

## Natural and anthropogenic disturbances shape benthic phototrophic and heterotrophic microbial communities in the Po River Delta system

Tamara Cibic<sup>a,\*</sup>, Stefano Fazi<sup>b</sup>, Federica Nasi<sup>a</sup>, Lorenzo Pin<sup>b</sup>, Francesca Alvisi<sup>c</sup>, Daniela Berto<sup>d</sup>, Luigi Viganò<sup>e</sup>, Annamaria Zoppini<sup>b</sup>, Paola Del Negro<sup>a</sup>

<sup>a</sup> Sezione di Oceanografia, Istituto Nazionale di Oceanografia e di Geofisica Sperimentale - OGS, via A. Piccard 54, I-34151, Trieste, Italy

<sup>b</sup> Water Research Institute, National Research Council (IRSA-CNR), Via Salaria km 29,300, I-00015, Monterotondo, Rome, Italy

<sup>c</sup> Institute of Marine Sciences, National Research Council (ISMAR-CNR), Via P. Gobetti 101, I-40129, Bologna, Italy

<sup>d</sup> Institute for Environmental Protection and Research, Località Brondolo, I-30015, Chioggia (Venice), Italy

<sup>e</sup> Water Research Institute, National Research Council (IRSA-CNR), Via Del Mulino 19, I-20861, Brugherio, Italy



### ARTICLE INFO

#### Keywords:

Microbial diversity  
Microphytobenthos  
Bacteria  
Coastal lagoon  
Freshwater input  
Organic enrichment  
Contamination  
Anoxia

### ABSTRACT

This study represents the first example in which benthic heterotrophic and photoautotrophic microbial communities (prokaryotes and microphytobenthos-MPB, respectively) were simultaneously investigated. To explore how they synergistically respond to multiple natural and anthropogenic stressors, in the framework of the Project RITMARE four lagoons of the Po River Delta were sampled in May 2016: two with more marine features, i.e. Scardovari (SC) and Caleri (CL), and two more directly affected by the Po River flow, i.e. Canarin (CN) and Vallona-Marinetta (MV). The abundance and structure of benthic communities were related to physical-chemical parameters i.e. grain-size, total N, total organic C, stable C and N isotopes, and synthetic organic contaminants. Stations were gathered into three distinct groups ( $R_{\text{anosim}} = 0.540$   $p < 0.001$ ) according to their different physical-chemical features: outer, inner-freshwater and inner-marine sites. Contamination levels did not seem to severely affect the microbial abundances that were overall stimulated by the combined effect of high organic and nutrient loads: prokaryotes up to +42% and MPB up to +93%. Bacteria and Archaea displayed high densities at sites directly influenced by the freshwater input and anthropogenically derived nitrogen. Delta- (30.8%), Alpha- (12.8%), Gamma- (11.8%) and Beta-Proteobacteria (7.1%) were the dominant classes at all sites. For both communities, a significant inter-lagoonal (among different lagoons) and intra-lagoonal (among stations within the same lagoon) pattern was highlighted by PERMANOVA. nMDS and SIMPER analyses revealed distinct assemblages in the inner and outer parts of the lagoons. We applied a novel functional approach based on diatom life modes. At inner and outer sites, different diatom living forms developed: epipsammic (on sand) nearby the lagoonal mouths, epipellic (on mud) at the innermost sites and non-benthic forms (planktonic, tychoepelagic and epiphytic) at sites directly influenced by freshwater. Distance-based Linear Models further indicated that salinity, clay and temperature were the significant drivers of the prokaryotic spatial distribution whereas clay, PAHs, PCBs and salinity best explained the MPB structure. The dominance of non-benthic diatom life modes in the more polluted lagoon (CN) suggests a negative influence of contamination on the MPB structure. Two diatom keystone species likely contribute to restore the oxic gradient in sediments frequently exposed to anoxia, allowing the subsequent microbial aerobic degradation and the recolonization of higher trophic organisms. The capacity of re-oxygenation after anoxia has important ecological and economic implications in lagoons exploited for aquaculture.

### 1. Introduction

In shallow water areas, particularly in estuaries and lagoons where the bottom substratum is soft, most important biochemical processes take place in sediments, where phototrophic and heterotrophic

microbial communities drive central ecosystem functions, such as primary production, decomposition and nutrient cycling (Larson and Sundbäck, 2008). In sediments, Bacteria and Archaea play a major role in geochemical processes as the main responsible for the degradation of organic matter, the incorporation of mobilized C into microbial biomass

\* Corresponding author.

E-mail address: [tcibic@inogs.it](mailto:tcibic@inogs.it) (T. Cibic).

<https://doi.org/10.1016/j.ecss.2019.04.009>

Received 22 November 2018; Received in revised form 27 February 2019; Accepted 7 April 2019

Available online 15 April 2019

0272-7714/ © 2019 Elsevier Ltd. All rights reserved.

and the transfer of matter and energy to higher trophic levels (Baker et al., 2015). Most of the phototrophic microbial organisms are eukaryotic (diatoms, dinoflagellates, phytoflagellates, etc.), but also prokaryotic photosynthetic organisms, such as cyanobacteria, contribute to the benthic community (MacIntyre et al., 1996). Microphytobenthos (MPB) are key to the carbon cycle in aquatic environments as producers of new organic matter that can enter the benthic and pelagic trophic web (Barranguet, 1997) and they constitute the only autochthonous source of primary production on sediment in the absence of macroscopic vegetation (Larson and Sundbäck, 2008). Moreover, in shallow estuaries and lagoons MPB primary production often exceeds that of phytoplankton (Underwood and Kromkamp, 1999). As the principal oxygen producer in coastal environments, MPB allow the aerobic degradation of both autochthonous and settled organic matter in sediments. Thus, microbenthic functions are crucial not only for the overall function of shallow-water sediments, but also for adjacent systems that depend on shallow areas for recruitment and food supply (Larson and Sundbäck, 2008).

The Po River Delta is subjected to multiple human-derived pressures resulting from many activities such as agriculture, industry, urban development and aquaculture. Through the delta high amounts of inorganic nutrients and contaminants are delivered from the Po River watershed, one of the most populated and productive areas in Europe, to the Adriatic Sea. Previously collected data on organic and inorganic pollutants in the Po River Delta system have shown high levels of contamination, mainly heavy metals and organic contaminants (Viganò et al., 2003). The delta systems are exposed simultaneously to combined stress factors of anthropogenic origin, including (1) a suite of chemical contaminants (e.g. metals, petroleum hydrocarbons, insecticides and/or herbicides), (2) organic enrichment (and oxygen depletion) and (3) elevated nutrient levels. In the transitional areas of the delta, water, sediments and biota are recipients and reactors in which nutrients and contaminants are metabolized, transformed and even taken up. Each stressor can uniquely affect species within a community and may produce synergistic effects when combined. Besides those of anthropogenic origin, benthic communities inhabiting the sediments of the Po River Delta lagoons are simultaneously exposed to natural disturbances, such as the stress caused by the alternation of flood river conditions with the lean-season flow, and major bottom currents that cause high turbidity. Indeed, in flood river conditions, the major bottom currents carry away not only the resuspended fine sediments but with them, also the microbial communities that inhabit the surface layers of delta sediments are recurrently swept away (Cibic et al., 2016b; Vidal-Durà et al., 2018). River discharge fluctuations have a great effect on bacterial community composition: hydrology (flood vs drought) defines specific environmental conditions modifying sediment characteristics (grain-size, organic matter, and C:N content) and quality of dissolved organic matter that affect both bacterial community composition and function in river sediments, and consequently the community in the receiving water bodies (Freixa et al., 2016). Further, in high temperature and low hydrodynamic conditions that often occur in the Po River lagoons, the major organic loads trigger hypoxia and anoxia events (Viaroli et al., 2010) with severe consequences for the lagoonal ecosystem.

Aquatic microbes are extremely sensitive to environmental changes because of their small size, fast growth rates and genome plasticity. Sediment contamination influences the composition of microbial communities, disfavoring sensitive bacterial phyla and favoring those involved in the degradation of pollutants. Consequently, the study of microbial community diversity, and their fluctuations over spatial and temporal scales, represents a useful tool to evaluate the consequences of the anthropogenic perturbation on the ecosystem health of aquatic systems (Quero et al., 2015). The development of molecular tools for the determination of the prokaryote taxonomic and functional diversity has enhanced our ability to investigate the relationships between prokaryotic biodiversity and ecosystem functioning (Danovaro and

Pusceddu, 2007). Moreover, Fluorescence *In Situ* Hybridization (FISH) techniques enable us to visualize and quantify specific microbial populations, providing crucial information regarding the spatial distribution of specific microbial lineages along environmental gradients (Fazi et al., 2007).

Marine microalgae are a particularly promising indicator of contamination since they are one of the most abundant life forms in aquatic environments and represent the base of the food chain (Torres et al., 2008). Diatoms, in particular, occur in all types of aquatic ecosystems; their assemblages are diverse according to the environmental status and therefore contain considerable ecological information. They are characterized by one of the shortest generation times of all biological indicators that allows them to rapidly respond to environmental changes. Consequently, diatoms have been increasingly used as indicators of different kinds of stress. For instance, benthic diatom response to different nutrient concentrations was studied for the assessment of the impact of river inflows (Cibic et al., 2012) and sewage discharge (Cibic et al., 2008). Furthermore, changes in the community structure have been observed in areas devoted to mussel farming (Franzo et al., 2014). Recently, the sensitivity of benthic diatom communities to metal and petroleum hydrocarbon contamination has been also reported (Potapova et al., 2016; Rubino et al., 2016; Rogelja et al., 2018). While the MPB response, in terms of community structure, to water quality parameters has been seldom investigated in coastal marine lagoons (Facca et al., 2002; Gambi et al., 2003; Facca and Sfriso, 2007; Rubino et al., 2016; Di Pippo et al., 2018), similar studies in river delta systems are even rarer (Cibic et al., 2012). Similarly, very little information is available on the distribution and diversity of benthic prokaryotes in relation to changes of environmental variables in these aquatic transitional ecosystems. So far, only a few studies have focused on microbial aspects in the lagoons of the Po River Delta: Manini et al. (2003) investigated the total microbial community whereas Danovaro and Pusceddu (2007) reported on the bacterial and archaeal community structure using FISH, and bacterial composition using Automated Ribosomal Intergenic Spacer Analysis (ARISA). Both studies were carried out at only one station of the Sacca di Goro. More recently, Pala et al. (2018) related the variation of bacterial and archaeal abundance, assessed by FISH, to physical-chemical variables at three sites of the same lagoon. However, as far as we are aware, none of the other lagoons of the Po River have been investigated in this regard. Therefore, the aim of this study was to contribute to the largely unknown benthic microbial communities and to investigate their response to cumulative effects of co-occurring natural and anthropogenic stressors in the Po River Delta. The assessment of the benthic microbial communities was carried out within the framework of the large Italian project RITMARE (la Ricerca Italiana per il MARE – Italian Research for the Sea). We hypothesized that the sediments in distinct lagoons of the Po River Delta harbor differently structured microbial communities (both prokaryotes and MPB). Specifically, in this study we seek to answer the following questions: 1) Does the structure of the prokaryotic and MPB community vary significantly in river-coastal lagoons that are differently affected by freshwater input? 2) Is it possible to highlight both an inter-lagoonal (among lagoons) as well as an intra-lagoonal (among stations within the same lagoon) pattern in microbial composition? 3) Which are the main physical-chemical drivers of the microbial community structure in this highly dynamic ecosystem? 4) What might be the ecological implications of a resilient MPB community in river-coastal lagoons frequently subjected to anoxia?

## 2. Material and methods

### 2.1. Study site and sampling

The Po River is the major Italian watercourse, which flows for about 650 km over a highly anthropized and industrialized area (estimated in  $114 \times 10^6$  equivalent inhabitants), collecting urban, industrial and

agricultural discharges of its watershed. It enters the Adriatic Sea spreading in nine branches and forming a delta originating about 50 km from the sea. This delta system, with a daily mean discharge of  $1500 \text{ m}^3/\text{s}$  (ranging from  $100 \text{ m}^3/\text{s}$  to  $11550 \text{ m}^3/\text{s}$ ) (Falcieri et al., 2014), is chemically contaminated, as the river carries land-derived nutrients, trace metals and organic micropollutants collected from the entire Po valley and the river tributaries (Viganò et al., 2003; Casatta et al., 2016). The River Po Delta is a complex system of flatlands and lagoons, traditionally exploited for different types of aquaculture, mainly mollusc farming that has become the main economic asset of the area (Abbiati et al., 2010). In particular, the clam *Ruditapes philippinarum* is being intensively farmed reaching very high production yields (Turolla, 2008) and making Italy the second-highest producer in the world after China (FAO, 2014).

Among the seven lagoons of the Po River Delta system that were sampled in the framework of the project RITMARE, in this particular study we focused on a subset of four lagoons, two characterized by more marine features (Scardovari and Caleri) and two more directly affected by the Po River flow (Canarin and Marinetta-Vallona). Following a north–south direction, the first investigated lagoon was Caleri ( $45.09^\circ \text{N}$ ,  $12.31^\circ \text{E}$ ). The hydrodynamics of this large ( $11.5 \text{ km}^2$ ) and shallow area (mean depth of 2.0 m; salinity varying from 15 to 35) primarily depends on the water exchange with the sea through a narrow mouth and only its easternmost part is devoted to clam aquaculture. Marinetta-Vallona ( $45.05^\circ \text{N}$ ,  $12.38^\circ \text{E}$ ), a shallow water body (area:  $10.0 \text{ km}^2$ ; average depth: 0.5–2 m; salinity: 10–30), is connected to the sea by a narrow mouth (about 100 m wide) and directly receives freshwater through a deltaic branch of the Po River (Po di Levante). This lagoon has been traditionally exploited for aquaculture, and about one-fifth of the total (about 200 millions €) northern Adriatic Sea production of clam (*R. philippinarum*) farming comes from the Vallona lagoon (Turolla, 2008; Abbiati et al., 2010). Canarin ( $44.92^\circ \text{N}$ ,  $12.49^\circ \text{E}$ ; area:  $10 \text{ km}^2$ ; mean depth: 0.5–1.8 m; salinity 14–32) currently presents only a small mouth to the sea in its northern part. It directly receives freshwater through minor distributaries originating from the primary branch of the Po River, the Po di Pila, which accounts for more than 50% of the freshwater discharge and sediment load of the Po River (Maicu et al., 2018). Water circulation in the entire southern area of the lagoon is very limited, whereas it is more effective in its northern part due to the strong and direct freshwater input. Canarin is subjected to eutrophication, with extensive growth of algae and frequent anoxic periods (ARPAV, 2004; Casatta et al., 2016). Among the four investigated lagoons, Scardovari ( $44.86^\circ \text{N}$ ,  $12.42^\circ \text{E}$ ) is the largest one ( $32 \text{ km}^2$ ), located between Po di Tolle and Po di Gnocca distributaries. The lagoon is connected to the Adriatic Sea through a wide mouth, partly obstructed by sand banks, with a depth varying from 0.5 to 2.8 m. Its northern area receives nutrient-rich agricultural run-offs, whereas the southern area is more influenced by seawater exchanges (salinity range: 25–28) and hosts extensive bivalve mollusc (clams and mussels) cultures (Abbiati et al., 2010; Munari and Mistri, 2008). In its northernmost part, less influenced by seawater exchanges, eutrophication causes seasonal blooms of opportunistic macroalgae (Natali and Bianchini, 2018) and hypoxia or anoxia events (ARPAV, 2016).

Among these four lagoons, Caleri was reported as the most pristine and Canarin as the most contaminated one (Casatta et al., 2016). Organic contaminants are thoroughly described in Viganò et al. (this issue) and Zoppini et al. (this issue). For the purposes of this study, the contaminant concentrations used in multivariate analyses are briefly listed in Table S1. In each lagoon, we sampled three stations located at increasing distance from the sea on one hand, and increasing freshwater influence on the other hand, in order to represent diverse environmental features inside each lagoon (Fig. 1). Sampling was carried out between May 3<sup>rd</sup> and 5<sup>th</sup> 2016 at stations CL1, CL2, CL3 in Caleri lagoon, MV5, MV6, MV7 in Marinetta-Vallona, CN10, CN11, CN12 in Canarin and SC15, SC16, SC17 in Scardovari (see Table S2 for station coordinates). At each station, sediment samples were collected by



Fig. 1. Study area and location of the sampling stations.

means of a manual box-corer in four-six replicates (surface area  $225 \text{ cm}^2$ ; sampled volume  $3375 \text{ cm}^3$ ). The uppermost layer (0–1 cm) was sampled, pooled and homogenized for the following analyses: grain-size, total organic C (TOC) and total N (TN), stable C ( $\delta^{13}\text{C}$ ) and N ( $\delta^{15}\text{N}$ ) isotopes, abundance and structure of prokaryotic and microphytobenthic communities. At each site, water temperature, salinity, pH and dissolved oxygen were recorded *in situ* by a multiparameter probe YSI ECO2 EXP 7 20014.

## 2.2. Physical and chemical analyses

Grain size analysis was carried out on bulk samples (10–25 g), collected at each station, oven-dried at  $55^\circ \text{C}$  for 48 h. After a treatment with hydrogen peroxide for 72 h, the muddy fraction was separated from sand, shells and shell fragments by wet sieving at  $63 \mu\text{m}$ . Mud was analyzed by means of a Micromeritics X-ray sedigraph to evaluate silt and clay concentrations after dispersion in a sodium hexa-metaphosphate 6‰ solution and subsequent ultrasonic disaggregation for 10–15 min. Sand was dry-sieved at  $2000 \mu\text{m}$  to separate shells and shell fragments. Shells, sand, silt and clay are expressed as percentage of the total dry weight of the four grain size classes.

TOC and TN contents were obtained by using Elemental Analyser Flash 2000 (Thermo Fisher Scientific, Germany). For TOC analysis, samples were acidified with HCl 1 N to remove carbonates and oven-dried at  $40^\circ \text{C}$  for 24 h (Hedges and Stern, 1984). The calibration was performed using acetanilide as a standard. The detection limit and the reproducibility of the method were  $0.4 \mu\text{mol}$  and 3%, respectively. TOC and TN concentrations were expressed as weight percentage of the element on the dry sediment.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were measured in bulk sediment samples. For  $\delta^{13}\text{C}$  analysis, samples were acidified to remove carbonates (Hedges and Stern, 1984).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the samples were determined using Isotope Ratio Mass Spectrometer Delta Plus coupled with an Elemental Analyser Flash 2000 (Thermo Fisher Scientific, Germany). Isotope ratios were expressed as parts per thousands (‰). The standard reference materials were Vienna Pee Dee Belemnite for carbon and atmospheric  $\text{N}_2$  for nitrogen. The analytical precision of measurements was 0.2% for carbon and 0.5% for nitrogen. Sucrose

IAEA-CH6 and glutamic acid were used as certified reference materials.

### 2.3. Prokaryotes

With the term ‘Prokaryotes’ we refer to heterotrophic microbial organisms (Bacteria and Archaea) whereas small phototrophic Cyanobacteria cells ( $< 2 \mu\text{m}$ ) were not included in this community. The total prokaryotic cell abundance was assessed by 4'-6-diamidino-2-phenylindole (DAPI, Vector Laboratories, USA) staining, following sediment extraction and detection procedures described elsewhere (Amalfitano and Fazi, 2008). Briefly, a subsample of fresh sediment was fixed in formaldehyde solution (fc: 1%), and amended with NaCl (fc: 0.85%), Tween 80 (fc: 0.5%) and sodium pyrophosphate (fc: 0.1 M). The resulting sediment slurry (10 mL) was divided into 1 mL aliquots, which were purified using Nycodenz density gradient centrifugation. Subsamples of the purified cell suspension (1 mL) were filtered onto 0.2  $\mu\text{m}$ -pore size polycarbonate membranes (47 mm diameter) and frozen at  $-20^\circ\text{C}$  until analysis.

Quantitative community composition analysis was performed by means of the Catalyzed Reporter Deposition - Fluorescent *In Situ* Hybridization (CARD-FISH) technique, according to the protocols previously described (Fazi et al., 2007, 2013). Horseradish peroxidase (HRP) labeled oligonucleotide probes (Biomers, Germany) were used to target Bacteria (EUB338 I-III), Archaea (ARCH915), Alpha-Proteobacteria (ALF968), Beta-Proteobacteria BET42a, Gamma-Proteobacteria (GAM42a), and Delta-Proteobacteria (DEL495 a-b-c). The hybridized filter sections were subsequently embedded in a mounting mixture containing DAPI at a final concentration of  $1 \mu\text{g mL}^{-1}$ . DAPI stained and probe hybridized cells were observed and quantified by epifluorescence microscopy (EM) (Leica DM LB 30, at 1000X magnification).

### 2.4. Abundance and community structure of microphytobenthos

In this study, with the term microphytobenthos – MPB, we refer to the microscopic eukaryotic algae (diatoms, dinoflagellates, phytoflagellates, etc.), and prokaryotic photosynthetic organisms, such as filamentous Cyanobacteria. For MPB analyses, three aliquots of homogenized sediment ( $2 \text{ cm}^3$ ) were withdrawn using a syringe and directly fixed with 10 mL of formaldehyde (4% final concentration) buffered solution  $\text{CaMg}(\text{CO}_3)_2$ , in pre-filtered bottom seawater (0.2  $\mu\text{m}$  filters). After manual stirring, 20- $\mu\text{L}$  aliquots of the sediment suspension were drawn off from the slurries and placed into a counting chamber. Only cells containing pigments and not empty frustules were counted under a Leitz inverted light microscope (Leica Microsystems AG, Wetzlar, Germany) using a  $\times 32$  objective ( $\times 320$  final magnification) (Utermöhl, 1958). When possible, at least 200 cells were counted *per* sample to evaluate rare species, too. The microalgal taxonomy was based on AlgaeBase (Guiry and Guiry, 2018) and WoRMS (WoRMS Editorial Board, 2018) websites. The qualitative identification of MPB assemblages was carried out using floras listed in Cibic and Blasutto (2011) as well as identification keys of freshwater microalgae by Canter-Lund and Lund (1995). Diatom life modes were distinguished based on literature (Round, 1971; Cibic et al., 2007; Rogelja et al., 2016; Rubino et al., 2016) into the following living forms: planktonic (truly planktonic species which settled on the substratum from the water column), tychopelagic (diatoms that are loosely associated with the sediment, commonly found in the water column) and benthic species. The latter were further divided into: epiphytic (attached to macroalgae, phanerogams or other substrata), epipsammic (living on sand) and epipelagic (living freely on soft sediments) forms. Quantitative data are reported as cells  $\text{cm}^{-3}$  of wet sediment (cells  $\text{cm}^{-3}$ ) and as Relative Abundance (RA).

### 2.5. Statistical analysis

To highlight relationships between abiotic and biotic variables a Spearman rank correlation analysis (R) was performed using STATISTICA v.7. Only statistically significant results are presented and discussed. Two biological data matrices were constructed, one for prokaryotes (based on the abundance of Archaea and total Bacteria, and four classes of Proteobacteria: Alpha-, Beta-, Gamma- and Delta-Proteobacteria), and one for MPB (based on the abundance of taxa identified at the species and genus level, excluding higher taxonomic levels). Univariate and multivariate analyses were performed using the PRIMER v7 software package (Clarke et al., 2014). Univariate diversity analysis was applied to MPB abundances considering richness (d, Margalef, 1986), equitability ( $J'$ , Pielou, 1966), diversity ( $H'(\log_e)$ , Shannon and Weaver 1949) and dominance ( $\lambda$ , Simpson, 1949). Comparison among stations was complemented by a visual representation of diversity, using k-dominance curves (Lambhead et al., 1983): species abundances (average of three replicates) were ranked (in log) in decreasing order of dominance and plotted cumulatively.

To highlight inter- and intra-lagoonal variability in the distribution of prokaryotic and MPB assemblages, a PERMANOVA test was used. The factors ‘lagoon’ and ‘in-out’ (i.e. stations located in the inner and outer, marine, part of the lagoon) were applied as fixed factors in a one-way analysis. Further, a two-way analysis (‘lagoon  $\times$  in-out’) (PERMANOVA test) was performed, in which ‘lagoon’ and ‘in-out’ were fixed factors, too. For both analyses, unrestricted permutation of raw data and 9999 permutations were applied and Monte Carlo p-values were considered. PERMANOVA was performed on two replicate samples *per* station for prokaryotes and three replicate samples for MPB.

Before each multivariate analysis, the MPB matrix was square root transformed; whereas none transformation was applied to the prokaryotic matrix. The data were then analyzed using cluster analysis (performed with the complete linkage clustering algorithm) and for both matrices, a Bray-Curtis similarity was applied.

To visualize differences in microbial assemblages among the 12 stations, a non-metric multidimensional scaling ordination (nMDS) was performed separately for prokaryotes and MPB. For prokaryotes, a Similarity Profiles (SIMPROF) analysis was used to gather significant ( $p < 0.05$ ) groups of stations that were overlaid on the nMDS ordination plot. For MPB, to highlight which taxa mainly contributed to the spatial variation of MPB assemblages, diatom genera with a Pearson correlation ( $r \geq 0.4$ ) were overlaid on the nMDS plot. Further, the relative contribution of each MPB taxon to average dissimilarities between pairs of lagoons was calculated using a one-way similarity percentage procedure (SIMPER, cut-off percentage: 50%). nMDS ordination was performed on two replicate samples *per* station for prokaryotes whereas for MPB, nMDS and SIMPER analyses were carried out on average (of three replicates) abundance values.

On both microbial communities, we performed multivariate analyses to examine spatial distributions in relation to environmental factors. The following abiotic variables were considered: i) water physical parameters (bottom temperature and surface salinity – as a signature of the freshwater input); ii) sediment features (sand, silt and clay fractions, TOC, TN,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ); iii) organic contaminants (Polycyclic Aromatic Hydrocarbons-PAHs, Polychlorobiphenyls-PCBs, degradation products of DDT-DDXs, and flame retardants, i.e. Polybrominated diphenyl ethers-PBDEs). Firstly, to define a possible correlation between abiotic variables and the two microbial matrices, the RELATE routine was used, separately for the two communities. The biotic and abiotic data were compared using the RELATE function, and 9999 random permutations were applied. This analysis uses a Spearman rank correlation test to generate a coefficient,  $\rho$ , which measures the similarity of the rank order between two matrices of the same samples. A statistic is then derived empirically by comparing to values calculated after randomizing one of the matrices. Possible  $\rho$  scores range from 1 (identical) to  $-1$  (opposite), with random values clustered around 0.

Subsequently, a BIO-ENV analysis, based on Spearman's coefficient, was used as a first explanatory approach, to assess which environmental variables correlated best with the pattern of taxa at sampling stations (Clarke and Ainsworth, 1993). Prior to RELATE and BIO-ENV analysis, data were normalized and Euclidean distance was applied. The main physical and chemical parameters correlated with the two assemblages (prokaryotes and MPB) were further used to perform Distance-based Linear Models (DistLM, Anderson et al., 2008) that assessed which variables explained differences ( $p < 0.05$ ) in microbial spatial variability. The data were normalized and options 'All specified' and R2 were used as the selection procedure and criterion, respectively.

A Principal Component Analysis (PCA) was carried out on environmental data in order to visualize the spatial distribution of main abiotic variables (surface salinity and bottom temperature, sand, silt and clay fractions, TOC, TN,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ). Organic contaminants (i.e. PAHs, PCBs, DDXs and PBDEs) and the biotic components (i.e. Archaea and Bacteria subgroups; and diatom living forms) 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 biotic variable.

Further, to test any spatial variations in abiotic factors among the sampling sites, stations were gathered into groups based on their features and position in the lagoon: outer stations, i.e. those near the lagoonal mouths, inner freshwater stations, i.e. those near the freshwater input, and inner marine stations, i.e. those with typical lagoonal features. The differences among groups were tested by ANOSIM (ANALYSIS Of SIMilarity). The ANOSIM statistic R is based on the difference of mean ranks between groups and within groups. The statistical significance of observed R is assessed by permuting the grouping vector to obtain the empirical distribution of R under null-model. ANOSIM tests *a priori*-defined groups (i.e. 'outer', 'inner-marine' and 'inner-freshwater' stations) against random groups in ordinate space. A zero ( $R = 0$ ) indicates that there is no difference among groups, while a one ( $R = 1$ ) indicates that all the samples within groups are more similar to one another than any samples from different groups (Clarke et al., 2014). Data were normalized and the Euclidean distance was applied. PCA and ANOSIM analyses were performed using STATISTICA 7 and PRIMER 7 software, respectively.

### 3. Results

#### 3.1. Physical and chemical data

The lowest temperatures, both at the bottom and at the surface, were recorded at MV5, whereas the highest ones were registered at CN11. A steeper salinity gradient was observed at the shallower stations of Canarin, with the lowest salinity (14.4) at the surface layer of CN12 and the highest one (32.7) at the bottom of CN10, while consistently higher salinities were registered in Caleri and Scardovari (Table S2).

Sampled stations were grouped into three main clusters considering the sand/mud ratio and shell (gravel) concentration according to Folk and Ward (1957): 1) muddy stations with sand concentration  $< 10\%$  and without shell fragments (CN10, CN12, SC16 and SC17); 2) sandy muddy stations with shell fragments increasing from traces (MV6), to 2–5% (CL1 and CL2) up to 19% at MV7; 3) muddy sandy stations (CL3 and CN11), slightly gravelly (SC15 and MV5). In the gravel grain size class, both shell fragments and whole shells were present. The sandy fraction between 1 and 2 mm was composed only of shell fragments, while the inorganic debris was present in particle sizes  $< 1$  mm. The highest percentage of shells and shell fragments present at MV7, and to a lesser extent at CL2 and CL1, was due to the intensive clam farming in these lagoons. Sediments displayed higher concentrations of sand at all lagoonal mouths, i.e. at MV5, SC15, CN11 and CL3 (Table 1), likely due to higher bottom current velocities. The higher clay fraction found at CN12, CN10, SC16 and SC17 indicates lower hydrodynamic conditions in Canarin and in Scardovari.

TOC displayed the highest value at CL3 and contents  $> 1\%$  were measured in Marinetta-Vallona and Canarin, while C/N ratios  $> 12$  were obtained at stations with more freshwater features, particularly CN11, CL3 and MV5, in which more negative  $\delta^{13}\text{C}$  values were obtained, and confirmed by the negative significant relationship between C/N and  $\delta^{13}\text{C}$  values ( $R_2 = 0.557$ ,  $p < 0.05$ ). Higher  $\delta^{15}\text{N}$  values observed at CN12, MV6 and MV7 could derive from major nitrogen loads of anthropogenic origin (Table 1).

#### 3.2. Benthic prokaryotic community structure

Total prokaryotic abundance, as determined by DAPI counts, ranged from  $1.13 \times 10^9 \pm 0.15 \times 10^9$  cells  $\text{g}^{-1}$  at SC16 to  $2.43 \times 10^9 \pm 0.32 \times 10^9$  cells  $\text{g}^{-1}$  at CN12. Comparable average abundances (as mean of three stations  $\pm$  SD) were obtained in Caleri and Scardovari ( $1.44 \times 10^9 \pm 0.20 \times 10^9$  cells  $\text{g}^{-1}$  and  $1.46 \times 10^9 \pm 0.43 \times 10^9$  cells  $\text{g}^{-1}$ , respectively) that were up to 42% lower than those measured in Canarin ( $2.04 \times 10^9 \pm 0.35 \times 10^9$  cells  $\text{g}^{-1}$ ) (Fig. 2). We could affiliate to Archaea 8.5%  $\pm$  0.5% and to Bacteria 87.3%  $\pm$  4.5% of total DAPI stained cells. Overall, the highest densities of Bacteria and Archaea were observed at sites with the highest clay content (67.3%, 47.6%, 63.4% and 52.8% for CN12, MV6, CN10 and SC17, respectively) and a significant correlation between these communities and clay was highlighted ( $R = 0.64$  and  $0.66$ ,  $p < 0.05$  for Bacteria and Archaea, respectively.) The lowest abundances were observed at sites with the highest sand content, close to the lagoonal mouths (CL3, MV5, SC15).

The direct quantification of cells belonging to four subgroups of Proteobacteria showed that Delta-Proteobacteria represented 30.8% of bacterial cells followed by Alpha- (12.8%), Gamma- (11.8%) and Beta-Proteobacteria (7.1%) (Fig. 2). Delta- and Gamma-Proteobacteria followed the same pattern of the total bacterial cells, being more abundant in Canarin and at sites with the highest clay content. Beta-Proteobacteria displayed the highest abundances at sites characterized by the lowest salinity (MV6 and CN12). Alpha-Proteobacteria displayed the highest percentages in the northern lagoons (17.3%, 15.5%, 10.0% and 8.4% in CL, MV, CN and SC, respectively) showing the highest percentages (about 20% of bacterial cells) at CL1 and MV5.

The PERMANOVA main-test performed on prokaryotic assemblages revealed that microbial communities significantly differed among lagoons (Pseudo-F = 5.14;  $p$ -value  $< 0.01$ ) and between their inner and outer parts (i.e. 'in-out' factor) (Pseudo-F = 4.8506;  $p$ -value  $< 0.05$ ), whereas no significant differences emerged for 'lagoon  $\times$  in-out' factors. The prokaryotic distribution in Canarin significantly differed from that in Caleri, Marinetta-Vallona and Scardovari (PERMANOVA pairwise test:  $t = 3.80, 2.26, 2.90$ ;  $p$ -value  $< 0.05, 0.01, 0.05$ , respectively).

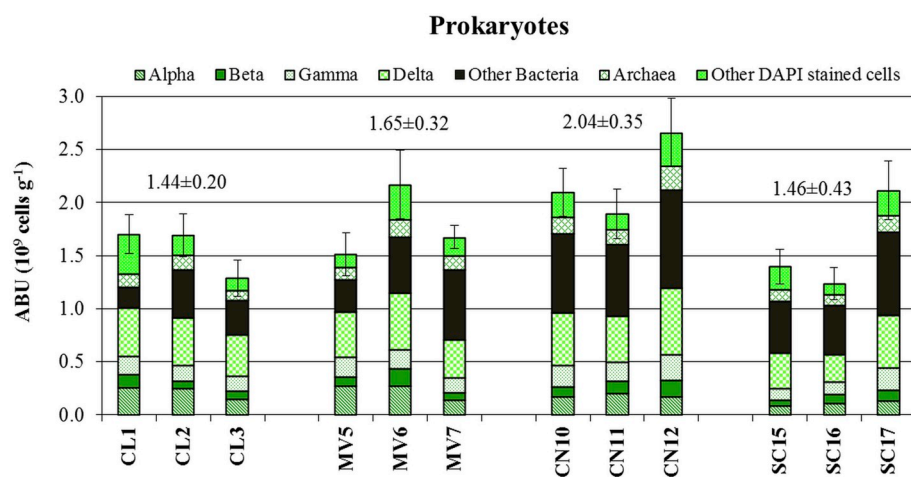
The nMDS analysis showed clear spatial differences among stations (Fig. 3). The SIMPROF test evidenced three significantly different ( $p < 0.05$ ) groups of samples that were gathered according to decreasing prokaryotic densities, going from the right to the left side of the plot. The group on the right comprised stations with the highest abundances, i.e. CN12, CN11, MV6, SC17 and CN10, while the group on the leftmost part of the plot gathered samples of SC15, SC16 and CL3 with the lowest abundances.

Although the RELATE routine did not highlight significant differences between the prokaryotic matrix and that of the abiotic variables as a whole, the BIO-ENV analysis revealed that the microbial assemblage was best correlated with surface salinity and bottom temperature, sand, silt and clay fraction, and  $\delta^{15}\text{N}$  ( $R = 0.35$ ). Therefore, in order to ascertain the role of physical and chemical variables on the structure of the prokaryotic community, the same six significant variables, obtained from BIO-ENV output, were used for the DistLM analysis. DistLM (best model,  $R^2 = 0.66$ ) showed that salinity, clay and temperature were the significant drivers of the prokaryotic distribution since they explained 38%, 37% and 35%, respectively, of the variability (Table 2).

**Table 1**

Grain-size, expressed as percentage of dry mass, porosity and sediment organic matter features at the sampling sites: Total Organic C (TOC), Total N (TN), C and N stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ).

	shells	sand	silt	clay	Porosity	TOC	TN	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C/N
	< -1 $\phi$	-1-4 $\phi$	4-8 $\phi$	8-12 $\phi$		%	%	‰	‰	
CL1	2.8	32.4	34.5	30.3	0.70	0.82	0.11	-22.39	3.91	8.6
CL2	4.3	41.3	29.0	25.4	0.66	0.68	0.10	-20.66	4.26	7.8
CL3	0.0	56.9	21.4	21.7	0.66	2.00	0.18	-26.16	2.73	12.8
MV5	1.8	69.8	10.3	18.1	0.63	1.22	0.11	-26.55	2.33	12.6
MV6	0.3	22.3	29.8	47.6	0.78	1.71	0.19	-25.53	4.09	10.4
MV7	19.0	34.3	15.6	31.2	0.70	1.08	0.16	-24.01	4.09	8.0
CN10	0.0	0.7	35.9	63.4	0.76	1.14	0.15	-25.60	2.02	9.1
CN11	0.2	62.1	13.8	23.9	0.66	1.30	0.10	-26.95	1.62	14.7
CN12	0.0	0.7	32.0	67.3	0.81	1.32	0.20	-25.02	4.33	7.8
SC15	1.2	68.8	10.4	19.6	0.56	0.56	0.07	-25.50	0.15	10.0
SC16	0.0	7.9	44.0	48.1	0.76	0.87	0.13	-23.81	2.32	7.9
SC17	0.0	1.1	46.2	52.8	0.74	1.03	0.13	-25.08	1.24	9.3

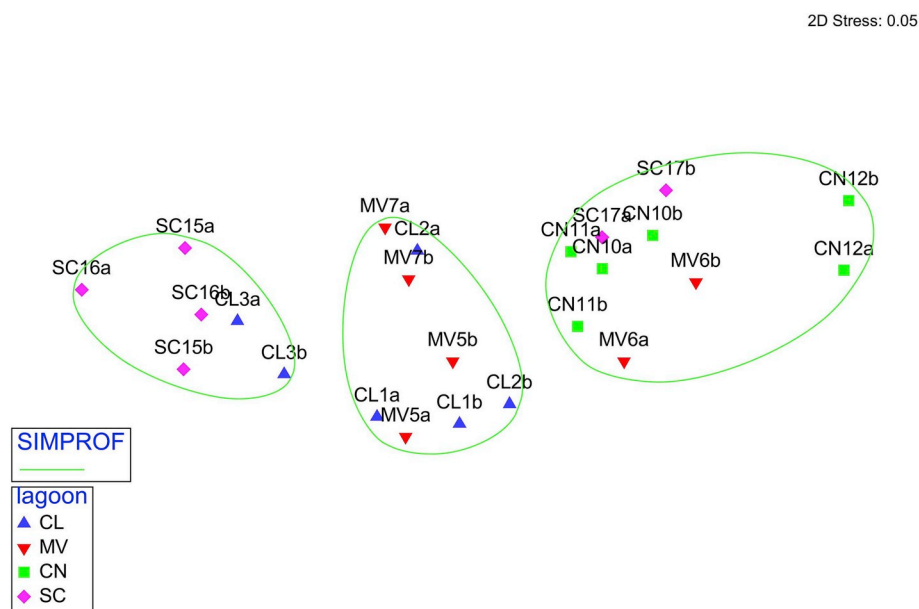


**Fig. 2.** Prokaryotic abundance and percentage of Alpha-Proteobacteria (Alpha), Beta-Proteobacteria (Beta), Gamma-Proteobacteria (Gamma), Delta-Proteobacteria (Delta) with respect to total Bacteria (other Bacteria); and total DAPI cell counts (other DAPI stained cells). Percentage of Archaea with respect to total DAPI cell counts measured at each sampling station. Above the histograms is indicated the mean value of three stations  $\pm$  SD for each lagoon.

**3.3. Microphytobenthic community structure**

Total MPB abundance ranged from  $22950 \pm 4031$  cells  $\text{cm}^{-3}$  at SC15 to  $139500 \pm 12304$  cells  $\text{cm}^{-3}$  at CN12. Overall, the average abundance (as mean of three stations  $\pm$  SD) was almost double in

Marinetta-Vallona ( $81600 \pm 22735$  cells  $\text{cm}^{-3}$ ) and Canarin ( $81200 \pm 50938$  cells  $\text{cm}^{-3}$ ) compared to Caleri ( $48700 \pm 10086$  cells  $\text{cm}^{-3}$ ) and Scardovari ( $42350 \pm 19131$  cells  $\text{cm}^{-3}$ ) (Fig. 4). The microalgal community was dominated by diatoms, which contribution varied between 78.8% and 95.5% of the total community



**Fig. 3.** Non-metric MultiDimensional Scaling (nMDS) analysis based on two replicate samples (indicated with an “a” and “b”) of prokaryotic abundance data from the twelve sampling sites. On the nMDS ordination plot, groups of stations significantly ( $p < 0.05$ ) gathered by the SIMPROF test are superimposed. Stations of distinct lagoons are indicated with different colors and symbols: Blue triangles = Caleri (CL); Red inverted triangles = Marinetta-Vallona (MV); Green squares = Canarin (CN); Pink diamonds = Scardovari. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**

Output of the DistLM analysis on prokaryotes and microphytobenthos (MPB), reporting results of the marginal test. SS: sum of square; Prop.: proportion of variance explained by each variable. Statistically significant p values are highlighted in bold.

	Marginal test				
	Variable	SS(trace)	Pseudo-F	p	Prop.
Prokaryotic community	Salinity	541.20	6.11	<b>0.02</b>	0.38
	Temperature	504.77	5.48	<b>0.03</b>	0.35
	Sand	339.06	3.12	0.09	0.24
	Silt	98.03	0.74	0.43	0.07
	Clay	531.01	5.93	<b>0.03</b>	0.37
	$\delta^{15}\text{N}$	171.01	1.36	0.26	0.12
MPB community	Salinity	2533.50	1.68	<b>0.02</b>	0.14
	Shells	1893.20	1.20	0.14	0.11
	Clay	2691.30	1.80	<b>0.01</b>	0.15
	$\Sigma$ PAHs	2576.10	1.71	<b>0.02</b>	0.15
	$\Sigma$ PCBs	2512.60	1.66	<b>0.02</b>	0.14
	$\Sigma$ DDXs	2217.20	1.44	0.08	0.13

at CL2 and CL1, respectively. Cyanobacteria were the second most abundant group, accounting for up to 17.1% of the whole community at CL2 and 15.9% at CN10 (Fig. 4). At the latter station, *Oscillatoria* sp. reached up to 6300 cells  $\text{cm}^{-3}$ , and it was consistently observed at almost all stations. Chlorophyceae were well represented at MV5 and CN11, with a Relative Abundance (RA) of 5.9% and 5.1%, respectively, particularly due to the presence of *Closterium* cf. *parvulum* and *Scenedesmus* sp.

Focusing on diatoms and considering all the sampled stations, a total of 55 taxa belonging to 35 genera were identified (Appendix 1). In Fig. 5 diatom genera with an RA  $\geq 2\%$  are shown. Overall, *Nitzschia* was the most abundant genus, reaching high abundances particularly at SC17 (RA = 59.0%), CL1 (RA = 55.9%) and MV7 (RA = 48.6%). To this genus belonged the highest number of species (S = 15), among them *N. sigma* and *N. fasciculata* were observed in high densities at SC17, *N. cf. dubia* at CL1 and *N. tryblionella* at MV7. The genus *Gyrosigma* dominated in Scardovari, particularly at SC16 and SC15 (RA = 53.9% and 39.0%, respectively) but it was well represented also at MV6 (RA = 27.4%). While *G. spencerii* prevailed at SC17 and MV6, *G. acuminatum* was dominant at S16. *Melosira*, the third most abundant genus, was found in high densities in Canarin, particularly at CN10 and CN12 (RA = 48.8% and 33.8%, respectively), as well as at MV6 (RA = 27.4%). Two species were distinguished in this genus, namely the highly abundant *M. nummuloides* and *M. jurgensii*.

According to their life mode, diatoms were divided into planktonic, tychopelagic, epiphytic and benthic, and the latter were further

distinguished into epipsammic and epipelagic forms (Fig. 6). The latter prevailed in Scardovari (RA = 76.47% and 59.44% at SC17 and SC16, respectively) and at CL1 (RA = 62.69%), while epiphytic diatoms were highly abundant at CN12, where *Grammatophora marina* reached an RA of 40.43%, and at MV5 mainly on the account of *Synedra pulchella* (RA = 27.65%). Tychopelagic diatoms, namely *Melosira nummuloides* and *M. jurgensii*, were observed in high densities in Canarin and Marinetta-Vallona and together accounted for up to 39.07% of the total diatom abundance at CN10. Epipsammic forms were well represented in Caleri and MV5 with the genus *Mastogloia* whereas freshwater planktonic specimens, i.e. *Fragilaria* cf. *construens* and *Fragilaria* cf. *crotonensis*, were found at CN11 (Appendix 1).

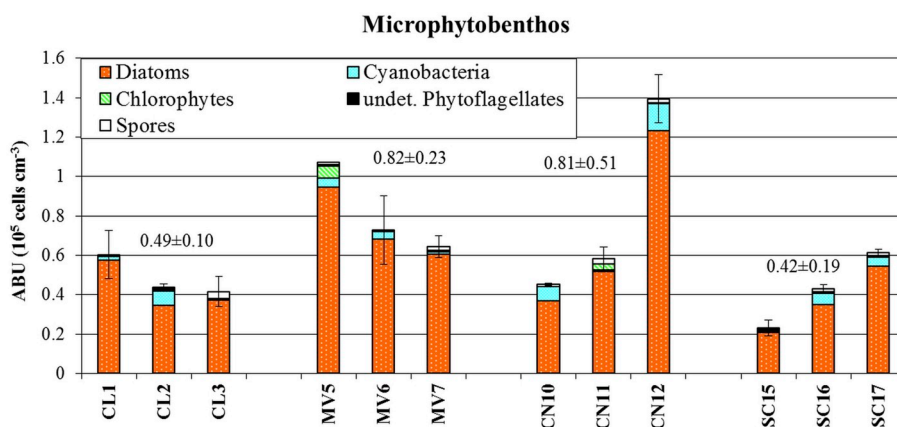
The highest richness (d = 2.91) of the MPB community was observed at MV5 due to the major number of taxa (S = 34) whereas the highest diversity was found at station CL3 (H' = 2.97) in which the MPB community was also more evenly distributed, as indicated by the highest equitability (J' = 0.87) (Table 3). In contrast, the lowest diversity (H' = 1.56) as well as the major dominance ( $\lambda = 0.33$ ) were obtained at CN12, likely due to the prevalence of *Grammatophora marina* at this site.

The k-dominance plots with typical semi-sinusoidal curves were obtained at the outer stations, i.e. CL3, MV5 and CN11, characterized by more marine features (Fig. 7). In contrast, k-dominance curves at CN10 and CN12 revealed the characteristic cut-off form of an altered community, due to the dominance of a few epiphytic and tychopelagic species with an RA > 40%.

The PERMANOVA main test performed on MPB taxa highlighted that the microalgal communities significantly differed among lagoons as well as among inner vs outer stations of the four lagoons (Pseudo-F = 6.87, 5.77; p < 0.001, respectively). PERMANOVA pair-wise tests confirmed highly significant differences for all couples of lagoons (varying from t = 2.00 p < 0.001 for Canarin and Marinetta-Vallona to t = 2.78, p < 0.001 for Caleri and Canarin). Further, significant differences in MPB distribution were obtained considering 'lagoon  $\times$  in-out' factor (Pseudo-F = 3.81; p < 0.001). In addition, MPB communities significantly differed between Canarin and Caleri, both at the inner and outer stations (pair-wise test on 'lagoon  $\times$  in-out' design: t = 3.53; p < 0.01 and t = 4.62 p < 0.05, for levels 'in' and 'out', respectively).

Since PERMANOVA carried out on three MPB replicate samples for each station was highly significant, averaged data were used for nMDS analysis. The nMDS plot showed clear spatial differences among lagoons and stations (Fig. 8). By superimposing the main diatom genera on the nMDS plot, different assemblages were highlighted at the inter-lagoonal level.

Further, SIMPER analysis carried out on MPB species pointed out the highest dissimilarity between CL and CN (69.67%), i.e. the most



**Fig. 4.** Microphytobenthic total and relative abundance of the microalgal groups at the twelve stations. Above the histograms is indicated the mean value of three stations  $\pm$  SD for each lagoon.

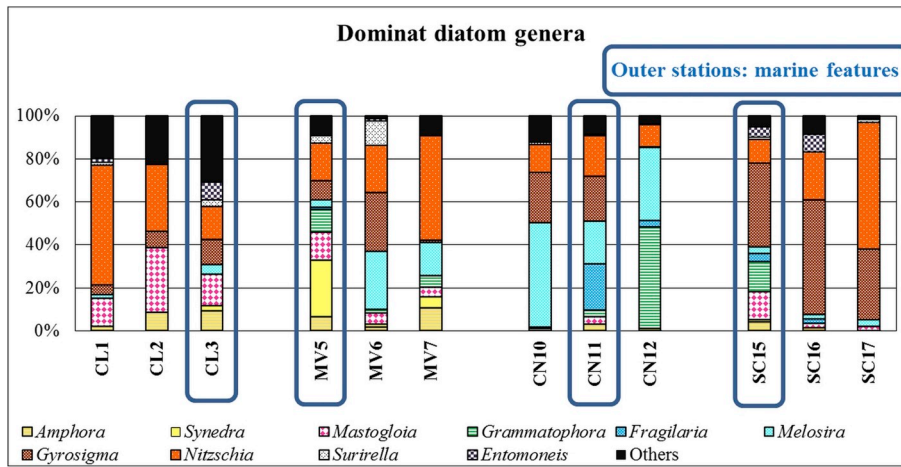


Fig. 5. Relative abundance (RA) of the main diatom genera at the sampling sites. Only genera with an RA  $\geq 2\%$  are shown in the graph. Stations located at the outer lagoonal mouths are circled in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

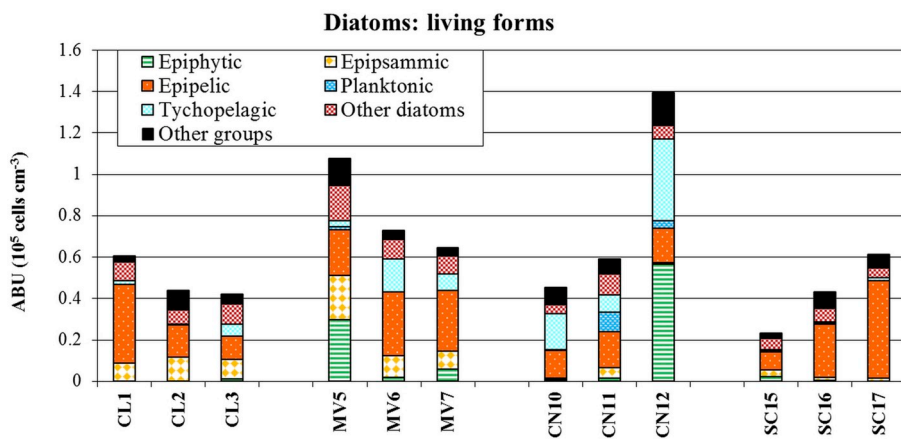


Fig. 6. Distinction and abundance of diatom living forms at the twelve stations. For more details, see the text.

Table 3

Diversity indices applied to the microphytobenthic community at the twelve sampling stations: S = number of taxa; N = number of organisms; d = richness;  $J'$  = equitability;  $H'$  = diversity;  $\lambda$  = dominance. The highest values are indicated in bold, the lowest ones in italics.

	S	N	d	$J'$	$H'(\log_e)$	$\lambda$
CL1	22	49500	1.94	0.78	2.42	0.14
CL2	18	31800	1.64	0.81	2.34	0.13
CL3	30	28000	2.83	<b>0.87</b>	<b>2.97</b>	<i>0.07</i>
MV5	<b>34</b>	83700	<b>2.91</b>	0.83	2.92	0.08
MV6	21	61800	1.81	0.78	2.38	0.14
MV7	23	51900	2.03	0.84	2.62	0.10
CN10	17	39000	1.51	0.68	1.93	0.23
CN11	28	44700	2.52	<b>0.87</b>	2.90	<i>0.07</i>
CN12	21	119100	1.71	0.51	1.56	<b>0.33</b>
SC15	21	15150	2.08	0.74	2.25	0.18
SC16	23	29400	2.14	0.72	2.27	0.20
SC17	18	51300	1.57	0.73	2.12	0.17

pristine and the most contaminated lagoon, mainly due to higher abundances of *Melosira nummuloides* (Contribut.% = 10.26) and *Grammatophora marina* (Contribut.% = 6.33) in CN while *Mastogloia* sp. (Contribut.% = 5.46) and *Psammodyction panduriforme* (Contribut.% = 3.24) showed higher densities in CL. The lowest dissimilarities were found between CL and MV (59.92%), i.e. the two northernmost lagoons, again on the account of the same two species of CN (*M. nummuloides*: Contribut.% = 5.64; *G. marina*: Contribut.% = 4.65) and

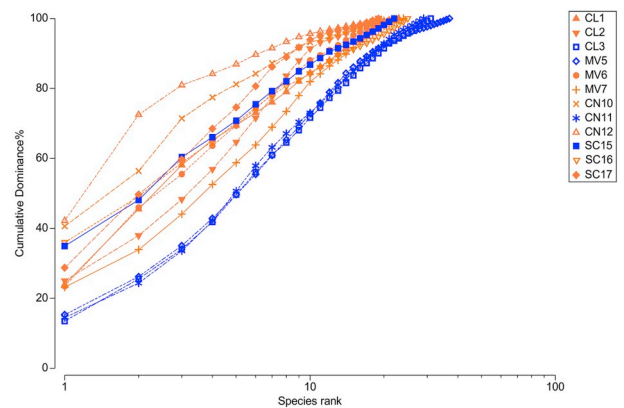
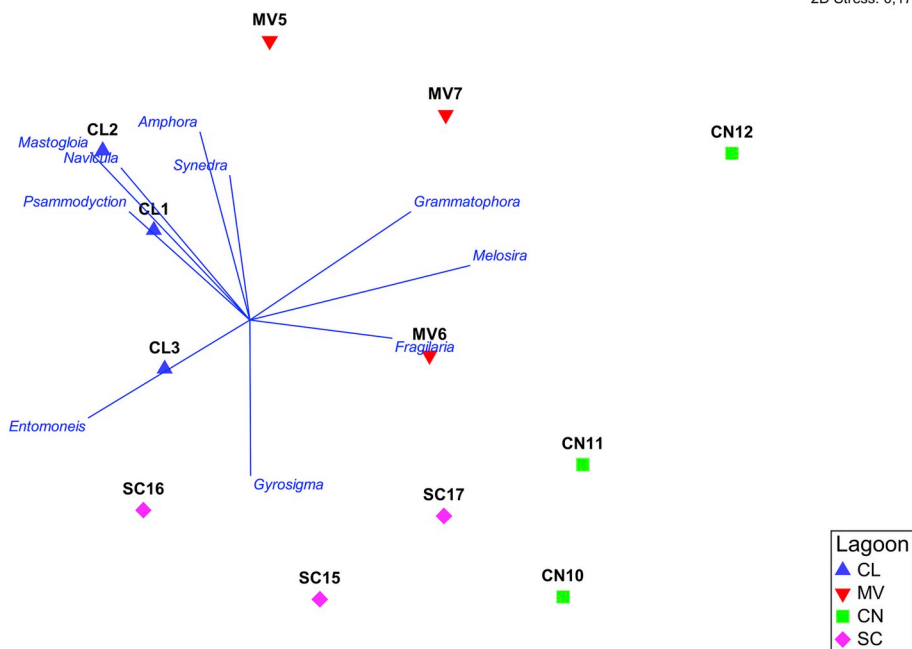


Fig. 7. k-dominance curves obtained from average diatom abundance data of the three replicate samples at the twelve sites. Stations located at the outer lagoonal mouths are marked in blue: CL3 (open square); MV5 (open diamond); CN11 (asterisk); SC15 (full square). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

*Synedra pulchella* and *Gyrosigma spencerii*, observed in MV only (Contribut.% = 5.31 and 4.20, respectively).

The RELATE routine revealed that the MPB community structure was highly correlated with the abiotic variables considered in this study ( $\rho = 0.43$ ,  $p < 0.001$ ). In addition, the BIO-ENV analysis indicated that





**Fig. 8.** Non-metric MultiDimensional Scaling (nMDS) analysis based on microphytobenthic abundance data (mean of three replicate samples) from the twelve sampling sites. Stations of distinct lagoons are indicated with different colors and symbols: Blue triangles = Caleri (CL); Red inverted triangles = Marinetta-Vallona (MV); Green squares = Canarin (CN); Pink diamonds = Scardovari. On the nMDS ordination plot, diatom genera with a correlation (Pearson) coefficient  $\geq 0.4$  are overlaid. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the MPB assemblage was best correlated with six abiotic variables, namely surface salinity, shells, clay, PAHs, PCBs and DDXs ( $R = 0.57$ ). Further, in order to ascertain the role of different physical and chemical variables on the structure of the MPB community, the six significant variables obtained from the BIO-ENV output were used for the DistLM analysis. DistLM (best model,  $R^2 = 0.66$ ) showed that four variables, i.e. clay, PAHs, PCBs and salinity were significant drivers of the MPB spatial distribution since they explained, respectively, 15%, 15%, 14% and 14% of the variability (Table 2).

### 3.4. Influence of abiotic variables and contaminants on the structure of benthic microbial communities

To integrate all the considered variables in a comprehensive manner, a Principal Component Analysis (PCA) was carried out (Fig. 9). The ordination plot accounted for 67.83% of total variance, the principal component axis 1 (PC1) explained 41.34% of total variance whereas the component axis 2 (PC2) explained 26.49% of the remaining variance. Clay and sand were the predominant elements of the first factor, while the major contributors to the second one were  $\delta^{13}\text{C}$  and TOC. On the PCA plot, stations were separated into three groups: those nearby the lagoonal mouths ('out': CL3, MV5, CN11 and SC15) were placed in quadrants I and II, in correspondence with a high percentage of sand. The inner stations of Scardovari and Caleri, characterized by more marine features ('in-marine'), were gathered in quadrants I and IV, nearby high salinity and  $\delta^{13}\text{C}$  values. The last group, located between quadrant III and IV, comprised the inner stations ('in-freshwater') of Marinetta-Vallona and Canarin influenced by fine grain-size and high organic loads (Fig. 9b). All pairwise comparisons between these three groups were significantly different, varying from  $R_{\text{anosim}} = 0.540$   $p < 0.001$  for 'out' vs 'in-marine' stations to  $R_{\text{anosim}} = 0.542$ ,  $p < 0.001$  for 'in-marine' vs 'in-freshwater' stations. The position of organic contaminants on the biplot, opposite to salinity and  $\delta^{13}\text{C}$ , is a clear indication of their freshwater origin. Archaea and all the considered bacterial subgroups were placed in quadrant III, directly stimulated by the freshwater input and the consequent nitrogen and organic enrichment of these clayey sediments (Fig. 9a). Different diatom living forms were separated on the biplot: epipsammic - nearby the lagoonal mouths; epipellic - in correspondence with finer sediments of the innermost sites; non-benthic forms (planktonic, tychopelagic and

epiphytic) at stations directly influenced by freshwater (Fig. 9c).

## 4. Discussion

In this study, the analytical effort was focused on the microbial phototrophic and heterotrophic components of the benthic compartment. Sediments, in fact, act as a repository of both natural and anthropogenic compounds from the water column and can be used as a record of recent and past disturbance factors that have affected a particular area. In addition, benthic organisms, due to their limited mobility, are exposed simultaneously to combined stressors of natural and anthropogenic origin and respond to them both at the individual and community level through the variation in the total numbers, the selection of taxa and the elimination of the sensitive ones (Solis-Weiss et al., 2001). Most of the available information on the relation between biodiversity and ecosystem status in coastal lagoons has been historically obtained from studies on macroscopic components, and this holds true also for the Italian transitional waters (Munari and Mistri, 2008; Mistri et al., 2018). Notwithstanding, also benthic microbial communities are useful bioindicators of the ecosystem health and are increasingly being used in biological monitoring (for bacteria: Sun et al., 2012; Quero et al., 2015, for MPB and diatoms: Facca and Sfriso, 2007; Franzo et al., 2014, Potapova et al., 2016). So far, few surveys, focused on the structure of microbial phototrophic and heterotrophic communities, have been carried out in the lagoons of the Po River Delta, even less in its northern part.

### 4.1. Microbial inter-lagoonal variability

Four lagoons of the Po River Delta system were investigated in this study, two with more marine features (Caleri and Scardovari) and two more influenced by the freshwater inflow (Canarin and Marinetta-Vallona). Indeed, the different physical-chemical features of the four lagoons greatly affected the abundance and structure of heterotrophic and autotrophic microbial communities. We found the highest Bacteria and Archaea abundances in correspondence to the lowest salinity, higher percentage of clay and the organic matter entrapped onto these fine particles, as clearly indicated by the PCA. Similarly, the almost double MPB abundances displayed in Marinetta-Vallona and Canarin, compared to Scardovari and Caleri, were likely due to high organic and

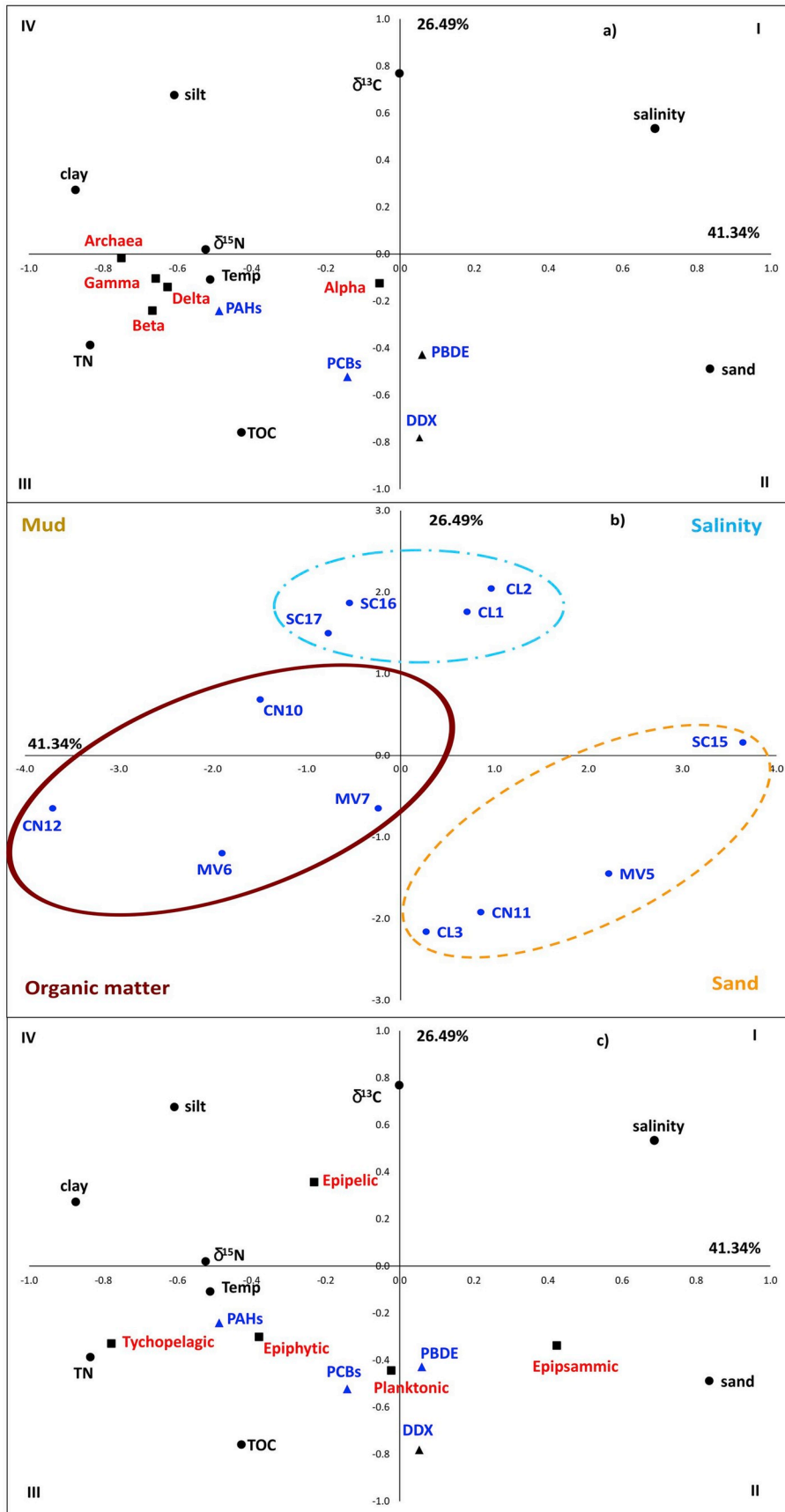


Fig. 9. Principal component analysis (PCA) ordination diagram based on the selected physical and chemical structural parameters, plotted in black full circles: (a) surface salinity and bottom temperature (Temp), sand, silt, clay, Total Organic C (TOC), Total N (TN),  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Organic contaminants, plotted in blue triangles: Polychlorobiphenyls (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs), Polybrominated diphenyl ethers (PBDEs) and degradation products of DDT (DDXs), and the main prokaryotic groups, plotted in red squares: Archaea, Alpha-Proteobacteria (Alpha), Beta-Proteobacteria (Beta), Delta-Proteobacteria (Delta) and Gamma-Proteobacteria (Gamma) are projected on the factor plane as additional variables without contributing to the results of the analysis. (b) Scatter diagram plotting factors 1 and 2 of the twelve stations sampled in the four investigated lagoons. Yellow dashed circle encloses the outer stations; brown full circle encloses the inner freshwater stations; light-blue dashed and dotted circle encloses the inner marine stations, respectively. (c) PCA ordination diagram based on the selected variables - the same as in (a) on which diatom living forms (red squares) are projected as additional variables. For more details, see material and methods. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

nutrient loads of anthropogenic origin, inferred by high  $\delta^{15}\text{N}$ . Mean TOC and TN contents were higher in the former two lagoons than in the latter ones (Table 1), highly stimulating the microalgal development, as highlighted by the PCA.

Overall, our total bacterial abundances were one order of magnitude higher than those recently reported for the Goro lagoon (Pala et al., 2018) and Manfredonia gulf (Molari et al., 2012), but in line with those reported for the Venice lagoon (Borin et al., 2009), as well as for Goro, Lesina and Marsala lagoons (Manini et al., 2003). At the larger geographical scale, our abundances were higher than those obtained from the sediments of the Bay of Cadiz in Spain (Köchling et al., 2011) but comparable to those estimated from the coastal lagoons in Japan (Tsuboi et al., 2013). Although our archaeal abundances were in line with those reported by Pala et al. (2018), the authors found a larger contribution of Archaea to the microbial community (< 20% in the uppermost sediment layer) compared to our results (< 10%). Our Archaea:Bacteria ratios were similar to those found by Borin et al. (2009) reported for the Venice lagoon and Vidal-Durà et al. (2018) obtained along the salinity continuum of the Humber estuary (UK).

Proteobacteria were consistently the most abundant group within the assemblages in the four lagoons. They are typically present in conditions of high concentrations of dissolved organic carbon and colonize both water column and sediments (Zwisler et al., 2003). In our study, Delta-Proteobacteria represented 30.8% of bacterial cells followed by Alpha- (12.8%), Gamma- (11.8%) and Beta-Proteobacteria (7.1%). The dominance of Proteobacteria in coastal environments, with a typically higher relevance of Gamma- and Delta-Proteobacteria as opposed to Alpha-, Beta- and Epsilon-Proteobacteria was already reported in the Venice lagoon (Quero et al., 2017), the lagoonal system of the Mar Piccolo of Taranto (Quero et al., 2015; Franzo et al., 2016), a Mediterranean lagoonal complex in the Ionian Sea (Pavlouidi et al., 2016), the Bizerte lagoon in Tunisia (Ben Said et al., 2010), an enclosed shallow coastal lagoon of the Gulf of St. Lawrence in Canada (Mohit et al., 2015) and in a tropical coastal lagoon in India (Behera et al., 2017).

This distributional pattern of Proteobacteria could be the result of the typical anoxic condition of sediments in these transitional environments (Borin et al., 2009). Indeed, the lagoons of the Po River Delta suffer from frequent hypoxia and anoxia events that in 2016 were registered already in February, particularly in Scardovari, and in spring and summer in the other lagoons as well (ARPAV, 2016). Molari et al. (2012) highlighted the importance of the redox potential and trophic resources as the main drivers for heterotrophic microbial assemblages in coastal sediments. Although by applying different molecular techniques, and at different level of taxonomic resolution, our results are fully in line with these authors that found that the microbial communities were dominated by anaerobic groups of the Proteobacteria phylum, families of the Deltaproteobacteria subphylum (Desulfobacterales and Desulfuromonadales) responsible for sulphur and sulphate reduction. In addition, the family of Chromatiales belonging to the Gammaproteobacteria subphylum, abundant in the Venice lagoon, is believed to be involved in the complete sulphur cycle in the sediments (Borin et al., 2009). In contrast to the findings of Borin et al. (2009) and Quero et al. (2017) for the Venice lagoon, the lower importance of Gammaproteobacteria in the lagoons of the Po River could be ascribed to the lower urban impact in these lagoons.

As highlighted by PERMANOVA, the prokaryotic distribution differed among lagoons, and particularly the microbial community in Canarin significantly differed from those of the other three lagoons. Indeed, in Canarin the prokaryotic assemblage was likely stimulated by the combined effect of high organic and nutrient loads, up to +42% by comparing Canarin to Caleri. The highest densities of Bacteria and Archaea were observed at sites directly influenced by the freshwater input. The four investigated lagoons differed for the occurrence of the analyzed bacterial classes, as highlighted by nMDS analysis and SIMPROF test. Delta- and Gamma-Proteobacteria followed the same

pattern of the total bacterial cells, being more abundant in Canarin and at sites with the highest clay contents in which organic matter is entrapped. In coastal sediments, sulphur cycling community composed of sulphur-oxidizing Gammaproteobacteria and sulphate-reducing Deltaproteobacteria was reported to be of particular importance in the degradation of organic matter (Mohit et al., 2015). According to these authors, this process is accelerated with increased input of fresh organic matter in regions with shellfish aquaculture. This is in accordance with our results, since we found higher percentages of Gamma- and Delta-Proteobacteria in Marinetta-Vallona, intensively exploited for clam farming, and particularly at MV5, in correspondence to a more negative value of  $\delta^{13}\text{C}$  and a high C:N ratio, a clear signature of organic matter of allochthonous (riverine-terrestrial) origin (Thornton and McManus, 1994). Sediments in Marinetta-Vallona and Canarin displayed higher concentrations of organic contaminants, mainly PCBs and PAHs. We found a significant relation between the concentration of total PAHs, and Beta- and Gamma-Proteobacteria ( $R = 0.61$  and  $0.58$ ,  $p < 0.05$ , respectively). The ability of Beta- and Gamma-Proteobacteria to use aliphatic and aromatic compounds has been previously established (Ben Said et al., 2008; Korlevic et al., 2015). Members of the Gamma-Proteobacteria group in particular are reported to aerobically degrade PAHs (Brooijmans et al., 2009). The dominance of Gamma-Proteobacteria in PAH-contaminated sediments was reported by Ben Said et al. (2008); 75% of PAHs degrading strains isolated from contaminated sediments of the Bizerte lagoon were associated with members of the genera *Pseudomonas* described as hydrocarbonoclastic strains.

Overall, our total MPB abundances were comparable to that reported at sites with similar salinity of the Grado-Marano lagoonal system (Cibic et al., 2012) but about one order of magnitude lower than the microphytobenthic densities found in the Lesina lagoon (Gambi et al., 2003) and the Venice lagoon (Facca et al., 2002; Facca and Sfriso, 2007). Further, compared to other Mediterranean lagoons, our MPB densities were similar to those observed in a Turkish lagoon connected with the Marmara Sea (Polge et al., 2010) and those reported for the Homa lagoon (Izmir Bay) (Çolak Sabancı, 2011).

In terms of the MPB composition, in the four investigated lagoons of the Po River Delta system, the microalgal community was largely dominated by diatoms while Cyanobacteria were the second most abundant group. This is in accordance with previous findings from other lagoonal systems (Gambi et al., 2003; Polge et al., 2010; Cibic et al., 2012) and with the microphytobenthic taxonomic report by Di Pippo et al. (2018) on the Cabras lagoon (western Mediterranean). Regarding Cyanobacteria, *Oscillatoria* sp. was highly abundant in Canarin, particularly at CN10, with an RA of 13.9%, likely due to N input of freshwater origin. This is in accordance with Underwood et al. (1998) who reported high densities of *Oscillatoria limosa* and *O. princeps* in muddy estuarine sediments following  $\text{NO}_3^-$  and  $\text{NH}_4^+$  enrichment. In mesotrophic lakes, mass occurrence of cyanobacteria or green algae are known to be directly linked to high water column nutrient loadings (Aberle and Wiltshire, 2006). In our study, chlorophytes, namely *Closterium* cf. *parvulum* and *Scenedesmus* sp. were observed in Marinetta-Vallona and Canarin only, at sites exposed to a direct riverine inflow. These are typical freshwater species (Guiry and Guiry, 2018) and their occurrence at these sites is a clear signature of the major river input. In more marine features, freshwater chlorophytes are usually not encountered and were not observed in sites with higher salinities (Gambi et al., 2003; Polge et al., 2010).

The four investigated lagoons differed mainly for the occurrence of diverse diatom genera, as highlighted by PERMANOVA and nMDS analyses. Interestingly, the dominant diatom taxa in the four lagoons displayed different life modes. Averaging the abundance data of the three stations, in Canarin the tychopelagic diatom *Melosira* prevailed (RA = 32.0% of the total MPB), followed by the epiphytic *Grammatophora* (RA = 27.6%), while also the planktonic *Fragilaria* (RA = 6.3%) was well represented. High densities of tychopelagic

forms indicate low hydrodynamic conditions: indeed, with high bottom velocities these species would be likely swept away by the current (Cibic et al., 2016b) and may not develop. In contrast, the dominance of *Grammatophora marina* was linked to the dense cover of the red algae *Gracilaria* sp., that was not observed in the other sampling sites, representing an optimal substrate for this epiphytic diatom. The occurrence of freshwater planktonic diatoms, such as *Fragilaria* spp., was instead due to the major river inflow in this lagoon and could be related to high nutrient availability. In fact, *Fragilaria crotonensis*, observed at CN11 and CN12, has been reported as an important component of the spring bloom in eutrophic lakes (Reynolds, 1983). *Fragilaria* is an opportunistic, fast-reproducing genus with a broad tolerance to many environmental gradients, which makes them competitive under unstable, changing conditions (Weckström and Juggins, 2005).

Epiphytic and tychopelagic forms were observed also in Marinetta-Vallona, but they co-occurred with other epipsammic and epipelagic diatoms. Epiphytic and epipsammic life modes, such as those belonging to the genera *Synedra* (RA = 11.9%) and *Amphora* (RA = 5.7%) were linked to the extremely high amount of mussel shell and fragments (up to 19% at MV7) as well as the presence of the nitrophilous macroalgae *Ulva* sp. Epiphytic and epipsammic diatoms are able to attach to these substrata through the production of mucous stalks, discs and apical pads (Round, 1971). In Marinetta-Vallona, with an RA of 26.3%, *Nitzschia* was the most abundant diatom genus. Particularly at MV7, where the total C reached 4.74% (Zonta et al., 2019) as a consequence of the intensive clam farming, the species *Nitzschia tryblionella* (RA = 19.1%) prevailed. This species has been previously described as a nutrient-loving species thriving under high organic matter conditions (Agatz, 1999).

On sandy sediments of Caleri lagoon, typical epipsammic forms developed, such as *Mastogloia* and *Amphora* that were encountered next to epipelagic diatoms belonging to the genera *Psammodictyon* and *Nitzschia*. Again, the genus *Nitzschia* dominated, reaching 36.6% of the total MPB abundance, mainly due to the presence of the following species: *N. tryblionella*, *N. lorenziana*, *N. dubia*, *N. fasciculata* and *N. vermicularis*. *N. dubia*, in particular, with an RA of 17.9%, was well represented at CL1. This brackish species was previously reported in high numbers in the second inlet of the Mar Piccolo of Taranto (Rubino et al., 2016) that displays more lagoonal features.

The great majority of the observed diatoms in Scardovari displayed an epipelagic life mode. Interestingly, in this lagoon only two diatom genera accounted for 78% of the total MPB: *Nitzschia* (RA = 39.2%) and *Gyrosigma* (38.8%). *Nitzschia sigma* was the dominant species and co-occurred alongside other *Gyrosigma* species, namely: *G. spencerii*, *G. acuminatum* and *G. fasciola*. The innermost part of Scardovari is frequently subjected to hypoxia/anoxia events (ARPAV, 2016; Maicu et al., 2018). Benthic diatoms have shown remarkable resistance to anoxic conditions (Admiraal, 1984; Larson and Sundbäck, 2008). There is experimental evidence that although sulphide-rich conditions decrease total microalgal biomass by 50%, diatom biomass remains unaltered (Sundbäck et al., 1990). In the innermost sites of Scardovari, *G. spencerii* and *N. sigma* were the two keystone species (RA = 18.6% and 25.5%, respectively). If, on one hand, *G. spencerii* has been described as a sulphide tolerant species and *N. sigma* as a sensitive one, on the other hand only the latter is able to use organic substrates for heterotrophic growth (Admiraal and Peletier, 1979). These different capacities may be responsible for the abundance of both species in diatom assemblages in estuaries and lagoons subjected to anaerobic conditions, as often occur in Scardovari. It is worth pointing out that the same dominance of a very few taxa in Scardovari has been also observed for higher trophic levels, i.e. the nematofauna (Franzo et al., 2019) and the macrofauna (Nasi et al. this issue), indicating that all benthic communities inhabiting the inner part of this lagoon are highly adapted to frequent anoxia events.

#### 4.2. Microbial intra-lagoonal variability

A clear intra-lagoonal pattern was evidenced by the PCA performed on abiotic variables that allowed us to separate three distinct groups of stations: the outer marine, the inner marine and the inner freshwater one. The different grain-size,  $\delta^{13}\text{C}$  and TOC, that largely explained the variance of the first two axes in the PCA, were responsible for this dissimilarity. These groups of sites had different physical-chemical features, as confirmed by the ANOSIM test for all pairs of groups, which selected specific microbial assemblages. This was further corroborated by the PERMANOVA main-test that pointed out significantly different prokaryotic assemblages between the inner and outer parts of the lagoons. The highest prokaryotic densities were observed in sites located in the inner parts of the lagoons characterized by fine grain-size (CN12, MV6, CN10 and SC17). Indeed, Bacteria and Archaea were significantly correlated with the clay content. In contrast, the lowest abundances were observed at sites with the highest sand content, close to the lagoonal mouths (CL3, MV5, SC15). These results suggest that moving away from the lagoonal mouths, the muddier sediments exerted a greater retention of sediment-bound organic matter. While Delta- and Gamma-Proteobacteria followed the same pattern of the total bacterial cells, being more abundant at sites with the highest clay content, Beta-Proteobacteria displayed the highest abundances at sites characterized by the lowest salinity (MV6, CN12 and CN11). Indeed Beta-Proteobacteria subclass is more easily found in freshwater and typically accounts for up to 4% in marine and up to 30% in freshwater assemblages (Glockner et al., 1999; Sekar et al., 2004). These contributions are in line with our results, since in the Po River Delta Beta-Proteobacteria ranged from 5.4% at the marine CL2 to 9.6% at the inner MV6. They are also in agreement with the findings of Vidal-Durà et al. (2018) who reported Beta-Proteobacteria varying from < 3% to 9% in the outer and inner parts of the Humber estuary, respectively.

The structure of the MPB community differed not only among the four lagoons, but also among stations of the same lagoon. This intra-lagoonal variability, corroborated by the PERMANOVA test, was likely ascribable to the confinement gradient, a hydrological parameter linked mainly to water exchange/renewal (Guelorget and Perthuisot, 1983), present inside each lagoon that selected different microalgal assemblages. Diversity indices and k-dominance curves revealed that the MPB community was more biodiverse at stations closest to the sea, compared to the innermost stations where the lowest biodiversity was found. This is in accordance with the findings of Miho and Witkowski (2005) from Albanian coastal lagoons and Rubino et al. (2016) for the Mar Piccolo. In the Po River Delta system, the highest richness was observed at MV5, the highest diversity at CL3 and the highest equitability at CN11. The higher biodiversity at the lagoonal mouths is likely due to the concomitant presence of typically marine taxa, such as *Biddulphia biddulphiana*, *Dimeregramma* sp., *Nitzschia distans*, and typically brackish ones. Moreover, due to higher bottom velocities at the outer stations, the seabed was constituted by coarser sediments (Table 1) that favored particularly epiphytic (e. g. *Grammatophora marina*, *Synedra* sp.) and epipsammic (e. g. *Navicula cancellata*, *Nitzschia angularis*, *N. commutata*) life modes that were well represented at these sites (Appendix 1). Some of these diatoms live attached to sand grains that prevents them from being carried away by strong bottom currents. Therefore, they may thrive also under high hydrodynamic conditions (Cibic et al., 2016b). Several other diatom life modes and microalgal groups, from green algae to Cyanobacteria (*Oscillatoria* sp., *Spirulina* sp.), co-occurred at these outer stations, leading to the highest biodiversity values. Further, lower contamination levels at the outer stations, due to a higher dilution effect induced by seawater might have favored the development of more sensitive taxa that at higher contamination levels would not develop (Rubino et al., 2016), enhancing the overall biodiversity at these sites.

### 4.3. Main physical-chemical drivers of the microbial community structure

According to the DistLM analysis, clay, PAHs, PCBs and salinity were found to be the significant drivers of the MPB structure. Our results are partially in accordance with those of Ribeiro et al. (2013) who reported that mud content alone was the environmental variable most correlated to the benthic diatom assemblage in the Tagus estuary. Indeed, the grain size is considered one of the most important factors influencing the colonization of sediments by MPB (Round et al., 1992). Salinity is also a significant structuring factor for diatoms living in brackish environments (Weckström and Juggins, 2006). Recently, Sawai et al. (2016) found a clear relationship between diatom species and salinity in Oregon and Washington estuaries. Similarly, epipelagic diatom species were correlated with salinity along the Colne Estuary in Great Britain (Underwood et al., 1998). Regarding the other two drivers, higher levels of PAHs and PCBs were reported by Casatta et al. (2016) particularly in Canarin, and lowest ones in Caleri. Indeed, MPB communities significantly differed between Canarin and Caleri, both at the inner and outer stations (PERMANOVA pair-wise test on ‘lagoon × in-out’ design). In addition, the SIMPER analysis carried out on MPB species pointed out the highest dissimilarity between these two lagoons. Interestingly, the MPB community in Canarin was principally constituted by epiphytic, tychoepelagic and planktonic diatom forms, i.e. life modes that are loosely or not at all associated with the sediments. In contrast, proper benthic forms, i.e. epipsammic and epipelagic, were less represented. Diatoms with these life strategies, i.e. thriving just above the surface sediments rather than within them, were the only forms observed in a highly contaminated site of the Mar Piccolo of Taranto (Rubino et al., 2016). According to these authors, subsurface sediments were likely too contaminated to become a favourable environment to be colonized by benthic diatoms. This could represent an adaptation strategy of the benthic diatom assemblage to severe contamination. Similarly, the paucity of benthic diatoms compared to their planktonic counterparts was recently highlighted by Potapova et al. (2016) in lagoonal sediments contaminated by heavy metals and PAHs.

In Canarin, the contamination levels are not so high as in sediments of the Mar Piccolo (Cibic et al., 2016a). However, diatoms loosely or not at all associated with the sediments prevailed over the typically benthic ones, suggesting a negative influence due to contaminants accumulated in surface lagoonal sediments. Yet, had the structure of the MPB community and the functional aspects deriving from the diatom life modes not been considered in this study, a different picture would have emerged. Taking into account the total MPB abundance only, the higher contamination levels in Canarin did not seem to severely affect the microalgal community that was overall stimulated by the combined effect of high organic and nutrient loads, up to +93% comparing Canarin to Caleri. According to the DistLM analysis, organic contaminants were more important drivers of the MPB community than either TOC, TN,  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ . Benthic diatom biomass is stimulated by anthropogenic N load (Mallin et al., 2007), but there is an objective difficulty in separating the effect of contaminants from other factors, especially eutrophication (Potapova et al., 2016). There is still little information concerning the combined effects of pollutants + nutrients on diatoms. Nutrient-rich systems are less sensitive to stressors: increased nutrient load may decrease the toxicant effect due to high nutrient status of organisms and dilution of the toxicant in increasing amounts of biomass (Sundbäck et al., 2007). Further, according to the authors, benthic diatom mats are rich in extracellular polymeric substances that may function as a protective barrier against toxic compounds, as well as enhance the uptake of favourable ones. This feature could decrease the vulnerability of microbial mats to toxic compounds. We believe that the toxic effect that might have been induced by organic contaminants on diatoms was hidden, to some extent, by the stimulating effect of the nutrient load.

According to the BIO-ENV output, the microbial heterotrophic assemblage was best correlated with surface salinity, bottom temperature,

the three grain-size fractions, and  $\delta^{15}\text{N}$ . The DistLM analysis further indicated that salinity, clay and temperature were the significant drivers of the prokaryotic distribution. Our findings are consistent with those of Pavlouli et al. (2016) and Vidal-Durà et al. (2018) who, by applying the BIOENV analysis, found that salinity was the environmental factor that best correlated with the bacterial community composition in a Mediterranean lagoon and in the Humber estuary. Grain-size was reported to be among the most important drivers shaping the sedimentary microbial community structure in an estuary of East China Sea (Ye et al., 2016). The DistLM model applied by Quero and co-workers. (2017) to their dataset revealed that the variation in benthic microbial composition was mainly associated with temperature which alone explained 19% of the variance within the Venice lagoon. In contrast, TN displayed the highest partial contribution to the variation in sediment bacterial biodiversity in a coastal lagoon of Canada (Mohit et al., 2015).

Interestingly, none of the organic pollutants considered in the BIOENV analysis showed to be correlated with the prokaryotic community composition, i.e. none of them seemed to directly affect the prokaryotic spatial distribution, at least at a broad taxonomic resolution, as they did for the MPB community. This could be due to the fact that several bacterial strains are able to utilize PAHs containing up to four benzene rings as sole carbon source (Ben Said et al., 2008). Members of Gamma- and Alpha-Proteobacteria in particular are key-players in oil hydrocarbon degradation, oxidizing more reactive components such as n-alkanes and more recalcitrant ones such as PAHs (Korlevic et al., 2015). In contrast to our findings, the DistLM analysis carried out by Quero et al. (2015) revealed that contaminants significantly shaped the community composition in the chronically polluted sediments of the Mar Piccolo of Taranto. Their results were, however, based on Illumina sequencing of 16S rRNA gene amplicons and, therefore, the analysis was performed at a finer taxonomic resolution. Moreover, the contamination levels in the Mar Piccolo were much higher than those reported for the Po River Delta lagoons: PCBs levels up to 100 times higher (Cibic et al., 2016a), and PAHs up to 42 times higher (Bellucci et al., 2016). Consequently, the microbial community was likely highly adapted to contaminant levels in the Mar Piccolo, and PCB- and PAH-degrading taxa (Quero et al., 2015; Franco et al., 2016) have been potentially selected in this historically polluted site. However, Lear and Lewis (2009) highlighted how difficult it is to find significant relationships between the anthropogenic impact and microbial community structure, given the synergistic effect of multiple stressors and the highly dynamic nature of riverine environments. Overall, our study underlines how in the Po River Delta lagoons, salinity, grain-size, temperature, anthropogenically derived N and C loads seem to overcome other drivers shaping the prokaryotic community.

## 5. Conclusions

This study represents the first example in which benthic heterotrophic and photoautotrophic microbial communities, in terms of abundance and diversity, were simultaneously investigated. We applied a novel functional approach based on diatom life modes that allowed us to highlight a negative influence of contaminants on the phototrophic community that would have not emerged with the traditional taxonomic analysis. Our results further revealed the presence of two diatom keystone species in sediments frequently exposed to anoxia. This has important ecological implications, since these diatoms likely contribute to rapidly restore the oxic gradient in the uppermost sediments, allowing the subsequent microbial aerobic degradation and the recolonization of higher trophic organisms. The future study of these microbial communities in relation to their capacity of recovery after an anoxic event is of paramount importance for an efficient management and sustainable use of coastal resources, and in particular lagoons that are exploited for aquaculture.

## Acknowledgements

The activities were funded by the Flagship Project RITMARE - La Ricerca Italiana per il MARE - The Italian Research for the Sea, coordinated by the Italian National Research Council and funded by the Ministry for Education, University and Research. We would like to thank Dr. Carlotta Mazzoldi and Andrea Sambo for their kind hospitality at the Stazione Idrobiologica in Chioggia. We are also grateful to Dr. Rocco Auriemma for statistical support. Special thanks go to Dr. Roberto Zonta as the scientific leader of the project.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2019.04.009>.

## References

- Abbiati, M., Mistri, M., Bartoli, M., Ceccherelli, V.U., Colangelo, A., Ferrari, C.R., Giordani, G., Munari, C., Ponti, M., Rossi, R., Viaroli, P., 2010. Trade-off between conservation and exploitation of transitional water ecosystems of the northern Adriatic. *Chem. Ecol.* 26, 37–41.
- Aberle, N., Wilthire, K.H., 2006. Seasonality and diversity patterns of microphytobenthos in a mesotrophic lake. *Arch. Hydrobiol.* 167, 447–465.
- Admiraal, W., 1984. The ecology of estuarine sediment-inhabiting diatoms. *Prog. Phycol. Res.* 3, 269–322.
- Admiraal, W., Peletier, H., 1979. Sulphide tolerance of benthic diatoms in relation to their distribution in an estuary. *Br. Phycol. J.* 14 (2), 185–196.
- Agatz, M., Asmus, R.M., Deventer, B., 1999. Structural changes in the benthic community along a eutrophication gradient on a tidal flat. *Helgol. Mar. Res.* 53, 92–101.
- Amalfitano, S., Fazi, S., 2008. Recovery and quantification of bacterial cells associated with streambed sediments. *J. Microbiol. Methods* 75, 237–243.
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA A+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, pp. 214.
- ARPAV, 2004. Le lagune del Delta del Po: ecosistemi fragili. Rilevamento in continuo della qualità delle acque. ISBN 88-7504-103-2. ARPAV, Rovigo, pp. 33 (in Italian).
- ARPAV, 2016. Rapporto di sintesi sull'andamento dei principali parametri ambientali delle acque di transizione del Veneto. ARPAV, Padova, pp. 4. (in Italian). [http://www.arpa.veneto.it/temi-ambientali/acqua/file-e-allegati/documenti/acque-di-transizione/Rapporto\\_Transizione\\_GIUGNO\\_2017.pdf](http://www.arpa.veneto.it/temi-ambientali/acqua/file-e-allegati/documenti/acque-di-transizione/Rapporto_Transizione_GIUGNO_2017.pdf).
- Baker, B.J., Lazar, C.S., Teske, A.P., Dick, G.J., 2015. Genomic resolution of linkages in carbon, nitrogen, and sulfur cycling among widespread estuary sediment bacteria. *Microbiome* 3 (1), 14.
- Barranguet, C., 1997. The role of microphytobenthic primary production in a Mediterranean mussel culture area. *Estuar. Coast Shelf Sci.* 44, 753–765.
- Behara, P., Mahapatra, S., Mohapatra, M., Kim, J.Y., Adhya, T.K., Raina, V., et al., 2017. Salinity and macrophyte drive the biogeography of the sedimentary bacterial communities in a brackish water tropical coastal lagoon. *Sci. Total Environ.* 595, 472–485.
- Bellucci, L.G., Cassin, D., Giuliani, S., Botter, M., Zonta, R., 2016. Sediment pollution and dynamic in the Mar Piccolo di Taranto (southern Italy): insights from bottom sediment traps and surficial sediments. *Environ. Sci. Pollut. Res.* 23, 12554–12565.
- Ben Said, O., Goñi-Urriza, M., El Bour, M., Dellali, M., Aissa, P., Duran, R., 2008. Characterization of aerobic polycyclic aromatic hydrocarbon-degrading bacteria from Bizerte lagoon sediments, Tunisia. *J. Appl. Microbiol.* 104, 987–997.
- Ben Said, O., Goñi-Urriza, M., El Bour, M., Aissa, P., Duran, R., 2010. Bacterial community structure of sediments of the Bizerte lagoon (Tunisia), a southern Mediterranean coastal anthropized lagoon. *Microb. Ecol.* 59, 445–456.
- Borin, S., Brusetti, L., Daffonchio, D., Delaney, E., Baldi, F., 2009. Biodiversity of prokaryotic communities in sediments of different sub-basins of the Venice lagoon. *Res. Microbiol.* 160 (5), 307–314.
- Brooijmans, R.J.W., Pastink, M.I., Siezen, R.J., 2009. Hydrocarbon-degrading bacteria: the oil-spill clean-up crew. *Microb. Biotechnol.* 2 (6), 587–594.
- Canter-Lund, H., Lund, J.W.G., 1995. Freshwater Algae. Biopress Ltd, Bristol, pp. 360.
- Casatta, N., Stefani, F., Pozzoni, F., Guzzella, L., Marziali, L., Mascolo, G., Viganò, L., 2016. Endocrine-disrupting chemicals in coastal lagoons of the Po River delta: sediment contamination, bioaccumulation and effects on Manila clams. *Environ. Sci. Pollut. Res.* 23, 10477–10493.
- Cibic, T., Blasutto, O., 2011. Living marine benthic diatoms as indicators of nutrient enrichment: a case study in the Gulf of Trieste. In: Compton, J.C. (Ed.), *Diatoms: Classification, Ecology and Life Cycle*. Nova Science Publishers Inc, New York, pp. 169–184.
- Cibic, T., Blasutto, O., Fonda Umani, S., 2007. Biodiversity of settled material in a sediment trap in the Gulf of Trieste (northern Adriatic Sea). *Hydrobiologia* 580, 57–75.
- Cibic, T., Acquavita, A., Aleffi, F., Bettoso, N., Blasutto, O., De Vittor, C., Falconi, C., Falomo, J., Faresi, L., Predonzani, S., Tamberlich, F., Fonda Umani, S., 2008. Integrated approach to sediment pollution: a case study in the Gulf of Trieste. *Mar. Pollut. Bull.* 56, 1650–1657.
- Cibic, T., Franzo, A., Celussi, M., Fabbro, C., Del Negro, P., 2012. Benthic ecosystem functioning in hydrocarbon and heavy-metal contaminated sediments of an Adriatic lagoon. *Mar. Ecol. Prog. Ser.* 458, 69–87.
- Cibic, T., Bongiorno, L., Borfecchia, F., Di Leo, A., Franzo, A., Giandomenico, S., Karuza, A., Micheli, C., Rogelja, M., Spada, L., Del Negro, P., 2016a. Ecosystem functioning approach applied to a large contaminated coastal site: the study case of the Mar Piccolo di Taranto (Ionian Sea). *Environ. Sci. Pollut. Res.* 23, 12739–12754.
- Cibic, T., Rogelja, M., Querin, Segarich, M., Del Negro, P., 2016b. Microphytobenthic community development under different hydrodynamic conditions nearby the rocky outcrops of the northern Adriatic Sea. *Biol. Mar. Mediterr.* 23 (1), 174–177.
- Clarke, K.R., Ainsworth, M., 1993. A method of linking multivariate community structure to environmental variables. *Mar. Ecol. Prog. Ser.* 92, 205–219.
- Çolak Sabancı, F., 2011. Relationship of epilithic diatom communities to environmental variables in Homa lagoon (Izmir, Turkey). *Aquat. Biol.* 13, 233–241.
- Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M., 2014. An Approach to Statistical Analysis and Interpretation. *Change in Marine Communities. PRIMER-E*, Plymouth, pp. 260.
- Danovaro, R., Pusceddu, A., 2007. Biodiversity and ecosystem functioning in coastal lagoons: does microbial diversity play any role? *Estuar. Coast Shelf Sci.* 75, 4–12.
- Di Pippo, F., Magni, P., Congesti, R., 2018. Microphytobenthic biomass, diversity and exopolymeric substances in a shallow dystrophic coastal lagoon. *J. Mar. Microbiol.* 2, 6–12.
- Facca, C., Sfriso, A., 2007. Epipellic diatom spatial and temporal distribution and relationship with the main environmental parameters in coastal waters. *Estuar. Coast Shelf Sci.* 75, 35–49.
- Facca, C., Sfriso, A., Socal, G., 2002. Changes in abundance and composition of phytoplankton and microphytobenthos due to increased sediment fluxes in the Venice lagoon, Italy. *Estuar. Coast Shelf Sci.* 54, 773–792.
- Falcieri, F., Benetazzo, A., Sclavo, M., et al., 2014. Po River plume pattern variability investigated from model data. *Cont. Shelf Res.* 87, 84–95.
- FAO, 2014. The State of World Fisheries and Aquaculture. pp. 223 Rome.
- Fazi, S., Amalfitano, S., Pizzetti, I., Pernthaler, J., 2007. Efficiency of fluorescence in situ hybridization for the identification of bacterial cells in river sediments with contrasting water content. *Syst. Appl. Microbiol.* 30, 463–470.
- Fazi, S., Butturini, A., Casamajor, E., Amalfitano, S., Vazquez, E., 2013. Stream hydrological fragmentation drives bacterioplankton community composition. *PLoS One* 8 (5), e64109.
- Folk, R.L., Ward, W.C., 1957. A study in the significance of grain-size parameters. *J. Sediment. Petrol.* 27, 3–26.
- Franzo, A., Cibic, T., Del Negro, P., Solidoro, C., 2014. Microphytobenthic response to mussel farm biodeposition in coastal sediments of the northern Adriatic Sea. *Mar. Pollut. Bull.* 79, 379–388.
- Franzo, A., Auriemma, R., Nasi, F., Vojvoda, J., Pallavicini, A., Cibic, T., Del Negro, P., 2016. Benthic ecosystem functioning in the severely contaminated Mar Piccolo of Taranto (Ionian Sea, Italy): focus on heterotrophic pathways. *Environ. Sci. Pollut. Res.* 23, 12645–12661.
- Franzo, A., Ascoli, A., Roscioli, C., Patrolecco, L., Bazzaro, M., Del Negro, P., Cibic, T., 2019. Influence of natural and anthropogenic disturbances on foraminifera and free-living nematodes in four lagoons of the Po delta system. *Estuar. Coast Shelf Sci.* <https://doi.org/10.1016/j.ecss.2019.02.039>.
- Freixa, A., Ejarque, E., Guarch-Ribot, A., Crognale, S., Amalfitano, S., Fazi, S., Butturini, A., Romani, A.M., 2016. Spatial and temporal variation of microbial function and structure in river sediments. *Limnol. Oceanogr.* 61, 1389–1405.
- Gambi, C., Totti, C., Manini, E., 2003. Impact of organic loads and environmental gradients on microphytobenthos and meiofaunal distribution in a coastal lagoon. *Chem. Ecol.* 00, 1–17.
- Glockner, F.O., Fuchs, B.M., Amann, R., 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl. Environ. Microbiol.* 65, 3721–3726.
- Guelorget, O., Perthuisot, J.P., 1983. Le domaine paraliq: expressions géologiques, biologiques et économiques du confinement. Presses de l'Ecole normale supérieure, Paris.
- Guiry, M.D., Guiry, G.M., 2018. AlgaeBase. World-wide Electronic Publication. National University of Ireland, Galway, Accessed date: 3 September 2018.
- Hedges, J.I., Stern, J.H., 1984. Carbon and nitrogen determinations of carbonate-containing solids. *Limnol. Oceanogr.* 29, 657–663.
- Köchling, T., Lara-Martín, P., Gonzalez-Mazo, E., Amils, R., Sanz, J.L., 2011. Microbial community composition of anoxic marine sediments in the Bay of Cádiz (Spain). *Int. Microbiol.* 14, 143–154.
- Korlević, M., Zucko, J., Najdek Dragić, M., Blažina, M., et al., 2015. Bacterial diversity of polluted surface sediments in the northern Adriatic Sea. *Syst. Appl. Microbiol.* 38 (3), 189–197.
- Lambhead, P.J.D., Platt, H.M., Shaw, K.M., 1983. The detection of differences among assemblages of marine benthic species based on an assessment of dominance and diversity. *J. Nat. Hist.* 17, 859–874.
- Larson, F., Sundbäck, K., 2008. Role of microphytobenthos in recovery of functions in a shallow-water sediment system after hypoxic events. *Mar. Ecol. Prog. Ser.* 357, 1–16.
- Lear, G., Lewis, G.D., 2009. Impact of catchment land use on bacterial communities within stream biofilms. *Ecol. Indic.* 9 (5), 848–855.
- MacIntyre, H.L., Geider, R.J., Miller, D.C., 1996. Microphytobenthos: the ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. distribution, abundance and primary production. *Estuaries* 19, 186–201.
- Maicu, F., De Pascalis, F., Ferrarin, C., Umgieser, G., 2018. Hydrodynamics of the Po river-delta-sea system. *J. Geophys. Res.: Oceans* 123. <https://doi.org/10.1029/2017JC013601>.
- Mallin, M.A., Cahoon, L.B., Toothman, B.R., Parsons, D.C., McIver, M.R., Ortwin, M.L., Harrington, R.N., 2007. Impacts of a raw sewage spill on water and sediment quality in an urbanized estuary. *Mar. Pollut. Bull.* 54, 81–88.

- Manini, E., Fiordelmondo, C., Gambi, C., Pusceddu, A., Danovaro, R., 2003. Benthic microbial loop functioning in coastal lagoons: a comparative approach. *Oceanol. Acta* 26, 27–38.
- Margalef, R., 1986. *Ecologia*. Omega, Barcellona, pp. 951.
- Miho, A., Witkowski, A., 2005. Diatom (Bacillariophyta) flora of Albanian coastal wetlands taxonomy and ecology: a review. *Proc. Calif. Acad. Sci.* 56 (12), 129–145.
- Mistri, M., Borja, Aleffi, I.F., Lardicci, C., Tagliapietra, D., Munari, C., 2018. Assessing the ecological status of Italian lagoons using a biomass-based index. *Mar. Pollut. Bull.* 126, 600–605.
- Mohit, V., Archambault, P., Lovejoy, C., 2015. Resilience and adjustments of surface sediment bacterial communities in an enclosed shallow coastal lagoon, Magdalen Islands, Gulf of St. Lawrence, Canada. *FEMS Microbiology* 91, fiv038.
- Molari, M., Giovannelli, D., d'Errico, G., Manini, E., 2012. Factors influencing prokaryotic community structure composition in sub-surface coastal sediments. *Estuar. Coast Shelf Sci.* 97, 141–148.
- Munari, C., Mistri, M., 2008. Biodiversity of soft-sediment benthic communities from Italian transitional waters. *J. Biogeogr.* 35, 1622–1637.
- Nasi, F., Ferrante, L., Alvisi, F., Bonsdorff, E., Auriemma, R., Cibic, T., 2019. Macrofaunal Bioturbation Attributes in Relation to Riverine Influence in Four Lagoons of the Po River Delta System (Northern Adriatic Sea, Italy). (This issue).
- Natali, C., Bianchini, G., 2018. Natural vs anthropogenic components in sediments from the Po River delta coastal lagoons (NE Italy). *Environ. Sci. Pollut. Res.* 25, 2981–2991.
- Pala, C., Molari, M., Nizzoli, D., Bartoli, M., Viaroli, P., Manini, E., 2018. Environmental drivers controlling bacterial and archaeal abundance in the sediments of a Mediterranean lagoon ecosystem. *Curr. Microbiol.* 75 (9), 1147–1155.
- Pavloudi, C., Oulas, A., Vasileiadou, K., Sarropoulou, E., Kotoulas, G., Arvanitidis, C., 2016. Salinity is the major factor influencing the sediment bacterial communities in a Mediterranean lagoonal complex (Amvrakikos Gulf, Ionian Sea). *Mar. Genomics* 28, 71–81.
- Pielou, E.C., 1966. Shannon's formula as a measure of specific diversity: its use and misuse. *Am. Nat.* 100, 463–465.
- Polge, N., Sukatar, A., Soylu, E.N., Gönülol, A., 2010. Epipelagic algal flora in the Kükükçekmece lagoon. *Turk. J. Fish. Aquat. Sci.* 10, 39–45.
- Potapova, M., Desianti, N., Enache, M., 2016. Potential effects of sediment contaminants on diatom assemblages in coastal lagoons of New Jersey and New York States. *Mar. Pollut. Bull.* 107, 453–458.
- Quero, G.M., Cassin, C., Botter, M., Perini, L., Luna, G.M., 2015. Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). *Front. Microbiol.* 6, 1053.
- Quero, G.M., Perini, L., Pesole, G., Manzari, C., Lionetti, C., Bastianini, M., Marini, M., Luna, G.M., 2017. Seasonal rather than spatial variability drives planktonic and benthic bacterial diversity in a microtidal lagoon and the adjacent open sea. *Mol. Ecol.* 26, 5961–5973.
- Reynolds, C.S., 1983. A physiological interpretation of the dynamic responses of populations of a planktonic diatom to physical variability of the environment. *New Phytol.* 95, 41–53.
- Ribeiro, L., Brotas, V., Rincé, Y., Jesus, B., 2013. Structure and diversity of intertidal benthic diatom assemblages in contrasting shores: a case study from the Tagus estuary. *J. Phycol.* 49, 258–270.
- Rogelja, M., Cibic, T., Pennesi, C., De Vittor, C., 2016. Microphytobenthic community composition and primary production at gas and thermal vents in the Aeolian Islands (Tyrrhenian Sea, Italy). *Mar. Environ. Res.* 118, 31–44.
- Rogelja, M., Cibic, T., Rubino, F., Belmonte, M., Del Negro, P., 2018. Active and resting microbenthos in differently contaminated marine coastal areas: insights from the Gulf of Trieste (northern Adriatic, Mediterranean Sea). *Hydrobiologia* 806 (1), 283–301.
- Round, F.E., 1971. Benthic marine diatoms. *Oceanogr. Mar. Biol. Annu. Rev.* 9, 83–139.
- Round, F.E., Crawford, R.M., Mann, D.G., 1992. *The Diatoms*. Cambridge University Press, Avon, pp. 747.
- Rubino, F., Cibic, T., Belmonte, M., Rogelja, M., 2016. Microbenthic community structure and trophic status of sediments in the Mar Piccolo of Taranto (Mediterranean, Ionian Sea). *Environ. Sci. Pollut. Res.* 23, 12624–12644.
- Sawai, Y., Horton, B.P., Kemp, A.C., Hawkes, A.D., Nagumo, T., Nelson, A.R., 2016. Relationships between diatoms and tidal environments in Oregon and Washington, USA. *Diatom Res.* 31 (1), 17–38.
- Sekar, R., Fuchs, B.M., Amann, R., Penthaler, J., 2004. Flow Sorting of marine bacterioplankton after fluorescence in situ hybridization. *Appl. Environ. Microbiol.* 70 (10), 6210–6219.
- Shannon, C.E., Weaver, W., 1949. *The Mathematical Theory of Communication*. University of Illinois Press, IL, pp. 117.
- Simpson, E.H., 1949. Measurement of diversity. *Nature* 163, 688.
- Solis-Weiss, V., Rossini, P., Aleffi, F., Bettoso, N., Orel, G., Vrišer, B., 2001. Gulf of Trieste: sensitivity areas using benthos and GIS techniques. In: Özhan, E. (Ed.), *Proceedings of the Fifth International Conference on the Mediterranean Coastal Environment*. MEDCOAST 01, Ankara, pp. 1567–1578.
- Sun, M.Y., Dafforn, K.A., Brown, M.V., Johnston, E.L., 2012. Bacterial communities are sensitive indicators of contaminant stress. *Mar. Pollut. Bull.* 64, 1029–1038.
- Sundbäck, K., Jönsson, B., Nilsson, P., Lindström, I., 1990. Impact of accumulating drifting macroalgae on a shallow-water sediment system: an experimental study. *Mar. Ecol. Prog. Ser.* 58, 261–274.
- Sundbäck, K., Petersen, D.G., Dahllöf, I., Larson, F., 2007. Combined nutrient-toxicant effects on a shallow-water marine sediment system: sensitivity and resilience of ecosystem functions. *Mar. Ecol. Prog. Ser.* 330, 13–30.
- Thorthon, S.F., McManus, J., 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay Estuary, Scotland. *Estuar. Coast Shelf Sci.* 38, 219–233.
- Torres, M.A., Barros, M.P., Campos, S.C.G., Pinto, E., Rajamani, S., Sayre, R.T., Colepicolo, P., 2008. Biochemical biomarkers in algae and marine pollution: a review. *Ecotoxicol. Environ. Saf.* 71, 1–15.
- Tsuboi, S., Amemiya, T., Seto, K., Itoh, K., Rajendran, N., 2013. The ecological roles of bacterial populations in the surface sediments of coastal lagoon environments in Japan as revealed by quantification and qualification of 16S rDNA. *World J. Microbiol. Biotechnol.* 29 (5), 759–774.
- Turolla, E., 2008. La venericoltura in Italia. In: Lovatelli, A., Farías, A., UriarteI (Eds.), *Estado actual de cultivo y manejo de moluscos bivalvos y su proyección futura: factores que afectan su sustentabilidad en América Latina*. Taller Técnico Regional de la FAO. FAO Actas de Pesca y Acuicultura, no 12. FAO, Rome, pp. 177–188 (in Italian).
- Underwood, G.J.C., Kromkamp, J., 1999. Primary production by phytoplankton and microphytobenthos in estuaries. *Adv. Ecol. Res.* 29, 93–153.
- Underwood, G.J.C., Phillips, J., Saunders, K., 1998. Distribution of estuarine benthic diatom species along salinity and nutrient gradients. *Eur. J. Phycol.* 33, 173–183.
- Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Int. Ver. Theor. Angew. Limnol.* 9, 1–38.
- Viaroli, P., Azzoni, R., Bartoli, M., Giordani, G., Naldi, M., Nizzoli, D., 2010. Primary productivity, biogeochemical buffers and factors controlling trophic status and ecosystem processes in Mediterranean coastal lagoons: a synthesis. *Adv. Oceanogr. Limnol.* 1 (2), 271–293.
- Vidal-Durà, A., Burke, I.T., Mortimer, R.J.G., Stewart, D.L., 2018. Diversity patterns of benthic bacterial communities along the salinity continuum of the Humber estuary (UK). *Aquat. Microb. Ecol.* 81, 277–291.
- Viganò, L., Stefani, F., Casatta, N., Mascolo, G., Murgolo, S., Roscioli, C., Zonta, R., 2019. Contamination Levels and Spatial Distribution in the Lagoons of the Po River Delta: Are Chemicals Exerting Toxic Effects? This Issue.
- Viganò, L., Arillo, A., Buffagni, A., Camusso, M., Ciannarella, R., Crosa, G., et al., 2003. Quality assessment of bed sediments of the Po River (Italy). *Water Res.* 37, 501–518.
- Weckström, K., Juggins, S., 2005. Coastal diatom-environment relationships from the gulf of Finland, Baltic sea. *J. Phycol.* 42 (1), 21–35.
- WoRMS Editorial Board, 2018. *World register of marine species*. Available from: <http://www.marinespecies.org.at.VLIZ>, Accessed date: 4 September 2018.
- Ye, Q., Wu, Y., Zhu, Z., Wang, X., Li, Z., Zhang, J., 2016. Bacterial diversity in the surface sediments of the hypoxic zone near the Changjiang Estuary and in the East China Sea. *MicrobiologyOpen* 5 (2), 323–339.
- Zonta, R., Cassin, D., et al., 2019. Grain Size and Heavy Metal Concentrations in the Surface Sediment Layer of the Po River Delta Lagoons (Italy). (This issue).
- Zoppini, A., Ademollo, N., Patrolocco, L., Bazzaro, M., Cibic, T., Franzo, A., Melita, M., Zonta, R., Amalfitano, S., 2019. Sediment Quality and Benthic Microbial Community Characteristics: Observations on the Po River Coastal Lagoons. (This issue).
- Zwisler, W., Selje, N., Simon, M., 2003. Seasonal patterns of the bacterioplankton community composition in a large mesotrophic lake. *Aquat. Microb. Ecol.* 31, 211–225.