Structural and functional response of coastal macrofaunal community to terrigenous input from the Po River (northern Adriatic Sea)

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ABSTRACT

To assess the influence of river-derived material on the benthic ecosystem in the Po River prodelta area, we investigated the structural and functional features of macrofaunal invertebrates in 14 sites. Biodiversity, species composition, *response* and *effect* traits of the community were determined and related to the main sediment physical-chemical and nutritional features (biopolymeric carbon, BPC; protein to carbohydrate ratio, Prt/Cho ratio; phaeopigment to chlorophyll-*a* ratio, Phaeo/Chl-*a*), as well as contaminants. We found significant spatial differences in community structure and its functional expression between northern and southern stations, and between nearshore and offshore ones. The shallower stations located nearby the main river mouth displayed higher densities, diversity and functional redundancy compared to southern stations. The main discriminating factors for the community were the higher percentage of sand and Prt/Cho nearby the river mouth, and fine grain-size and high Phaeo/Chl-*a* at the southernmost and deeper stations. Contaminant concentrations did not seem to severely affect the macrofaunal community. Changes in community structure were due to the dominance of few species, which occurrence varied according to grain-size and organic matter quality that in turn were linked to the different magnitude of depositional loads in the investigated area.

Focusing on *response* traits, we observed a clear influence of high loads nearby the main river mouth, through the dominance of *sessile* and *tube-builder* trait-categories at stations with a higher sand content. In contrast, we detected a major expression of *burrowers* and *interface crawlers*, and *swimmers* at southern and principally offshore muddy stations. *Effect* traits revealed that in the northern part of the prodelta prevailed *suspension*, *surface deposit feeders* and *conveyor* invertebrates that are, respectively, fundamental players in the benthic-pelagic coupling and elemental cycling within benthos. In the southern part, *subsurface deposit feeder* and *biodiffuser* organisms dominated. The latter are able to rework huge amounts of sediments also in the deepest layers, amplifying the elemental cycling and decomposition. In highly dynamic systems, as delta areas, the presence of invertebrates able to exploit different resources through bioturbation activities may enhance the functions of riverine costal ecosystems.

1. Introduction

Estuaries and deltas are transitional zones between land, freshwater habitat and sea (Levin et al., 2001). These areas of freshwater influence are subjected to several natural and anthropogenic factors that determine highly dynamic temporal and spatial patterns of both abiotic and biotic components (Kennish et al., 2014). Major terrestrial inputs in estuaries lead to high concentrations of nutrients, primary and secondary production (Lohrenz et al., 1990; Herman et al., 1999) but also

land-derived contaminants that may cause the destruction of habitats and depletion of species in these marine ecosystems (Lotze et al., 2006).

Sediments are a pivotal component of aquatic ecosystems where important transformation and exchange processes take place (Snelgrove et al., 2000). Indeed, river plumes supply sediments with phytodetritus that influences the composition, abundance and dynamics of benthic communities located beneath the plume (Danovaro et al., 2002; Salen-Picard et al., 2003). Macrofaunal organisms, due to their high biomass and species diversity (Snelgrove, 1998), could be considered a key

biological component, which drive important processes in marine sediments, such as nutrient cycling, sediment reworking, bio-irrigation, organic matter decomposition and secondary production (Widdicombe et al., 2004; Bremner et al., 2006; Olsgard et al., 2008).

Macrofaunal spatial distribution patterns have been investigated worldwide in relation to several environmental variables (e.g. Snelgrove, 1998; Levin et al., 2001; Gray and Elliott, 2009). In particular, studies quantifying the impacts of deposition of material from river plumes on macrofaunal communities are well documented (e.g. Thrush et al., 2004; Forrest et al., 2007; Hermand et al., 2008; Akoumianaki and Nicolaidou 2007). However, these studies have focused on the structural aspects of species' assemblages, such as abundance, biomass and diversity, but they have rarely assessed the functional adaptation to the environment. Lately, the scientific interest in understanding the influence of impacts on the functioning (e.g. productivity, transfer of energy and nutrient regulation) of benthic assemblages has increased, since the magnitude of the response is highly dependent on the functional features of the community (Hale et al., 2014). Indeed, species interact with and respond to their physical and chemical environment in various ways depending on their ability to do so. Therefore, the use of species composition alone might be inadequate for investigating processes that sustain an ecological system (Díaz and Cabido, 2001), since the ecosystem processes are determined by the functional characteristics of the organisms, rather than by their taxonomic identity (Grime, 1997). In this regard, the use of biological traits allows to gain insights into the relative presence of certain functional properties related to life history, and behavioural or morphological characteristics of macrofaunal communities in response to habitat constrains (Southwood, 1977; Solan et al., 2004).

Biological Trait Analysis (BTA) is a useful analytical approach that looks beyond the mere identity of communities' taxa and focuses more on their functional roles in shaping the ecosystem properties. In fact, BTA combines structural data of a macrofaunal community (i.e. species abundance or biomass) with the information on functional features of species (Bremner et al., 2006; Beauchard et al., 2017). Hence, by applying this approach it is possible to implement the following functional indices: i) functional diversity that represents the variety of function developed by organisms in a community, comprising a component of biodiversity; ii) functional redundancy used to define the resilience of ecosystem functions. In addition, ecosystem properties should primarily depend on the identity of dominant species and their functional traits. In fact, functional identity indicates the role of a single species in the ecosystem and depends on the diversity and distribution of traits within a community (Mouillot et al., 2011; Sigala et al., 2012; Gladstone-Gallagher et al., 2019). Recently, an increasing number of studies have applied this approach to marine benthic assemblages in relation to several environmental variables (e.g. Breine et al., 2018; Villnäs et al., 2018), hypoxia events (Gogina et al., 2014), dumping of dredged sediments (Bolam et al., 2016; Hussin et al., 2012), sewage discharges (Gusmao et al., 2016; Krumhansl et al., 2016) and chemical contamination (e.g. Nasi et al., 2018; Egres et al., 2019). However, these studies have rarely distinguished between *response* and *effect* biological traits (Hooper et al., 2005; Petchey et al., 2009). This concept has been shown to be of fundamental importance for the application of BTA in the terrestrial realm (Díaz and Cabido, 2001), in freshwater aquatic systems (Engelhardt, 2006) and, more recently, in marine ecosystems as well (i. e. Bolam et al., 2016). *Effect* traits concern ecosystem properties, e.g. increased mobility within the benthos will facilitate oxygen penetration through bioturbation. In contrast, *response* traits concern the response to changes in the physical environment, e.g. deep borrowing species are less likely to be affected by disturbance at the sediment-water interface. The distinction between these two groups of traits could give more insights in determining the impact of environmental change on ecosystem processes, since *response* traits may vary independently from *effect* traits (Violle et al., 2007; Bolam et al., 2016).

Investigations on the effects of river discharge on invertebrate

biological features and consequent changes in the ecosystem functioning are still rare, particularly in delta areas. In this study, we focused on macrofaunal invertebrates inhabiting the coastal area adjacent to the Po River delta (northern Adriatic Sea). In this prodelta area, a major flood event is able to transfer to the Adriatic Sea an amount of solid material and organic matter comparable to the annual sediment transport (Miserocchi et al., 2007; Giani et al., 2009). Moreover, the Po River watershed is subjected to multiple human-derived pressures resulting from many activities such as agriculture, industry and urban development. Therefore, the Po River carries yearly tons of anthropogenic chemicals collected from the river basin and its distributaries into the sea (Viganò et al., 2003). Previous studies highlighted that under both moderate and peak discharge regimes, the relative fractions of terrigenous organic carbon carried and released by the river is transported from its main distributary mouth (i.e. Po di Pila) southward along the shelf (Tesi et al., 2011). Several studies have described the macrozoobenthic community of this particular area (e.g. Occhipinti-Ambrogi et al., 2002, 2005; N' Siala, 2008), others investigated the impact of river-derived material on this community (Simonini et al., 2004; Bongiorni et al., 2018) or estimated the secondary production of selected macrofaunal invertebrates in relation to the river regime (e.g. the polychaete *Owenia fusiformis*, Ambrogi et al., 1995; the bivalve *Spisula subtruncata* Ambrogi and Occhipinti-Ambrogi, 1987). However, to the best of our knowledge, the influence of river discharge on macrofaunal community functional diversity and traits composition in the Po River prodelta area has not been investigated yet.

In this study, we hypothesized that: i) river-derived material influences species composition and functional diversity of coastal benthic invertebrates; ii) the different river-plume depositions significantly modify the macrofaunal community structure and functional expression of *response* and *effect* traits; iii) sediment loads influence the functional identity and shape ecosystem properties. To test these hypotheses, samples were collected in the Po River prodelta area, along one parallel north-south transect (from the main mouth Po di Pila, southwards) and along two coast-offshore transects (Po di Tolle and Po di Gnocca), during two sampling campaigns. The structure and function of benthic invertebrate assemblages were analysed and related to the sediment grain-size, organic matter quantity and quality, and contamination levels.

2. Material and methods

2.1. Study site

With an average discharge of 1500 m^3s^{-1}, the Po River is one of the largest rivers of the Mediterranean Sea in terms of freshwater fluxes (Ludwig et al., 2009). It extends over 685 km^2 and most of the drainage basin runs through a wide low-gradient alluvial plain (Tesi et al., 2011). Formed by seven river branches (178 km), several lagoons and wetlands, its delta system is located in the north-western boundary of the Adriatic Sea (Fig. 1). Overall, the river is considered one of the main vectors for the transport of nutrients, trace metals and other compounds to the northern Adriatic Sea (Giani et al., 2012). Characterized by two annual floods (>5000 m^3s^{-1}), associated with rainfall in autumn and snowmelt in spring (Boldrin et al., 2005), its total discharge is not equally distributed along the coast of the delta. Only 20% flows into the northern coast, 30% to the Pila tip (then driven southward by coastal currents) and the remaining 50% into the southern coast. This means that almost 80% of freshwater, sediments and river-borne compounds are driven offshore the coast of the southern delta lagoons (Maicu et al., 2018). During normal flow conditions, transported fine-grained sediments undergo a relatively rapid deposition nearby the mouths (~6 cm year^{-1} near the Pila distributary; Tesi et al., 2011). Conversely, during flood events, these particles may cover a wide distance before reaching the sea bottom. The plume is principally transported southward along the shelf due to the predominant cyclonic Western Adriatic Coastal Current –WAC (driven chiefly by the pressure gradient established
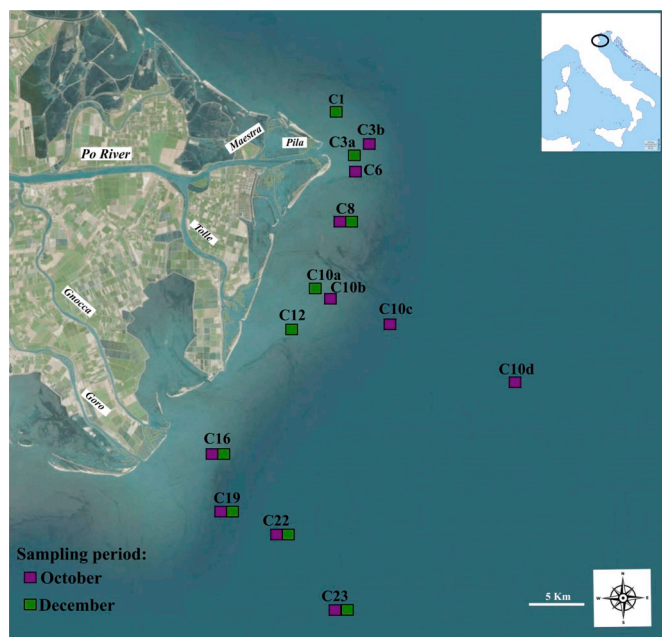
Fig. 1. Location of sampling sites and periods in the Po River prodelta.

Table 1

Coordinates and depth of sampling stations, and an indication of the sampling period as well as of the transect to which the station belongs (i.e. 'north-south'; 'distributary channel' and 'depth').

	Latitude	Longitude	Depth (m)	Sampling period	Transect
C1	44.993	12.556	13	December	north, nearshore
C3a	44.968	12.571	8	December	north, nearshore
C3b	44.974	12.581	19	October	north, offshore
C6	12.5732	44.956	10	October	north, nearshore
C8	44.929	12.558	11	October/ December	north, nearshore
C10a	44.891	12.538	15	December	south, Po di Tolle, nearshore
C10b	44.8867	12.55	17	October	Po di Tolle, offshore
C10c	44.8692	12.601	24	October	Po di Tolle, offshore
C10d	44.838	12.7	31	October	Po di Tolle, offshore
C12	44.867	12.519	14	December	south, nearshore
C16	44.7966	12.456	14	October/ December	south, Po di Gnocca, nearshore
C19	44.7636	12.462	19	October/ December	Po di Gnocca, offshore
C22	44.7511	12.506	25	October/ December	Po di Gnocca, offshore
C23	44.7082	12.555	28	October/ December	Po di Gnocca, offshore

between interior dense-water and coastal freshwater set up by the Italian rivers) and it is subjected to wind-induced resuspension events promoted principally by the north-easterly Bora wind (Frignani et al., 2005). Furthermore, the latter tends to confine the plume along the Italian coastline (Kourafalou, 1999), especially during winter when this katabatic wind is stronger while the south-easterly Scirocco drives riverine water northward (Pirazzoli and Tomasin, 2002).

2.2. Sampling design

Sediment samples were collected during two oceanographic campaigns on board of the R/V OGS-Explora and R/V Dalla Porta, in October and December 2014, respectively. The sampling sites, during both campaigns, were located at increasing depths (comprised between 9 and 21 m) and distance from the main distributary mouth (Po di Pila) along the southward river plume (Fig. 1). Seven out of the 14 stations were considered "nearshore" and seven "offshore" (Table 1). Moreover, to assess the possible influence of distinct distributary channels on the macrozoobenthic community features, stations were gathered along a 'north-south' transect (i.e. coastal stations from the northern part, nearby the Po di Pila to the southern area of the Po prodelta), and two coastal-offshore transects off Po di Tolle and Po di Gnocca.

During the first campaign, virtually undisturbed sediment cores for physical-chemical analyses were sampled by means of an automatic KC Haps bottom corer (KC-Denmark) equipped with polycarbonate sample tubes (internal diameter: 12.7 cm; surface area: 127 cm^2); whereas for macrofaunal community a van Veen grab (0.3 m^2) was used. In the second campaign, sediments sampled for macrofaunal and physical-chemical analyses were collected with a van Veen grab (0.1 m^2) in three replicates.

2.3. Sediment grain-size, organic matter, pigments and contaminants

At each sampling, for chemical variables (organic carbon, total nitrogen, biopolimeric carbon, pigments and contaminants), aliquots of 10–15 g of surface sediments (top 0–2 cm layer) were sampled.

For grain-size analysis, sediments were sieved at 2 mm and pre-treated with 10% hydrogen peroxide (60 °C for 24h) before being analysed with Malvern Mastersizer 2000 equipped with Hydro 2000s (Malvern Instruments Ltd., Malvern, UK). Data are expressed as

percentages of sand, silt and clay.

Organic carbon (Corg) and Total Nitrogen (TN) contents were determined using a CHNO–S elemental analyser (mod. ECS 4010, Costech, Italy) according to Pella and Colombo (1973). For Corg analysis, samples were acidified with increasing concentration of HCl (0.1N and 1N) to remove the carbonate fraction (Nieuwenhuize et al., 1994). Standard acetanilide (Costech, purity ≥ 99.5%) was used to calibrate the instrument and empty capsules were also analysed in order to correct for blank. The relative standard deviations for three replicates determination were lower than 3%. Corg and TN concentrations are expressed as percentage. Organic carbon to nitrogen molar ratio (C:N) was calculated and used as a proxy of the organic matter origin (Rumolo et al., 2011).

For biopolymeric carbon (BPC) analyses, subsamples of homogenised sediment were freeze-dried and processed in triplicates for the determination of carbohydrates, lipids and proteins. Colloidal and EDTA extractable carbohydrates (CHO) were analysed following the method described by Blasutto et al. (2005). Lipids (LIP) were analysed according to Bligh and Dyer (1959) while proteins (PRT) were determined following Hartree (1972). Data were converted to carbon equivalents using the conversion factors proposed by Fichez (1991) and their sum was referred as biopolymeric carbon (BPC). The protein to carbohydrate ratio (Prt/Cho) was used as a proxy of the "age" of the organic matter in sediments (Pusceddu et al., 2000).

Chloroplastic pigments (chlorophyll-a-Chl-a and phaeopigment-Phaeo) were analysed spectrofluorometrically following the procedures described by Lorenzen and Jeffrey (1980). Pigments were extracted overnight (4 °C, 90% acetone) from 0.2 to 0.5 g of wet sediment (in three replicates). After centrifugation the supernatant was used to determine the Chl-a, and acidified with 0.1 N HCl to estimate the amount of Phaeo. The phaeopigments to chlorophyll-a ratio (Phaeo/Chl-a) was used as a proxy of freshness/degradation of phyto carbon (Dell'Anno et al., 2002).

The concentrations of metals, except for mercury, were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Optima 2100DV, PerkinElmer, USA) (USEPA, 1994). The analyses of Hg were carried out by atomic absorption spectrophotometry by cold vapour (Analyst 100, PerkinElmer, USA) (USEPA, 1976). PAHs and PCBs analyses were conducted according to Cassin et al. (2018). For the purpose of this study, we used the sum of the analysed PAHs, and the sum of PCB congeners, referred to PAHs and PCBs in the text. In

Table 2
Functional trait-categories used in the present study, divided in *response* and *effect* traits. Codes of categories are also presented.

	Traits	Categories	Code	Relevant to river loads
Response traits	Sediment position	Endofauna	Endo	Typical living position in sediment profile. Taxa will generally need to re-establish their sediment vertical
		Epifauna	Epif	position following river loads to undertake their biological processes (e.g. feeding) (Bolam et al., 2016).
		Interface	Inter	
		Epibiont	Epib	
	Adult mobility	Sessile	Sess	Adult of faster moving species are more likely to evade burial following disposal, while those capable of
		Semi-motile	Smob	movement within the sediment may be capable of vertically migration thought deposited sediments (Bolam
		Motile	Mob	et al., 2016).
	Adult movement method	No muv.	Nom	Indicates potential for the adult stage to evade, or to be exposed to physical disturbance.
		Swimmer	Swim	
		Crawler	Craw	
		Tube-builder	Tub	
		Burrower	Burw	
Effect traits	Maximum size	< 5 mm	S	Smaller animals may be more able to take advantage of refugia from sedimentation at microhabitat scales.
		5-30 mm	M	
		30-80 mm	M/L	
		> 80 mm	L	
	Adult longevity	≤1 yr	Al1	Maximum reported life span of the adult stage. Indicates the relative investment of energy in rather than
		1-3 yrs	Al3	reproductive growth and the relative age of sexual maturity, i.e. proxy for relative *r*- or *K*-strategy (Ranta
		3-6 yrs	Al6	et al., 2002).
		6-10 yrs	Al10	
	Reproductive frequency	Semelparous	Sem	Reproductive frequency indicates role in community development.
		Iteroparous	Iter	
		Semi-continous	Scon	
	Adult feeding habit	Suspension feeder	Susp	Feeding habits indicates role in trophic pathway. Feeding habits may be affected by increased suspended
		Surface deposit feeder	Sdep	sediment (e.g. suspended feeders) (Thrush et al., 2004).
		Subsurface deposit feeder	Ssdep	
		Herbivore	Herb	
		Predation	Pred	
		Scavanger	Scav	
	Bioturbation	No. bio	Nob	Describes the ability of the organism to rework the sediments, influencing the oxygen concentration and
		Superficial modifier	Smod	nutrient cycling throughout sediment layers. Bioturbation mode has important implication for sediments-
		Biodiffuser	Bdif	water exchanges and sediment biogeochemical proprieties (Kristensen et al., 2012).
		Regenerator	Rege	
		Conveyor	Cony	

December 2014, sediment samples for contaminant analyses were not retrieved from C22 and C23 stations.

2.4. Macrofaunal community processing

The sediments collected for macrofaunal community analysis were sieved on a 1.0 mm mesh to retain the fraction of macrofaunal organisms. The retained sediment and organisms were immediately fixed with a formaldehyde solution (4% v/v final concentration in seawater). After washing, organisms were separated from the sediment by tweezers, divided into the main taxonomical phyla, and preserved in ethanol 70°. Macrofaunal invertebrates were identified under a stereomicroscope (Zeiss Discovery V.12, 8–110 × final magnification) and counted. For the taxonomic identification, the keys listed in Morri et al. (2004) were used.

2.5. Biological traits analysis

In this study, we considered 8 biological traits with 35 categories that are commonly used to describe variation in important morphological, behavioural and life history characteristics of marine benthic invertebrates (Törnroos and Bonsdorff, 2012). These traits were chosen also following previous studies with a similar aim (Reid et al., 2011; Bolam et al., 2016) (Table 2). The considered traits were then divided in two broad groups: (i) *response traits* i.e. those that affect a species response to changes in the environment, such as disturbance, resource availability or climatic shift; (ii) *effect* traits i.e. those that affect ecosystem properties (Bolam et al., 2016 and reference therein).

The taxa were coded based on their affinity for the chosen traits (Table S1) using the "fuzzy coding" procedure. Through this method, taxa can exhibit trait-categories to different degrees considering the interspecific variations in traits occurrences (Chevenet et al., 1994; Bremner et al., 2006). The fuzzy-coding procedure involves the assignment of a score ranging from a minimum of 0 (no affinity for the considered category) to a maximum of 3 (high affinity). Intermediate values, 1 and 2, represent a low and moderate-high importance for the selected trait, respectively. Traits for each taxon were derived from literature sources (i.e. Giangrande, 1997; Jumars et al., 2015; Queirós et al., 2013) and databases (i.e. www.marlin.ac.uk/biotic; www.poly-traits.lifewatchgreece.eu). Taxonomic resolution was kept at species level whenever possible but adjusted to genus or family when the information on traits was available only at a higher taxonomic level.

2.6. Data analysis

On traits profiles, trait-category richness was computed as the total number of categories expressed per sample. Two functional indices were calculated, functional diversity (expressed as FDiv) and functional redundancy (expressed as FDiv to Shannon-Wiener ratio-FDiv/H'; Van der Linden et al., 2012). FDiv is the degree to which abundance distribution in niche space maximises divergence in functional characters within the community (Mason et al., 2005), whereas functional redundancy represents a phenomenon for which multiple species representing a variety of taxonomic groups can share similar roles in ecosystem functionality (Díaz and Cabido, 2001). Hereby, a higher ratio value is indicative of lower functional redundancy. Further, to analyse the

functional identity (i.e. range of expression of functional traits), the community weighted trait means (CWM) method was applied. CWM is a widely used index that may reflect the trait strategies given by the species pool and environmental conditions of a site (Muscarella and Uriarte, 2016). For the calculation of CWM in our study, a community was defined as the species assemblage in each sample. CWM values represent the expression of a trait by species in a given community, weighted by the abundance of species expressing that specific trait. The FDiv and CWM values of the macrofaunal community in each sample were obtained using the FD library in the R program ver. 3.1.3 (Laliberté et al., 2014).

The physical-chemical parameters of Po River prodelta sediments, species composition and traits occurrences (CWM values) were compared among sampling sites using univariate and multivariate analyses, separately. First, to test the temporal variation of river loads, a one-way PERMANOVA main test was performed, where 'sampling period' (October *vs* December 2014) was selected as a fixed factor. In addition, spatial differences of stations gathered in transects were tested, where 'north-south' and 'distributary channel' were selected as fixed factors. Moreover, to test the influence of bathymetry on physical-chemical parameters in the sampling area, 'depth' (i.e. nearshore stations <16 m; offshore stations >16 m) was selected as factor (Table 1). Further, to assess whether the spatial differences among the investigated gradients were found during both sampling periods, a two-way PERMANOVA main test was carried out, where 'sampling period' were nested as random factor in 'north-south', 'distributary channel' and 'depth' factors, separately. When significant differences were obtained, PERMANOVA pairwise tests were performed. Unrestricted permutation of raw data and 9999 permutations were applied.

Univariate diversity analysis was applied to macrofaunal community considering the number of taxa, equitability (J' Pielou evenness) and), and diversity (H' Shannon-Wiener diversity) (Clarke et al., 2014).

In order to highlight differences among sites in the study area, variations in abiotic factors, macrofaunal community biodiversity and functional diversity indices were tested by the Mann-Whitney *U* test, where factors 'north-south', 'depth' and 'distributary channel' were applied. These analyses were carried out using STATISTICA 7 software.

In addition, to point out which taxa majorly contributed to spatial variation of macrofaunal assemblage, SIMPER (SIMilarityPERcentange) one-way analysis was used and factors 'north-south' and 'depth' were assigned. A cut-off at 60% was applied.

Environmental parameters were used to perform the Distance-Linear Modelling (DistLM) to assess which variables explained differences (p < 0.05) in species and traits composition (i.e. species abundance and CWM matrices, respectively). Prior to the analysis, the environmental data were normalized and the option "All specified" and R^2 were used as the selection procedure and criterion, respectively. This analysis was applied to physical-chemical data and contaminants, separately. Since contaminant data were not available for stations C22 and C23 in December, macrofaunal community data from these stations and period were not included in the DistLM model.

In order to visualize any spatial patterns of macrofaunal species composition within the study area, a non-metric multidimensional scaling ordination (nMDS) was performed. Abiotic variables (physical-chemical and contaminant data highlighted by DistLM models performed on both matrices; i.e. species composition and CWM values) were overlaid separately as supplementary variables (vectors) onto ordination space to investigate their relationship in this distribution.

For each multivariate analysis (i.e. PERMANOVA, SIMPER, DistLM and nMDS) the abiotic parameters were normalized and the Euclidean distance was applied; whereas the two matrices (i.e. species composition and CWM values) were square root and Log(x+1) transformed; the Bray-Curtis similarity and Euclidean distance were applied, respectively. These analyses were performed using PRIMER 7 (PRIMER-E Ltd. Plymouth, UK) (Clarke et al., 2014).

In this study, we applied the RLQ analysis (Dolédec et al., 1996) to look for relationships between traits-categories occurrences (for *response* and *effect* attributes, separately) and sediment variables. The RLQ analysis requires the generation of three different data tables: the R table, gathers information on the environmental variables (i.e. salinity, grain-size, Corg, C:N, BPC, Prt/Cho and Phaeo/Chl-*a* ratios) from all sites; the L table, constituted by the abundance of each species in each sampling site; and the Q table, composed of trait data provided by the fuzzy-coding procedure of the scoring of each taxa of the thirty-five different trait-categories (Table 2). Before running the RQL analysis, as a first step, we carried out the analysis separately on each of the following three tables: environmental variables (R), abundance (L), and traits (Q). Thus, a correspondence analysis (CA) was firstly conducted on the L table, while a principal component analysis (PCA) was performed on the R table. Regarding fuzzy-coded trait data, a fuzzy correspondence analysis (FCA) was conducted. These separate analyses were then combined with the RLQ analysis maximizing the covariation between environmental variables and taxonomical traits. This analysis was carried out with ade4 (Dray and Dufour 2007) package in the R program (ver. 3.1.3).

To highlight the spatial relationship between predictor variables (i.e. physical-chemical parameters and contaminants, separately) and traits-categories, linear regression was computed. By doing so, the predictive power of environmental parameters for each modality was discriminated via the coefficient R^2 and F-values. This analysis was carried out using STATISTICA 7 software.

3. Results

3.1. Physical-chemical parameters and contaminants in sediments

The lowest bottom salinity was registered at offshore stations located along the 'Po di Tolle' transect (i.e. 32.7 and 32.8 at C10c and C10b, respectively), whereas the highest salinity was observed at southernmost stations and the deepest ones of 'Po di Gnocca' transect, during both sampling periods (Table 3).

The sediments in the Po River prodelta area were characterized by high percentage of silt (average value: 69.2 ± 3.7%). The sand percentage varied from a minimum of 0.9% at C12 to a maximum of 19.9% at C8 in December. A decreasing pattern in sand fraction was evidenced, in particular in December, from northern to southern stations (10.6 and 0.9% at C3a and C12, respectively) and from C16 to offshore ones (5.8 and 1.2% at C16 and C23, respectively). The sand percentage significantly differed between nearshore and offshore stations (*U* test z = 2.4; p < 0.05). Conversely, higher values of clay were obtained at southern stations, in particular at C12 (33.5%). The clay percentage significantly increased from stations located nearby the Po di Pila mouth to southern ones (*U* test z = −2.3; p < 0.05) (Table 3).

During both campaigns, the lowest values of Corg were recorded at station C8 (0.8% and 1.0% in October and December, respectively), whereas the highest one was obtained at C19 during the first sampling period (1.6%). The C:N molar ratio over the sampling area and periods varied between 8.6 and 13.3 at C19 (in October and December, respectively), and significantly decreased toward the southern stations (*U* test z = 2.1; p < 0.05).

The lowest Phaeo/Chl-*a* ratio, considering both sampling periods, was found in front of Po di Pila (8.1 at C3a), and the highest one at the southernmost and deepest station (29.4 at C23 in December).

BPC values were slightly higher in October than in December. During the first campaign, BPC ranged between 1747.9 and 3583.9 μg g^{-1} at C3a and C10d, respectively. The Prt/Cho ratio, in both periods, was higher at stations nearby the main river mouth, displaying a maximum at C6 in October and minimum at C23 in December (2.3 and 0.4, respectively) (Table 3).

Overall, the PERMANOVA one-way main-test revealed that the physical-chemical data significantly differed between nearshore and offshore stations (Pseudo-F = 2.8; p < 0.05). A similar result was

Table 3

Physical-chemical parameters measured at sampling stations (from north to south). Corg (organic carbon); Prt/Cho (protein to carbon ratio); Phaeo/Chl-a (phaeopigments to chlorophyll-a ratio); C:N (carbon and nitrogen ratio).

	Salinity	Sand	Silt	Clay	Corg	C:N	Phaeo/Chl-a	BPC	PRT/CHO
		%			%			µg g^{-1}	
C1	36.8	5.9	72.0	22.1	1.2	10.5	8.6	2099	0.8
C3a	34.8	10.6	64.2	25.1	1.2	10.6	8.1	2637	1.6
C3b	37.3	1.8	75.6	22.6	1.6	9.6	9.7	3583	1.8
C6	33.0	8.9	71.9	19.2	1.4	11.8	10.8	3206	2.3
C8 (oct)	33.5	6.7	71.3	22.0	1.0	10.4	14.6	2101	1.3
C8 (dec)	35.5	19.9	63.7	16.4	0.8	10.7	14.1	2645	1.5
C10a	36.4	2.1	69.2	28.7	1.2	9.9	15.5	2275	1.4
C10b	32.8	7.5	73.9	18.6	1.1	10.7	14.0	2685	1.0
C10c	32.7	3.3	71.7	25.0	1.3	12.1	19.4	1866	0.8
C10d	37.9	4.7	71.6	23.7	1.2	10.1	13.7	1747	0.9
C12	36.2	0.9	65.6	33.5	1.2	9.3	12.8	2254	0.5
C16 (oct)	35.90	5.8	65.3	28.9	1.4	10.2	9.1	3331	1.7
C16 (dec)	36.0	10.7	64.4	24.9	1.0	9.2	19.3	3456	1.0
C19 (oct)	36.3	3.1	75.5	21.4	1.6	13.3	16.1	2313	0.7
C19 (dec)	36.6	4.2	67.4	28.3	1.1	8.6	20.2	2278	0.4
C22 (oct)	37.2	1.1	67.9	31.0	1.3	10.2	14.6	2307	1.1
C22 (dec)	37.3	1.8	67.3	30.9	1.3	9.6	19.4	2334	0.4
C23 (oct)	37.1	1.0	70.0	29.0	1.1	10.2	15.7	1943	1.0
C23 (dec)	37.9	1.2	66.7	32.2	1.0	9.0	29.4	1804	0.4

obtained by the two-way PERMANOVA main test, where the factor 'depth' significantly differed from the 'sampling periods' one (Pseudo-F = 4.8; p < 0.01), in particular in October (PERMANOVA pairwise test: t = 1.8, p < 0.01). On the contrary, no significant differences were noticed between sampling periods, northern and southern stations and between the two minor distributaries (i.e. Po di Tolle and Po di Gnocca).

Concentrations of heavy metals, PAHs and PCBs, measured in surface sediments of the study area, are listed in Table S2. C16 was the most contaminated station by Cr, Cu, Ni, Cd and Al. In contrast, the highest values of Hg (0.48 mg kg^{-1}), As (14.3 mg kg^{-1}) and Zn (142.7 mg kg^{-1}) were found at the deepest stations C23, C10c and C22, respectively, especially in October. The maximum concentration of PCBs was detected at C16 in December (27.8 µg g^{-1}). Conversely, the maximum value of PAHs (157.2 µg g^{-1}) was obtained at the offshore station C10d in October.

3.2. Macrofaunal structural and functional features

The lowest macrofaunal abundances in the Po River prodelta were observed at C23 (110 and 83.5 ind. m^{-2} in October and December respectively), whereas the highest densities were obtained in both sampling periods at C8 (2293.3 ind. m^{-2} in October and 5333.3 ind. m^{-2} in December). Stations positioned nearby the Po di Pila mouth displayed higher densities compared to southern stations. Nearshore stations displayed similar abundances, whereas the offshore ones displayed lower macrofaunal numbers. Polychaetes and molluscs were the dominant taxa (45% and 42% of the total abundance, respectively) followed by crustaceans (11%), echinoderms (2%) and other groups (anthozoans, sipunculids and nemertines, together 1%). Overall, polychaetes were dominant at stations located in front of the main river mouth, whereas at the southern ones molluscs and crustaceans considerably increased.

The PERMANOVA main test performed on macrofaunal community highlighted differences between stations located in the northern and southern area of the Po delta as well as nearshore vs offshore ones (Pseudo-F = 1.8; p < 0.05 and 3.7; p < 0.001, respectively). In addition, from the two-way PERMANOVA main test, the macrofaunal community at stations located at a depth of <16 m significantly differed from that inhabiting deeper sites (Pseudo-F = 2.3; p < 0.01), both in October and December (PERMANOVA pairwise test: t = 1.5, p < 0.05 for both periods). Conversely, no significant differences were obtained between either sampling periods or the two minor distributaries (i.e. Po di Tolle and Po di Gnocca).

A total of 142 taxa were observed in the study area. The highest

Table 4

Values of structural and functional diversity indices of macrofaunal community for sampling sites and periods. J': Pielou eveness; H': Shannon-Wiener diversity; FDiv: functional dispersion; FD/H': FDiv to Shannon-Wiener diversity ratio.

	N. taxa	J'	H'	N. trait-categories	FDiv	FD/H'
C1	43	0.75	4.10	33	0.72	0.18
C3a	23	0.59	2.66	31	0.86	0.32
C3b	21	0.27	1.20	31	0.64	0.53
C6	26	0.62	2.89	31	0.71	0.25
C8 (oct)	31	0.48	2.40	33	0.67	0.28
C8 (dec)	31	0.37	1.84	33	0.59	0.32
C10a	39	0.57	3.00	32	0.80	0.27
C10b	32	0.70	3.52	32	0.63	0.18
C10c	18	0.82	3.42	31	0.81	0.24
C10d	17	0.81	3.31	29	0.79	0.24
C12	15	0.87	3.40	30	0.81	0.24
C16 (oct)	33	0.51	2.59	33	0.85	0.33
C16 (dec)	20	0.67	2.90	31	0.66	0.23
C19 (oct)	22	0.47	2.09	29	0.68	0.33
C19 (dec)	18	0.44	1.85	31	0.54	0.29
C22 (oct)	24	0.59	2.69	33	0.85	0.32
C22 (dec)	17	0.85	3.47	30	0.75	0.22
C23 (oct)	12	0.84	3.03	29	0.72	0.24
C23 (dec)	8	0.77	2.30	29	0.69	0.30

number of taxa was observed at C1 (43), and the lowest one at C23 in December (8). As corroborated by U test, a significant spatial pattern of variation in the taxa richness was observed comparing nearshore vs offshore stations (U test z = 2.3; p < 0.05). The highest value of diversity (H' = 4.10) was obtained at C1, whereas the lowest values of diversity (H' = 1.20) and evenness (J' = 0.27) were obtained at C3b, likely due to the dominance of the polychaete *Sternaspis scutata* (750.0 ind. m^{-2}) at this site (Table 4). Overall, no significant patterns were noticed either along the 'north-south' transect or nearshore vs offshore sites.

Concerning the functional analyses performed on the communities, a significant spatial pattern of variation in the trait-category richness (average: 31.1 ± 1.5; range: 29–33) was observed from stations located at a depth of <16 m to deeper ones (U test z = 2.1; p < 0.05) (Table 4). On the contrary, no significant differences were noticed in FDiv between northern and southern stations, nearshore and offshore ones. The latter values ranged from a minimum of 0.54 at C19 in December to a maximum of 0.86 at C3a. As FDiv values, no significant differences were observed for functional redundancy values (FDiv/H') along the plume sedimentation gradient (i.e. north-south stations and nearshore-offshore

ones). However, a great variability in FDiv/H′ was instead observed at stations nearby the main river mouth (i.e. Po di Pila): C1 (0.2), C3a (0.3) and C3b (0.5). At southern stations, no spatial pattern in FDiv/H' was noticed (average value: 0.26 ± 0.04) (Table 4).

The SIMPER test showed high dissimilarity values in macrofaunal composition between stations located nearby the Po di Pila mouth and the southern ones (average dissimilarity = 71.6). The dissimilarity was mainly due to the presence of *Owenia fusiformis* (Contrib.% = 8.3) and the crustacean *Ampelisca intermedia* (Contrib.% = 4.3) that were dominant in the northern and southern sites, respectively. In addition, the difference in taxa between nearshore and offshore stations (average dissimilarity = 72.9) was attributed to the high abundance of *Corbula gibba*, *A. intermedia* and *O. fusiformis* at nearshore stations (Contrib.% = 8.2, 6.7 and 3.9, respectively), whereas *S. scutata* was dominant at the deeper ones (Contrib.% = 5.53) (Table 5).

The PERMANOVA main test performed on macrofaunal trait-categories occurrences highlighted differences between coastal stations located in the northern and southern area of the Po delta and between nearshore *vs* offshore ones (Pseudo-F = 1.8; p < 0.05 and 3.7; p < 0.001, respectively). In addition, from the two-way PERMANOVA main test, macrofaunal community significantly differed from stations located at a depth of <16 m to deeper ones (Pseudo-F = 3.9; p < 0.001), both in October and December (PERMANOVA pairwise test: t = 1.7, p < 0.05 and 2.0 p < 0.01, respectively). Since no significant differences in species composition and traits occurrences were tested among stations gathered in Po di Gnocca and Po di Tolle transects, this factor ('distributary channel') were not considered for the subsequent results.

The CWM values of the study area showed higher occurrences for categories: *endofauna*, *semi-motile*, and *iteroparous*. On the contrary, less represented categories (CWM values < 0.05) of traits were: *epifauna*, *adult longevity ≤ 1 year* and *6–10 years*, *herbivore*, *scavenger* and *regenerator* (Fig. 2).

In particular, significant variations in categories belonging to 'adult mobility', 'adult movement method', 'reproductive frequency', 'adult feeding habit' and 'bioturbation' traits, were observed between stations located nearby the Po di Pila mouth and the southern ones, and between nearshore *vs* offshore sites (Fig. 3). *Semi-motile* organisms were dominant at northern stations (z = 2.3; p < 0.05). On the contrary, sites located in the southern part of the Po River prodelta were characterized by high

expression of *swimmer*, *herbivore* and *predator* modalities (z = −2.1, −2.3, −2.1; p < 0.05) (Fig. 3a and b). High expression of *sessile* and *tube-builder* invertebrates was observed at nearshore stations, compared to *burrower* organisms that were dominant at deeper sites (z = 2.7, 2.7, −2.3; p < 0.05, respectively). As 'reproductive frequency' trait, the *semelparous* modality dominated at nearshore stations located in front of the Pila mouth (z = 2.2; p < 0.05, respectively). Further, a high expression of *suspension* and *surface deposit feeder* invertebrates was observed at stations closest to the shore (z = 2.2; p < 0.05 and z = 3.6 p < 0.01, respectively), whereas high expression of *subsurface deposit feeder* ones was noticed at deeper stations (z = −2.4; p < 0.05). Concerning the 'bioturbation' trait, higher values of *superficial modifier* and *conveyor* modalities were found at shallower stations (z = 3.2, 2.7; p < 0.01) whereas *biodiffuser* animals were more abundant at offshore ones (z = −3.5; p < 0.001, respectively) (Fig. 3c and d).

3.3. Taxa assemblage, traits occurrences and relationship with environmental variables

DistLM, applied to both structural and functional variables (R² = 0.5 for both matrices, separately) revealed that sand and clay were the main drivers of the species assemblage (Pseudo-F = 3.2, 2.5; p < 0.001, respectively), followed by Prt/Cho and Phaeo/Chl-*a* ratios, BPC and salinity (Pseudo-F = 2.3, 2.1, 2.1, 1.8; p < 0.05, respectively). Sand and salinity were the main drivers of traits occurrences (Pseudo-F = 3.8, p < 0.01 and 3.6, p < 0.05, respectively). Further, DistLM, applied to both species composition and CWM values (R² = 0.7 for both matrices), revealed that Hg, Pb, and As were somehow related to the species assemblage (Pseudo-F = 3.0, 2.4; p < 0.01 and 2.3; p < 0.05, respectively), and PAHs to community traits occurrences (Pseudo-F = 2.6; p < 0.05).

The vector fitting nMDS analysis based on the macrofaunal assemblage of the study area showed a spatial variation in taxa composition, in particular from stations located nearby the main river distributary mouth (Po di Pila) to southern and deeper ones. BPC, Prt/Cho and sand were placed close to the northernmost and shallower stations (left-hand and lower side of the ordination), whereas salinity, clay and Phaeo/Chl-*a* were located towards southern and offshore stations (right-hand and lower side of the plot) (Fig. 4a). A variation in species distribution from

Table 5

Taxa contribution to average dissimilarity between stations grouped into the transect 'north-south' and sea bottom depths (SIMPER analysis; cut-off at 60%). Av. = average abundance; SD = standard deviation; Contrib% = species contribution to average dissimilarity between groups.

| Taxa	North		South		Nearshore		Offshore		'North *vs* South'	'Near-' *vs* 'Offshore'
					ind. m⁻²				Contrib%	
	Av.	SD	Av.	SD	Av.	SD	Av.	SD		
Abra alba	38.9	66.2	4.2	6.3	27.8	55.0	24.3	75.8	2.0	2.1
Acmira assimilis	23.9	31.0	0.0	0.0	15.9	27.3	0.0	0.0	1.8	1.2
Acrocnida brachiata	10.6	16.4	8.3	5.8	10.7	13.0	0.3	1.1	1.4	1.8
Ampelisca intermedia	49.4	87.3	330.8	359.1	177.8	272.2	37.3	65.4	6.0	5.6
Chamelea gallina	36.7	66.2	0.0	0.0	24.4	55.5	5.7	17.9	1.9	1.5
Corbula gibba	342.2	441.8	225.0	227.8	327.8	365.7	33.3	101.9	6.1	8.9
Dosinia lupinus	13.3	18.5	1.7	3.3	9.6	15.8	4.7	14.8	–	1.2
Heteromastus filiformis	212.2	455.8	2.5	1.7	141.9	375.5	2.3	3.5	4.3	3.3
Kurtiella bidentata	11.7	25.4	5.8	7.9	10.4	20.6	22.7	38.9	–	2.0
Labioleanira yhleni	0.0	0.0	0.0	0.0	0.0	0.0	5.7	5.5	–	1.1
Moerella distorta	7.8	8.9	10.0	7.2	9.6	7.7	1.7	1.8	–	1.5
Nephtys hystricis	17.8	24.6	19.2	14.5	19.3	21.1	7.7	8.9	1.8	1.8
Nereis lamellosa	8.3	5.5	0.0	0.0	5.2	6.3	1.0	2.2	1.5	–
Notomastus aberans	1.7	2.8	11.7	11.4	6.3	8.9	0.0	0.0	–	1.2
Nucula nucleus	30.0	49.1	19.2	19.7	28.5	39.9	12.0	16.6	2.1	2.4
Owenia fusiformis	809.4	1162.8	0.8	1.7	540.0	1004.2	0.0	0.0	11.5	8.3
Polititapes aureus	29.4	59.6	0.0	0.0	19.6	49.3	6.3	20.0	1.7	1.4
Peronidia albicans	42.8	64.3	6.7	13.3	30.0	54.9	7.0	17.9	2.5	2.0
Pseudoleiocapitella fauveli	46.1	78.6	11.7	10.0	35.9	64.1	0.3	1.1	2.6	2.4
Spisula subtruncata	14.4	18.7	0.8	1.7	10.0	16.2	11.0	34.8	1.5	1.5
Sternaspis scutata	163.3	293.5	83.3	47.4	62.6	58.6	181.0	230.2	5.0	4.9
Striarca lactea	214.4	312.1	4.2	5.0	143.0	269.0	48.7	148.1	5.3	4.6
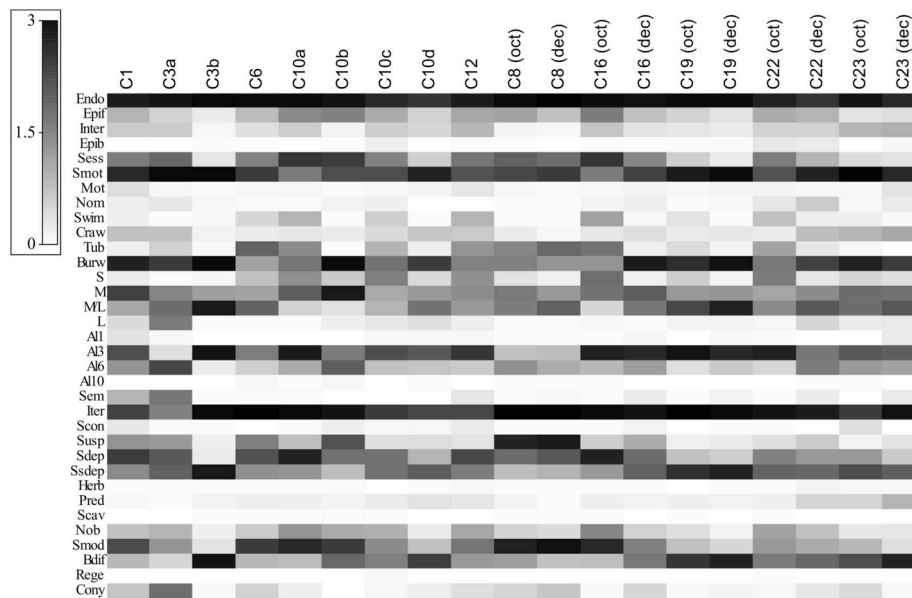
Fig. 2. Shade plot representing the trait-categories occurrences (community weighted means –CWM) of macrofaunal community at the sampling area and periods. Sites are displayed along a north-south direction. See Table 2 for trait labels.

the left side of the plot (i.e. C8 in both sampling periods) to the right lower part (i.e. C23 in December) was displayed. This spatial distribution was likely due to the dominance of the polychaetes *Heteromastus filiformis, Owenia fusiformis* and the bivalve *Chamelea gallina* at stations positioned nearby to main river distributary mouth, whereas the polychaetes *Labioleanira yhleni* and *Sternaspis scutata* characterized the offshore sites (Fig. 4b). Moreover, in the vector fitting nMDS performed with contaminants highlighted by DistLM; Hg, Pb, As and PAHs were plotted close to the offshore stations (Fig. S1).

Two RLQ combined analyses were carried out on trait-categories occurrences expressions belonging to *response* and *effect* attributes (Figs. 5 and 6). The RLQ analysis, performed on *response* traits accounted for 88.0%, of total variance (RLQ1: 73.2% and RLQ2: 14.8%; Fig. 5a). Clay and Corg were the predominant elements of the positive part of RLQ1 axis related with *interface* and *crawler* as trait-categories. These associations largely matched the characteristics of the polychaete *L. yhleni*. Conversely, in the negative part of RLQ1 increasing values of sand corresponded to high occurrences of *sessile* and *endofauna* modalities. The latter was associated with the polychaete *O. fusiformis* and the bivalves *Striarca lactea,* and *Corbula gibba*. The positive part of RLQ2 revealed that *burrower* modality, mainly expressed by the polychaete *S. scutata* and the bivalve *Nucula nucleus*, corresponded to high percentage of silt. The negative part of RLQ2 showed that Prt/Cho ratio was associated with *tube-builder* trait categories. The main corresponding taxa were the polychaetes *O. fusiformis* and *Melinna palmata*.

The RLQ carried out on *effect* traits clearly distinguished northern stations from southern and offshore ones (Fig. 6a). The first RLQ axis accounted for 73.3%, whereas the second axis represented 17.4% of the total variance of RLQ analysis (90.7%). In the positive part of RLQ1 and negative one of RL2 (right hand side of Fig. 6c), higher percentages of silt, clay, Corg and Phaeo/Chl-*a* ratios corresponded to high occurrences of *adult longevity 1–3 years*, *subsurface deposit feeder* and *biodiffuser* modalities, mainly expressed by the polychaete *S. scutata*, the bivalve *Kurtiella bidentata* and the echinoderm *Amphiura chiajei*. Sand and Prt/Cho ratio were the predominant elements of the negative part of RLQ1, related principally with *suspension feeder* and *superficial modifier* trait-categories. These associations largely matched the characteristics of the polychaete *O. fusiformis* and the bivalve *C. gibba*. In addition, the Q canonical weight plot (Fig. 6b) highlighted high occurrences of *conveyor* and *semelparous* modalities due to the major density of the polychaete

H. filiformis observed at C3a.

The linear regression highlighted significant relationships between modalities belonging to 'Sediment position', 'Mobility', 'Movement method', 'Feeding strategy' and 'Bioturbation' with salinity, grain-size, BPC, Prt/Cho and Phaeo/Chl-*a* ratios. Moreover, a significant relationship was found between endofauna surface deposit feeders and lower values of Hg and Pb. Significant linear regressions were observed between sessile, no bioturbation and superficial modifier and lower PAHs contents. On contrary, *biodiffuser* and *regenerator* as modalities showed a relation with As and Pb (Table S3).

4. Discussion

We explored the structural and functional features of the macrofaunal community influenced by high river loads. Grain-size, sand and clay in particular, were found to be the main environmental drivers related to taxonomic and functional features of macrofaunal assemblages at large scale (the entire investigated area). Conversely, at small spatial scale (in front of the Pila mouth), the quantity and quality of food supply influenced the structure of the community. In particular, at offshore stations the lower species numbers and diversity index values, indicating a less structured community, are likely related to the lower quality of the organic matter (high Phaeo/Chl-*a*). Moreover, we infer that the high variations in functional diversity and species composition observed in the Po River prodelta were likely due to the variability of river-derived inputs. However, high functional diversity and redundancy observed in the shallow area in front of the main river mouth could be related to the high efficiency of invertebrates to utilize available resources, principally of riverine origin. The clear influence of river loads was deduced by the occurrence of certain *response* traits. *Sessile* and *tube-builder* organisms were noticed at stations characterized by high superficial current velocity but low turbidity. On the contrary, a major expression of *burrowers, interface crawlers* and *swimmers* occurred at southern and principally offshore muddy stations. Focusing on *effect* traits, we observed those traits which have potentially affected ecosystem properties. At stations characterized by either high and continuous or intermittent deposition of fresh, river-derived material, *suspension feeders* (obtaining food by actively sweeping or holding out a filter), *surface deposit feeders* (obtaining food from the surface of the substratum) and *conveyor* invertebrates were well represented. All are
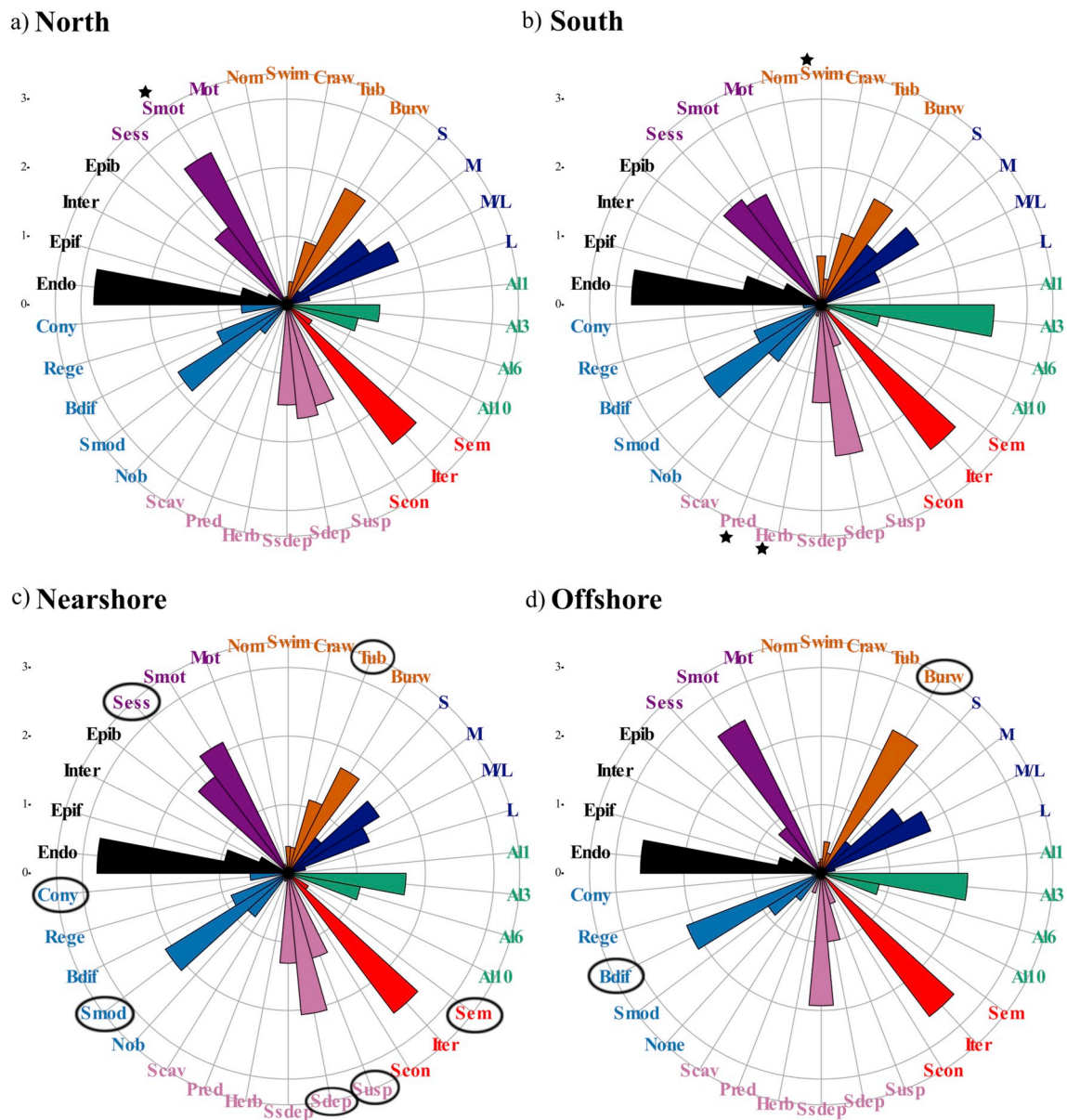
Fig. 3. Community-weighted mean of trait-categories occurrences (CWM), average values for sampling stations gathered as north (a), south (b), nearshore (c) and offshore (d) sites. Colour code and individual bars represent trait affiliation and trait-categories occurrences, respectively (See Table 2 for trait labels). Variables with a circle explain significant (*U* test) difference between northern and southern stations while those with a star explain significant difference between nearshore *vs* offshore ones. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

fundamental players in the benthic-pelagic coupling and elemental cycling within benthos. On the contrary, where lower and continuous river deposition occur, the elemental cycling within benthos and decomposition were amplified by *bioddiffuser* invertebrates that are able to rework huge amounts of sediments also in the deepest layers.

4.1. Environmental background

In this study, we sampled the coastal macrofaunal community in two periods characterized by different river flows. In October 2014, sampling was performed after a prolonged period of lean river flow. In the two weeks preceding the campaign, there was no substantial rainfall and the river outfall was therefore modest (Fig. S2). In contrast, in December 2014 a rapid-response survey was conducted following the most relevant flood of the year (November 19th) (Fig. S2, Braga et al., 2017). Despite these different river flow conditions, the PERMANOVA main-test performed on sediment grain size, organic matter features and

most of the considered contaminants did not highlight any statistical differences between the two periods. According to Tesi and co-authors (2011), during low-to-moderate flow conditions, sediment deposition is rapid and occurs within 2 km from the river mouth. Conversely, with high river run-off, sediments are transported further into the prodelta because a thick plume increases the resident time of particles in the water column. Therefore, since in December the campaign was carried out soon after a major plume (Braga et al., 2017), it is likely that there was not enough time to allow the deposition of river-driven material into the seabed. Moreover, only two months elapsed between the two sampling periods, which could not suffice to observe a clear temporal pattern in physical-chemical variables of surface sediments.

On the other hand, differences in physical-chemical variables between north *vs* south coastal stations, and between nearshore *vs* offshore ones, confirm that the spatial variation in the prodelta area is due to the decreasing amount of terrigenous material settled to the seabed with increasing distance from the Pila mouth (Boldrin et al., 2005; Tesi et al.,
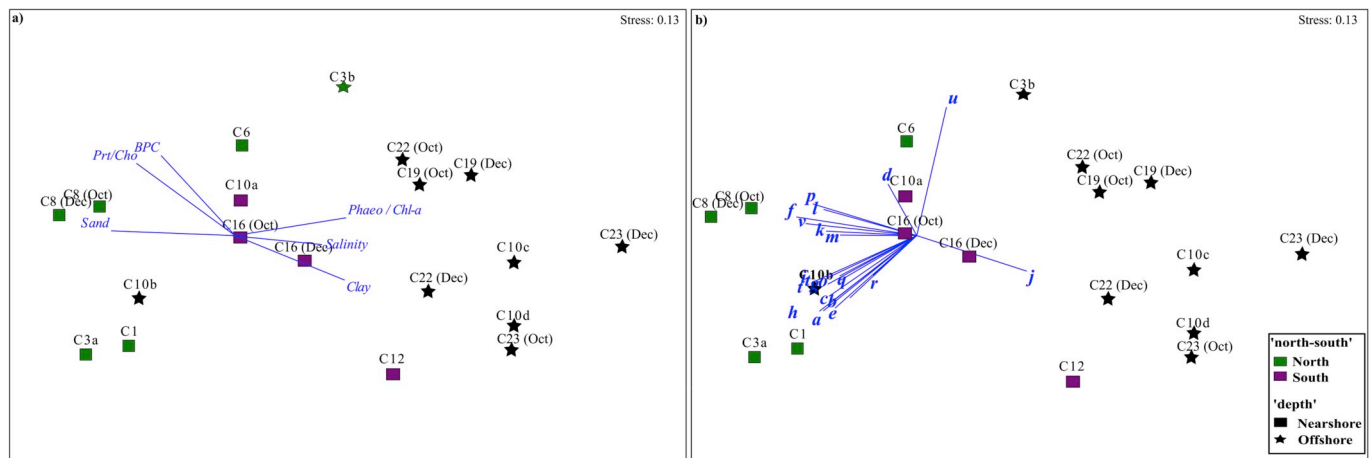
Fig. 4. Non-metric multidimensional scaling (nMDS) ordination plot of the macrofaunal community in the study area with the physical-chemical parameters a). Species with high cumulative percentage by SIMPER analysis (cut off 60%) are represented b) and denoted by a letter: a = *Abra alba*; b = *Acmira assimilis*; c = *Acrocnida branchiata*; d = *Ampelisca intermedia*; e = *Chamelea gallina*; f = *Corbula gibba*; g = *Dosina lupinius*; h = *Heteromastus filiformis*; i = *Kurtiella bidentata*; j = *Labioleanira yhleni*; k = *Moerella distorta*; l = *Nepthys hystricis*; m = *Nereis lamellosa*; n = *Notomastus aberans*; o = *Nucula nucleus*; p = *Owenia fusiformis*; q = *Paphia aurea*; r = *Peronida albicans*; s = *Pseudoleiocapitella fauveli*; t = *Spisula subtruncata*; u = *Sternaspis scutata*; v = *Striarca lactea*. Transects are indicated in different colours and 'nearshore or offshore' position in different shapes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2011). The C:N ratio (>10) was remarkably higher at the northern stations (C6, C8), suggesting a continental/plant origin of the organic matter rather than a marine one (Rumolo et al., 2011; Miserocchi et al., 2007). The dissimilarity between nearshore *vs* offshore stations was likely ascribable to different hydrodynamics and the grain-size spatial pattern, suggesting the presence of two diverse sedimentary matrices. Although mud (silt + clay) content prevailed at all stations, the percentage of sand was higher at the shallower stations and those located nearby the Po Pila mouth (the main distributary).

4.2. Macrofaunal community structure and spatial distribution in prodelta area

The benthic community structure of the Po River prodelta is typical of estuarine environments in congruence with studies worldwide (e.g. Hermand et al., 2008; Occhipinti-Ambrogi et al., 2005; Thrush et al., 2004). The high variability and the strong gradient of the environmental features may differently affect the spatial distribution of macrofaunal invertebrate species in the prodelta area. In fact, the sampling design was conceived to assess the influence of the river delta channels on the macrozoobenthic community features at increasing distance from the main distributary mouth (Po di Pila) along the southward river plume. As highlighted by PERMANOVA main–test, significant differences were found in macrofaunal community structure (i.e. species composition) between northern *vs* southern, and nearshore *vs* offshore stations, confirming the high spatial heterogeneity in this area. At a large scale, this difference is mainly due to the grain size distribution pattern, where sand and clay were the main drivers (by DistLM analysis). According to previous studies (Ambrogi et al., 1990; N'Siala et al., 2008), species such as the bivalves *Peronidia albicans, Spisula subtruncata, Chamelea gallina* and the polychaete *Owenia fusiformis* were found to be dominant in the area near the main distributary mouth (i.e. northern stations). These species are commonly observed in muddy-sand sediments and belong to the SFBC bioconeosis ("Sable Fins Bien Calibré", Pérès and Picard, 1964). In particular, *O. fusiformis* is typical of estuarine environments (Ambrogi et al., 1995; Dauvin, 1992; Pinedo et al., 2000), and is found especially nearby the riverine mouths. The synergic effect of higher hydrodynamics, led the deposition of sandy particles favouring the proliferation of this species. Conversely, at the southern and offshore stations, species inhabiting sediments with small grain-size particles (i.e. the polychaete *Sternaspis scutata,* the gastropod *Turritella communis* and

the echinoderm *Oestergrenia digitata*) were observed. These species are common in estuarine/delta areas, as they typically belong to the Mediterranean VTC ("Vaseux Terrigène Côtière", biocoenosis of terrigenous mud), above all *S. scutata*, commonly observed in sediments influenced by river-derived deposition (Hermand et al., 2008 and references therein).

At a smaller scale, other factors such as the quantity and quality of food supply are known to influence the macroinvertebrate distribution (Thrush et al., 2004). According to DIstLM model and nMDS ordination (see Fig. 4), species in northern-shallower sites were clearly distinguished from those inhabiting the offshore and southern ones, and the main drivers of this difference were BPC, Prt/Cho and Phaeo/Chl-*a* ratios. Nearby the main river mouth, the macrofaunal community was influenced by the supply of high-quality food, as suggested by the higher contribution of proteins compared to carbohydrates (Dell'Anno et al., 2002). In contrast, the organic matter at the southern deeper stations was more aged (lower Prt/Cho ratios). The high ratio of Phaeo/Chl-*a* could indicate more degraded organic matter that is less palatable for macrofaunal invertebrates (Bongiorni et al., 2018). Therefore, our results suggest that the lower species numbers and diversity values at southern stations, indicating a less structured community, could be related to a lesser quality of organic matter.

The high variations in biodiversity and species composition observed at stations located nearby the Po River mouth were likely due to the variability of terrigenous-river inputs. In fact, in our study area, the different river loads are important factors in the synecology of species. Braga et al., 2017 by using a Landsat 8 imagery, and Maicu et al. (2018), by applying an unstructured 3D numerical model revealed that the northern part of Po delta, in particular the Pila mouth and the central area (i.e. C6 and C8), are characterized by intermittent freshwater riverine pulses. In these water masses, different current velocities and turbidity levels are established that are strictly dependent on the magnitude of the flood event, but are usually highest in front of main mouth and lowest few km southward. In fact, in spite of a relatively short distance (i.e. 1 km) between the nearshore C3a and the offshore C3b, we observed remarkable differences in Shannon Wiener's diversity index and overall species composition (see Table 4 and Fig. 4). Station C3a was characterized by high macrofaunal density, in particular of the polychaete *Heteromastus filiformis* (>1000 ind. m^{-2}). Our result agrees with those of Selen-Picard et al. (2003) and Hermand et al. (2008), who related this taxon to high and continuous fresh organic matter
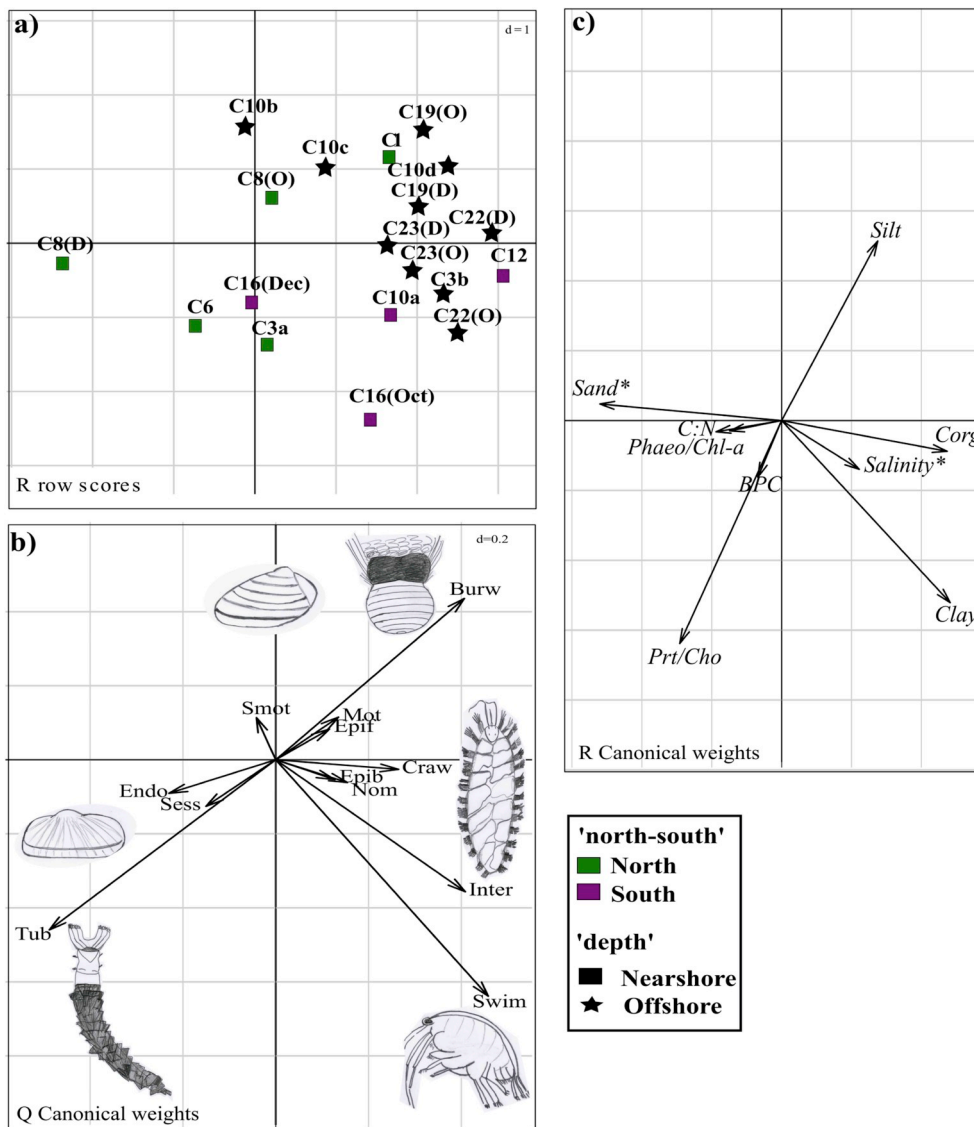
Fig. 5. RLQ diagram as defined by two first axes with a projection of sampling sites a), *response* trait categories b) and, environmental variables c). The d value in the upper right of the corner is the scale of each graph. Transects are indicated in different colours and 'nearshore or offshore' position in different shapes. Abiotic variables with asterisk explained the best variability (DisTLM analysis) in species and traits composition. See Table 2 for complete trait labels. The drawn species, that principally drive the traits occurrences (from top to bottom) are: *Sternaspis scutata, Labioleanira yhleni, Ampelisca intermedia, Owenia fusiformis, Striarca lactea* and *Nucula nucleus*. Prt/Cho (protein to carbon ratio); Phaeo/Cha-*a* (phaeopigments to chlorophyll-*a* ratio). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

deposition. Conversely, at C3b we observed a high dominance of a few species, especially *S. scutata*. This taxon reaches high density in fine sediments not subjected to grain-size variation, and influenced by lower but continuous deposition of river-derived material (Touzani and Giresse, 2002). Furthermore, at stations in the central southern part of the prodelta, we observed the simultaneous high abundance of sandy species (i.e. *P. albicans, S. subtruncata, C. gallina* and *O. fusiformis*), muddy species (i.e. *Abra alba* and *Nucula nucleus*) and a large dominance of the bivalve *Corbula gibba* (see Fig. 4b). The latter is widespread in soft bottoms of the northern Adriatic Sea and is commonly related to environmental instability. Our result agrees with Aleffi and Bettoso (2000) and Brenko (2006), who found high density of *C. gibba* at shallower stations of the delta Po River coastal area characterized by inconstant sediment deposition. In fact, this area is influenced by high organic loads from river distributaries and the lower deposition of fine suspended material from the southernmost Po di Pila plume (Tesi et al., 2011; Maicu et al., 2018). In addition, a different spatial pattern in community composition was highlighted between nearshore *vs* offshore stations (by PERMANOVA test). On one hand, organisms at stations nearby the coastline are subjected to an intermittent stress induced by flood events of different magnitude, leading to communities that are highly adapted to this kind of environmental stress. On the other hand, at deeper stations we observed a more stable community typical of areas influenced

by lower and continuous river-driven material deposition (Selen-Picard et al., 2003; Tesi et al., 2011).

4.3. Functional characteristics

The functional diversity did not show any relation with biodiversity, except for stations where a few species were dominant. In fact, the low values of H′ and FDiv, observed in front of the Pila mouth (C3b and C8 in December) and in the southern part of the prodelta (C19 in December), were due to the high density of polychaetes *Sternaspis scutata* (at C3b and C19) and *Owenia fusiformis* (in C6 and C8). The continuous river-plume deposition might have influenced the stability of the community at these stations, where a few species likely performed few biological functions. On the contrary, we obtained the highest value of FDiv at the shallower stations nearby the main distributary (i.e. C3a). The high functional diversity combined with a low H′ value at C3a indicates the presence of functional redundancy in this particular site meaning that several species perform the same functions and therefore the community might be less susceptible to changes in ecosystem functions caused by species loss (Gladstone-Gallagher et al., 2019). Furthermore, we observed a great variability of FDiv/H' at the three stations located in front of the Po di Pila compared to the southern ones (Table 4). In accordance with previous studies (i.e. Leung, 2015), the habitat heterogeneity at the main Po
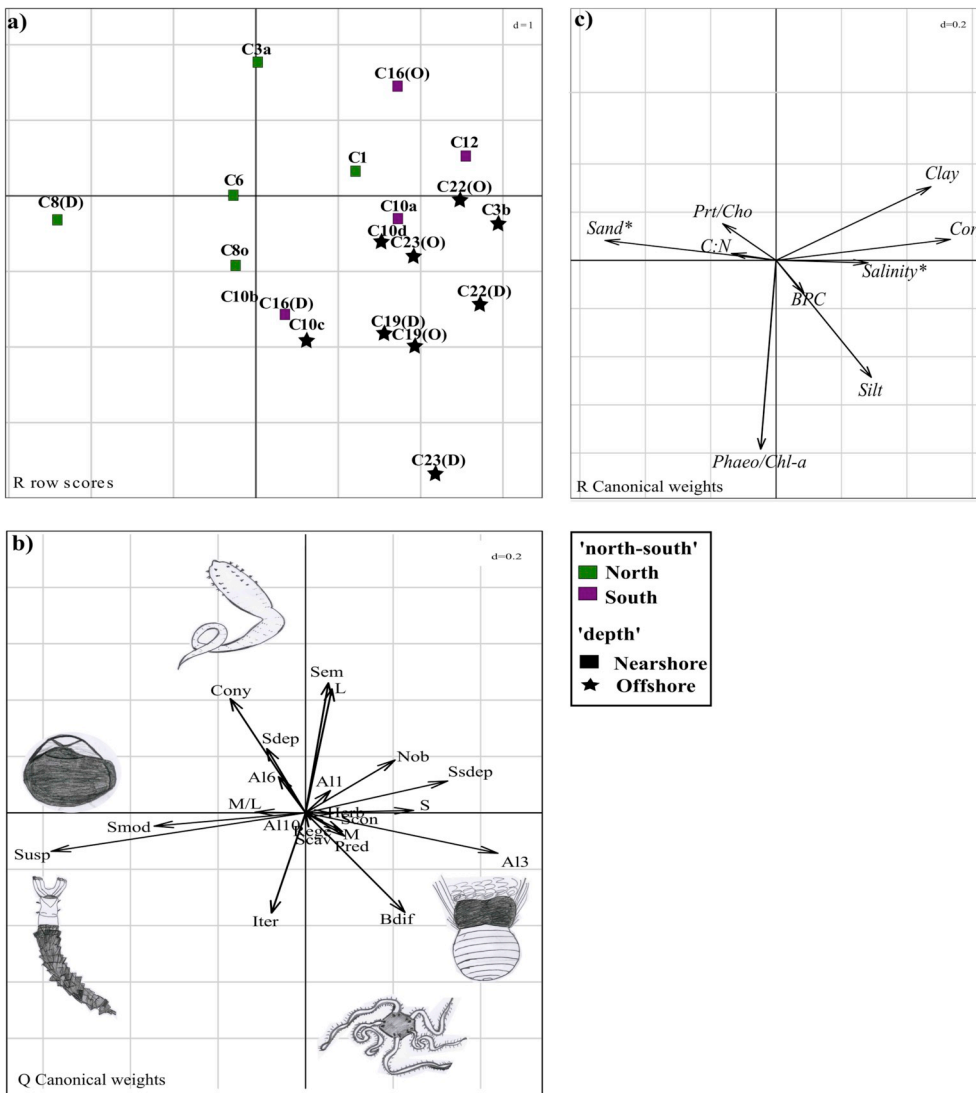
Fig. 6. RLQ diagram as defined by two first axes with a projection of sampling sites a), *effect* trait categories b) and, environmental variables c). The d value in the upper right of the corner is the scale of each graph. Transects are indicated in different colours and 'nearshore or offshore' position in different shapes. Abiotic variables with asterisk explained the best variability (DisTLM analysis) in species and traits composition. Transects are indicated in different colours and 'nearshore or offshore' position in different shapes. See Table 2 for complete trait labels. The drawn species, that principally drive the traits-occurrences (from top to bottom) are: *Heteromastus filiformis, Sternaspis scutata, Amphiura chiajei, Owenia fusiformis, Corbula gibba*. Prt/Cho (protein to carbon ratio); Phaeo/Cha-*a* (phaeopigments to chlorophyll-*a* ratio). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

River mouth could have affected the functional diversity at this site. The station dominated by *S. scutata* displayed lower FDiv and low functional redundancy, indicating a minor overlapping of functional niches among species, and thus this site was likely more susceptible to species loss than other habitats. The high functional diversity and redundancy obtained at C1 and C3a, and C10a as well, could be related to the high efficiency of invertebrates to utilize available resources, principally of river origin (Bongiorni et al., 2018) to perform their functions, thus shaping the ecosystem properties.

Regarding the functional identity, similarly to macrofaunal structural analysis, significant difference between northern *vs* southern coastal stations, and between nearshore *vs* offshore ones were observed. Since the BTA is based on species composition, some similarities between the traits occurrences and species composition were expected. However, Nasi et al. (2018) did not find any spatial difference in trait composition among sampling sites, in spite of the high variability in taxa composition. In the Po River prodelta, the significant spatial differences in taxa and functional expression further confirmed the high heterogeneity of this dynamic system.

Focusing on *response* traits, we observed a clear influence of high river loads on coastal macrofaunal community (see Fig. 5a). Following a diverse grain-size distribution pattern along the prodelta coastline, at northern sites, we detected a dominance of *sessile* and *tube-builder* due to the major abundance of *O. fusiformis* present especially at C8. This

species is strictly dependent on the grain-size distribution, since to construct its tube it needs fine sand with a certain percentage of silty-clay material (>5%) (Pinedo et al., 2000). In addition, *sessile* invertebrates are not able to live in areas influenced by continuous deposition of sediment particles (Thrush et al., 2004). Indeed, we noticed high occurrence of *sessile* invertebrates, in particular endofauna bivalves, such as *Striarca lactea* at stations characterized by low turbidity and higher surface current velocities (Braga et al., 2017). Conversely, at stations characterized by finer sediments (>70% of silt), *burrower* invertebrates dominated. *Burrower* species are very common in muddy sediments of coastal areas (Gogina et al., 2017; Nasi et al., 2020), since they are able to penetrate from 10 to 30 cm into sediments layers (Kristensen et al., 2012). In addition, we observed high occurrences of *interface*, *swimmer* and *crawler* modalities toward the southern stations and offshore ones, where increasing values of clay fraction and salinity were observed. The low river influence at these stations might have improved the sediment status and therefore the ability to live at the water-sediment interface.

Regarding the *effect* traits and their potential shaping of ecosystem properties of delta area, we evidenced a clear spatial pattern comparing northern *vs* southern stations, and nearshore *vs* offshore ones. Our results further revealed that the grain-size together with the quality of organic matter (i.e. Prt/Cho and Phaeo/Chal-*a*) were highly related with *effect* traits occurrences (see Fig. 6c). In the prodelta area, we observed a

shift from *conveyor, surface deposit feeder* modalities at northern and shallower stations in front of the Pila mouth, to *superficial modifier* and *suspension feeder* invertebrates at stations located in the central part of the delta system, and the occurrences of *biodiffuser* and *subsurface deposit feeder* categories at offshore stations. The modalities *surface deposit feeders* and *conveyors* at these stations were principally due to the highest abundance of the polychaete *Heteromastus filiformis*. This invertebrate is usually dominant in sandy-mud and, in particular, where high deposition of organic matter occurs (Hermand et al., 2008; Marchini et al., 2008), mostly displaying an opportunistic behaviour with fast growth, early maturity and high fecundity (Méndez et al., 2000). In particular, *semi-continuous* and *semelparous* trait-categories, highly dominant at stations in front of the Pila mouth, are features of short-lived invertebrates that increase in abundance when a fresh food supply is available (higher Prt/Cho), rapidly stimulating the growth, reproduction rate and survivorship of the species having these functional features (Cruz-Rivera and Hay, 2000; Gray and Elliott, 2009; Quijón et al., 2008). In addition, being *conveyor* organisms, they are able to feed on sinking particles from the water column, move sediment grains through their gut by ingestion and secretion, transfer particles from deeper to superficial layers and vice-versa, thus enhancing the organic matter remineralization (Kristensen et al., 2012).

The continuous and high load of terrigenous material from a river is known to affect suspension-feeding animals by clogging feeding structures, interfering with particles selection, and require the use of energy to clear away unvented particles (Thrush et al., 2004). In fact, in the central part of the Po delta system, where high current velocities and low turbidity were reported (Braga et al., 2017; Maicu et al., 2018), we observed a high dominance of *suspension feeder* on the account of *O. fusiformis* and the bivalve *Corbula gibba*. The sediment features of these stations (i.e. higher percentage of sand and Prt/Cho) coupled with intermittent freshwater riverine pulses might have led to the proliferation of these two species able to amplify the benthic-pelagic coupling. In fact, *suspension feeders* can capture large quantities of suspended organic matter and phytoplankton, and then incorporate them into the sediments through pseudo-faeces, stimulating the microbial loop (Törnroos and Bonsdorff, 2012).

Offshore stations were characterized by high expression of modalities *biodiffuser* and *deposit feeders,* on the account of *S. scutata* and the echinoderm *Amphiura chiajei*. *Biodiffusers* are dominant in muddy sediments since they are able to constantly and randomly biomix (both horizontally and vertically) the local sediments over a short distance, which results in particles transport (Queirós et al., 2015). Further, the presence of aged organic matter (high Phaeo/Chl-*a*) at southern stations did not prevent their survivorship. In fact, these animals, in particular polychaetes, are considered non-selective deposit feeders (Lopez and Levinton, 1987) able to feed on both fresh and aged organic matter, promoting nutrient cycling within sediment layers (Törnroos and Bonsdorff, 2012). Commonly, *S. scutata* is a deep burrowing subsurface deposit feeder living below 4 cm of depth and is able to use the food resource provided by river floods later on and over a longer period of time (Selen-Picard et al., 2003).

4.4. Structural and functional macrofaunal features in relation to contaminants

In coastal sediments of the Po delta system, we did not find any significant spatial pattern in most of the contaminants. The higher concentration of Cr and Ni detected along the Po River prodelta coastline is known to be due to leaching of sedimentary ophiolite complexes, which emerge in the Western Alps and some areas of the Italian Apennine. The elevated Cr and Ni backgrounds are therefore a geogenic character of the Po River alluvial sediments and likely unrelated to anthropogenic contamination (Bianchini et al., 2013; Lopes-Rocha et al., 2017). We observed higher concentrations of Hg, Pb, As and PAHs, at increasing distance from the main Po River mouth, confirming the high

amount of river-driven material deposition (principally of fine sediment particles) towards the southern and offshore area of the prodelta (Palinkas and Nittrouer, 2007; Tesi et al., 2011). This finding was previously reported in other studies (Readman et al., 2002; Silva et al., 2004; Yao et al., 2015) in which a clear spatial pattern in grain-size and contaminants distribution was found. In fact, heavy metals and PAHs tend to be adsorbed on suspended particles and their concentration generally increase as particle size decreases.

The co-variation of sedimentary variables with contaminants has emerged in other studies aimed to detect the effect of pollution or other anthropogenic disturbances on benthic assemblages (e.g. Egres et al., 2019). Disentangling the effect of contamination from other physical-chemical factors is challenging since the taxonomic and functional features of benthic assemblages are largely driven by sediment characteristics (Bolam et al., 2016; Breine et al., 2018; Oug et al., 2012). This limits our ability to distinguish responses of benthic communities to anthropogenic disturbances from those linked to natural variability. However, in our study area we observed low values of the considered contaminants, if compared with those measured in other estuarine areas (Pilò et al., 2016), coastal basins (Nasi et al., 2018) and harbours (Cibic et al., 2017; Trannum et al., 2004). Therefore, we can assume that the macrofaunal community of the Po delta system is not severely affected by contamination. Further, estuarine/delta areas are concomitantly subjected to stress of natural and anthropogenic origin. According to the Estuarine Quality Paradox, *sensu* Dauvin (2007), in naturally stressed estuarine ecosystems it is difficult to detect anthropogenic stress. The estuarine benthic communities that are well adapted to natural environmental instability display similar responses to those suffering from human-induced stress.

5. Conclusion

This study represents the first attempt to investigate the structural and functional aspects of the macrofaunal community in the Po River coastal area. Our results highlighted a clear dissimilarity in community structure and species composition among sites differently impacted by river-derived terrigenous material. Changes in community structure were due to the dominance of few species. The polychaetes *Heteromastus filiformis* was dominant at shallower stations characterized by high and continuous terrigenous depositions, whereas *Owenia fusiformis* and the bivalve *Corbula gibba*, indicators of environmental instability, were found in sediments influenced by intermittent river loads. At the offshore and southern muddy stations, high abundances of the polychaete *Sternaspis scutata*, influenced by lower but continuous deposition of river-derived material, were observed.

In addition, different river-plume depositions remarkably influenced the effect traits of the macrofaunal community whereas no clear spatial pattern was observed in response traits. However, as response traits, *sessile* and *tube-builder* invertebrates were noticed at stations characterized by high surface current velocities but low turbidity. On the contrary, a major expression of *burrowers*, *interface crawlers* and *swimmers* occurred at southern and principally offshore muddy stations. For *effect* traits, *suspension* and *surface deposit feeders* as well as *conveyors* modalities dominated in sandier sediments in the northern part of the prodelta, whereas *subsurface deposit feeders* and *biodiffusers* prevailed in the southern part, characterized by lower but continuous deposition of finer particles. In highly dynamic systems, as delta areas, the presence of *conveyor* and *biodiffuser* invertebrates able to exploit different resources through bioturbation activities may enhance the functions of riverine coastal ecosystems.

Our findings indicate that by combining the classic taxonomic approach, which considers the structural characteristics of the macrofaunal community, with a novel approach, based on the functional attributes of these organisms, more insight in the functioning of highly dynamic systems, such as delta areas, could be gained. The deepening of the knowledge on macrofaunal structural and functional attributes

might be integrated in marine monitoring programs for a better management of coastal ecosystems in particular of dynamic environments such as delta areas.

Declaration of competing interest

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

CRediT authorship contribution statement

Federica Nasi: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. **Rocco Auriemma:** Methodology, Formal analysis, Investigation, Writing - original draft. **Federica Relitti:** Formal analysis, Investigation, Resources. **Matteo Bazzaro:** Investigation, Resources. **Daniele Cassin:** Methodology, Investigation, Resources. **Tamara Cibic:** Writing - review & editing, Supervision, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2019.106548.

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