 1E). The brackish conditions in the inner part of the second inlet enhanced the presence of communities different from those observed at St. 1E, and the main distance from the navy arsenal contributed to a less impacted benthic ecosystem. Its functioning seemed mainly driven by the mussel farms. The fresh organic matter produced by bivalves partially fueled microbial processes and the mobilized C was transferred to the higher trophic levels mainly represented by paralic filter feeders.

Our results demonstrated that the evaluation of the benthic ecosystem functioning could allow to detect different environmental conditions that would be likely neglected using more traditional approaches as the sole chemical characterization of sediments. However, we warmly recommend further investigations, especially on a wider sampling grid and by deepening the study of the bacterial community structure, prior to any corrective action on the area. This latter aspect deserves to be developed extensively in order to understand the potential ability of specific prokaryotes in degrading persistent contaminants. The main findings by Matturo et al. (2015, this issue), in fact, suggest that the autochthonous microbial community (sampled at St. 1I) of the Mar Piccolo of Taranto showed to be potentially efficient in sustaining the biodegradation of PCBs. Although the study was performed in controlled anaerobic microcosms, which unlikely simulate the environmental conditions, these results provide a promising starting point for addressing corrective plans.

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