

# Macrofaunal bioturbation attributes in relation to riverine influence: What can we learn from the Po River lagoonal system (Adriatic Sea)?

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## ABSTRACT

Delta areas are highly dynamic transitional areas, important for burial and decomposition of terrestrial and marine organic matter (OM). In these environments, macrofaunal organisms are influenced by fluctuations of fresh OM inputs that modify several aspects of their growth, reproduction and behaviour. Macrofaunal bioturbation activities are significant processes performed by individuals, which directly influence the biogeochemical cycling and thus the ecosystem functioning. To assess the influence of terrigenous/freshwater OM on bioturbation attributes of macrofauna in a lagoonal system, we integrated the bioturbation and irrigation potential (BP<sub>c</sub> and IP<sub>c</sub>) community indices and functional traits linked to reworking and ventilation of sediments (using Biological Traits Analysis-BTA). The macrofaunal community was investigated in four lagoons (Caleri, Marinetta-Vallona, Canarin and Scardovari) of the Po River Delta in Italy (northern Adriatic Sea). We examined the macrofaunal biodiversity and bioturbation attributes in relation to grain-size distribution, carbon and nitrogen ratio (C:N), carbon and nitrogen stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ), and the radionuclides  $^{137}\text{Cs}$  and  $^7\text{Be}$ . The latter were used as proxies of the age of terrigenous/riverine-derived OM. The lowest values of BP<sub>c</sub> and IP<sub>c</sub> that corresponded to minimum values of benthic biodiversity were observed at the inner sampling stations of Scardovari. Here, low diversity values were likely ascribed to recent hypoxic/anoxic conditions exacerbated by the low water renewal that characterizes this lagoon, influencing the bioturbation processes. Grain-size fractions were significant drivers of the differences in BP<sub>c</sub> and IP<sub>c</sub>, and the major number of taxa in coarse sediments contributed to the highest values of bioturbation indices. A significant difference in IP<sub>c</sub> between sandy and muddy stations was due to the presence of *subsurface deposit feeders* with high burrowing depths and *blind-ended burrows* that enhanced the bio-irrigation in coarse sediments. We observed diverse spatial patterns of trait-categories belonging to sediment reworking activity at stations differently influenced by freshwater OM. *Semi-motile* and *conveyor* invertebrates, that are able to move sediment particles through their gut by ingestion and secretion, were dominant nearby the lagoon mouths where OM of freshwater origin was present. High occurrence of *motile* and *biodiffuser* invertebrates, that can randomly mix sediments layers, were found at stations characterized by old OM. Macrofaunal sediment reworking and ventilation processes are of paramount importance in the framework of an efficient management and sustainable use of lagoons, often exposed to anoxic and dystrophic events, that are strongly exploited for aquaculture.

## 1. Introduction

Marine transitional environments, represented mainly by estuaries, deltas and lagoons (Tagliapietra et al., 2009), are among the most dynamic and complex natural ecosystems in the world. They are ecotonal environments regulated by interactions between riverine inflows and tidal currents and characterized by wide seasonal and daily variability of

chemical-physical parameters (Elliott and McLusky, 2002). Therefore, these environments are frequently described as highly productive, due to high abundance and diversity of primary producers influenced by high inputs of organic matter (OM) transported by rivers (Nixon, 1986; Bongiorno et al., 2018). The mobilization and quality of OM, as well as the magnitude of its input within the estuary, are highly dependent upon the intensity and frequency of river discharge (Bonifacio et al., 2014).

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This terrestrial OM may be an important food source for deltaic/estuarine consumers as it rapidly accumulates on the riverbed and along flood plains of the alluvial system, supplying large amounts of allochthonous material (Antonio et al., 2010).

In estuarine and delta systems, benthic macrofaunal communities are principally influenced by fluctuations in organic inputs (Johnson et al., 2007; Viaroli et al., 2008). The arrival of fresh OM to the sea floor has been shown to modify several aspects of growth, reproduction and behaviour (e.g. feeding strategies, bioturbation modes) of benthic invertebrates (Quijón et al., 2008). Among the characteristics and ecologically important macrofaunal features, bioturbation activities are significant processes that occur in marine and lagoonal system (Boudreau, 1998). Bioturbation is defined as biogenic activities that cause sediment reworking including burrow and mound accumulation, particles ingestion/digestion during foraging, and water transport in and out of the sediment matrix (e.g. ventilation and burrow flushing) (Meysman et al., 2006; Kristensen et al., 2012). Sediment particles reworking and water ventilation through burrows (i.e. bioturbation activities) modify primary production, benthic-pelagic coupling, microbial activity and composition, metal cycling, redox conditions of sediments, grain-size, and carbon and nitrogen cycling (Na et al., 2008; Queirós et al., 2015; Valdemarsen et al., 2018). In fact, since these processes are supported by invertebrates' bioturbation activities, macrofaunal communities have been considered key biological components in soft sediments (Loreau et al., 2002; Naem and Wright, 2003).

Experimental studies that examined bioturbation processes were based on several tracer techniques, including the use of phaeopigment concentration profiles, radionuclides (Bentley and Nittrouer, 2003) and inert fluorescent sediment tracers (i.e. luminophores) (Gerino, 1998; Solan et al., 2004b). The latter techniques were used to quantify the vertical particles transport and to estimate the biodiffusion coefficient of single species (Dupont et al., 2006), and of multi-species communities (Dupont et al., 2007). Solan et al. (2004a), using photographic images of sediment profiles, performed parameterized models that predict how species extinction is likely to affect the biogenic mixing depth (BMD), an indicator of bioturbation. In addition, to estimate species contributions to the BMD, a new community bioturbation potential index ( $BP_c$ ) was proposed. This index combines structural characteristics of the investigated community with biological features such as functional traits, by considering each species' body size, abundance, degree of mobility and mode of sediment mixing (the single species' values are named individual bioturbation potential- $BP_i$ ). Overall, the macrofaunal bioturbation processes and their environmental modifications can be addressed not only through biogeochemical analyses but also considering ecological aspects of the species. The bioturbation intensity, estimated by Queirós et al. (2015) as the bioturbation distance (i.e. the average distance travelled by a particle in a random-walk bioturbation model) was related with the metric of community Bioturbation Potential ( $BP_c$ ). Therefore,  $BP_c$  could be considered as a proxy for the environmental biogenic modification capacity of a benthic community (Solan et al., 2004a; Queirós et al., 2015; Gogina et al., 2017).

The irrigation of sediments induced by organisms is another important aspect that influences the biogeochemical processes at the sea bottom. Bioirrigation is mostly driven by burrow-dwelling animals able to ventilate the sediments, creating a rapid exchange of water between the overlying water and subsurface sediment (Kristensen et al., 2012). This process is mostly driven by ventilation rates and suspension deposit feeding activities of benthic species (Aller, 1982). Consequently, the bioirrigation is primarily dependent on body mass and feeding type (Christensen et al., 2000). Since in the calculation of community bioturbation potential ( $BP_c$ ), the mobility trait likely underestimates the contribution of sessile species with low mobility but high bioirrigation efficiency, Wrede and co-authors (2018) modified the  $BP_c$  proposed by Solan et al. (2004a) into a new index, named community irrigation potential ( $IP_c$ ). In this index, Wrede et al. (2018) replaced the mobility and reworking traits with the functional characteristics of bioirrigation:

burrow and feeding types and injection pocket depth of burrows.

$BP_c$  has been successfully implemented in several studies and correlated with sedimentary processes such as biogeochemical cycling and sediment turnover (Foshtomi et al., 2015; Queirós et al., 2015; Wrede et al., 2017); alkalinity fluxes (Braeckman et al., 2014) and hypoxia events (Van Colen et al., 2012; Villnäs et al., 2012). Other studies underlined the spatial and temporal variability pattern in macrofaunal bioturbation activities, in particular related to species richness (Baldrighi et al., 2017; Brine et al., 2018), identification of key species contributing to bioturbation (Morys et al., 2017) and it was also used for modelling and mapping spatial differences of ecosystem functioning (Gogina et al., 2017). So far, the  $BP_c$  index has been usefully applied in many marine studies, whereas the new  $IP_c$  index has been less adopted in ecological surveys (Renz et al., 2018).

Natural and artificial short-lived radionuclides, i.e.  $^{137}\text{Cs}$  and  $^7\text{Be}$ , have been widely used in estuarine, coastal and marine areas to evaluate sediment accumulation rates across different spatial and temporal scales in environments influenced by anthropogenic activities (Battiston et al., 1988), by riverine loads (Frignani et al., 2005; Palinkas et al., 2005), as well as to trace recent terrestrial sedimentary contribution to coastal and marine environments (Alvisi et al., 2013). Generally, flood events occur over very short time-scales (from days to weeks), so radionuclides with monthly half-lives, such as  $^7\text{Be}$  appear to be appropriate tracers of recent flooding events (Feng et al., 1999; Saari et al., 2010). Conversely, the use of  $^{137}\text{Cs}$  ( $t_{1/2} = 30.2$  years) allows us to trace riverine-derived sediment deposition at sea/delta at a longer time scale and/or reworking processes according to mass accumulation rates.

The link between macrofaunal invertebrates and their bioturbation processes, and the spatial distribution of recent/older OM has been poorly documented, especially in lagoonal system. Therefore, we focused on the Po River Delta (northern Adriatic Sea), which is considered one of the major transitional systems in Europe. This deltaic system and coastal lagoons are ecotonal environments characterized by wide seasonal and daily variability of chemical-physical parameters, e.g. current speed and direction, salinity, temperature and fluvial inputs (Marchini et al., 2008). Furthermore, they are characterised by multiple physical-chemical and biological processes favouring inorganic and organic sedimentation and bioaccumulation (Hedges and Keil, 1995). Previous studies carried out in the Po River Delta lagoons mainly focused on the macrofaunal community structure i.e. biodiversity and species composition considering different spatial and temporal scales (Casellato, 1994; Sabetta et al., 2007), in relation to anthropogenic impacts (Munari and Mistri, 2014), or as an indicator of the benthic ecological status (Munari et al., 2009; Munari and Mistri, 2010). Therefore, the functional features of macrofaunal invertebrates regarding the bioturbation modes are still poorly documented, both in this area and in lagoonal environments in general. Few studies have applied the biological traits approach (using the Biological Trait Analysis-BTA) to investigate the functional features of the macrofaunal assemblage in lagoonal systems. Some of them considered macrofaunal traits occurrences in relation to species richness (Sigala et al., 2012; Faulwetter et al., 2015), species distribution (Marchini et al., 2008), metal contamination and sewage discharge (Piló et al., 2016), and impacts by chemical compounds (Egres et al., 2019).

To the best of our knowledge, this study represents the first attempt to investigate the influence of allochthonous terrigenous/freshwater OM on bioturbation modes in coastal lagoons, in terms of  $BP_c$  and  $IP_c$  indices, and biological traits occurrences. We hypothesized that the macrofaunal community, inhabiting coastal river lagoons of the Po River Delta, responds differently in terms of bioturbation attributes to uneven amounts of riverine material. We aimed to test the following specific hypotheses: (1) The zoobenthic community structure influences bioturbation processes in different sedimentary environments; (2) Macrofaunal bioturbation attributes display both an inter-lagoonal (among lagoons) and an intra-lagoonal (among stations within the same lagoon) pattern; and (3) The bioturbation attribute patterns are driven by specific sediment

physical-chemical variables.

## 2. Methods

### 2.1. Study area and sampling design

The Po River is the main Italian fluvial system, a waterway with a length of 650 km and with an annual mean flow rate of  $\sim 1500 \text{ m}^3/\text{s}$  (Boldrin et al., 2005). Overall, autumn–winter flood peaks exceed  $10\,000 \text{ m}^3/\text{s}$ , whereas in summer low water fluxes (few hundred  $\text{m}^3/\text{s}$ ) are registered (Correggiari et al., 2005; Stefani and Vincenzi, 2005). At the south-eastern end of the Veneto region, the Po River flows into the Adriatic Sea forming a Delta system that originates 50 km inland from the coast. The river divides into seven branches, spreading the high amount of freshwater and terrigenous material along the coastline (Correggiari et al., 2005; Stefani and Vincenzi, 2005). The Po River Delta system (ca.  $178 \text{ km}^2$ ), is one of the largest wetlands in the Mediterranean, second only to the Venice lagoon (ca.  $549 \text{ km}^2$ ). The Po Delta lagoons are traditionally exploited for different types of aquaculture, mainly mollusc farming, which has become the main economic asset of the area. In particular, the clam *Ruditapes philippinarum* is being intensively farmed reaching very high production yields (Turolla, 2008; Abbiati et al., 2010). Among the lagoons of the Po River Delta system investigated within the framework of the Italian project RITMARE (la Ricerca ITaliana per il MARE – Italian Research for the Sea), four lagoons, namely Caleri, Marinetta-Vallona, Canarin and Scardovari, were chosen for this study. While Caleri and Scardovari have more marine features, Marinetta-Vallona and Canarin are subjected to higher freshwater inputs. The geographical and hydrological features of these four lagoons are exhaustively presented by Maicu et al. (2018).

The macrofaunal community was sampled in May 2016 at three stations in each lagoon following an increasing distance from the sea and a different proximity to freshwater input, in order to represent diverse environmental features inside each lagoon (CL1, CL2, CL3 in Caleri; MV5, MV6, MV7 in Marinetta-Vallona; CN10, CN11, CN12 in Canarin and SC15, SC16, SC17 in Scardovari) (Fig. 1).

At each station, three replicates of sediments were sampled using a manual box-corer (sampling area:  $225 \text{ cm}^2$ ; 15 cm of depth). The sediment samples were sieved through a 1.0 mm mesh to retain the macrofaunal invertebrates and immediately fixed with formaldehyde solution (4% v/v final concentration in seawater). After washing, organisms were separated from the sediment, divided into the main taxonomic phyla and preserved in ethanol 70°. Macrofaunal organisms were identified at the lowest possible taxonomical level (up to the species level in most cases) using a stereomicroscope (Zeiss Discovery V.12, at 8–100x magnification) and counted. Biomass of each taxon was obtained by Wet Weight (WW) measurement. Subsequently, the WW values were converted in Ash-Free Dry Weight (AFDW) and, if shell-organisms were identified, their biomass was transformed in Shells-Free Dry Weight (SFDW) before calculating the AFDW. The conversion factors proposed by Ricciardi and Bourget (1998) and Brey (2001) were used.

The environmental variables considered in this study (i.e. shells, sand, silt and clay fractions, carbon and nitrogen molar ratio C:N,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  stable isotopes) were determined on the same samples, and are thoroughly described in Cibic et al. (2019). An aliquot of the collected samples was preserved at  $+4 \text{ }^\circ\text{C}$  for radioisotope analysis.

### 2.2. Radioisotopes analysis

$^7\text{Be}$  is a natural radionuclide with a half-life of 53.3 days and it is concentrated in terrestrial soils (Palinkas et al., 2005). The presence of  $^7\text{Be}$  would reflect the trajectory of particles as they travel from the source region to the ocean. In order to have detectable  $^7\text{Be}$  in marine sediments, the material should be rapidly eroded from soils and delivered to the marine environment with a storage in the drainage basin less

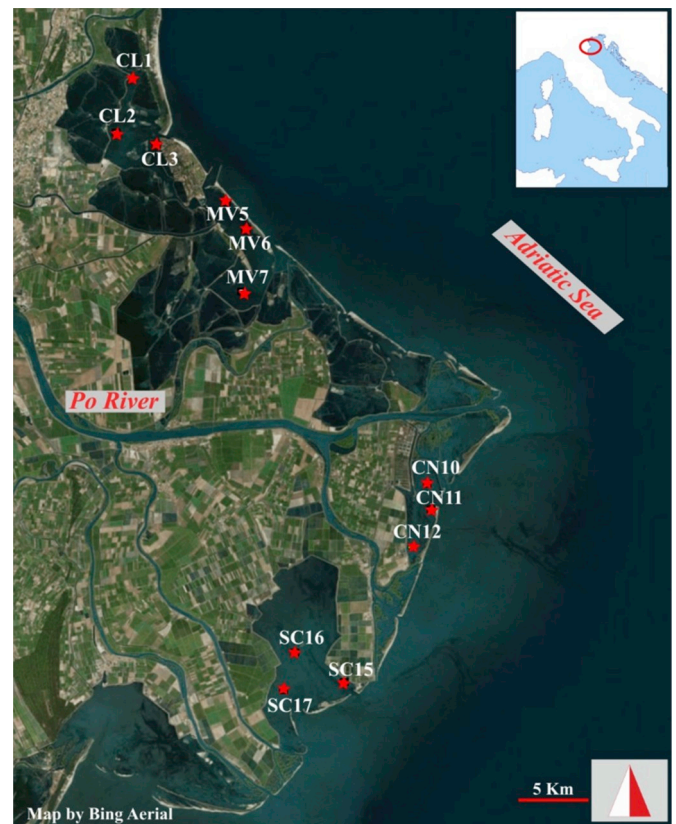


Fig. 1. Location of the sampling stations in the four lagoons (from N to S: Caleri, Marinetta-Vallona, Canarin, Scardovari) of the Po River Delta.

than 200 days, since recently eroded particles are rarely transported directly to the marine environment, but arrive after a series of depositional and erosional events occurring within the river channel and floodplains (Palinkas et al., 2005). The contemporary presence of  $^{137}\text{Cs}$  in the soils of the Po Plain as a result of the Chernobyl accident in 1986 (Frignani et al., 2004), allows us also to use this radioisotope to trace the deposition of riverine-derived sedimentary material at sea/delta at a decadal time scale. Moreover, since the  $^{137}\text{Cs}$  distribution in sediments also depends also on mixing and diffusion, we also wanted to test it as a tracer of these processes in the investigated lagoons.

Radioisotope analysis was performed on an aliquot of the same sediment samples (i.e. 0–1 cm surface layer) taken for the other considered environmental variables. They were dried at  $55 \text{ }^\circ\text{C}$ , and then slightly disaggregated for the analysis of radiotracers by pressing it in plastic jars in order to obtain a standard geometry (10 ml). The radioisotope abundances were measured using the peak at 661.7 keV and 477 keV for  $^{137}\text{Cs}$  and  $^7\text{Be}$ , respectively, and counted via gamma spectrometry using co-axial intrinsic germanium detectors (Ortec HPGe GMX-20195P and GEM-20200). By means of the GAMMAPLUS software, the obtained spectra were analysed and the radiotracer activity was calculated. Data from stations with a confidence level of the minimum detection activity (MDA) equal to 95% were considered as reliable, whereas data from stations below this limit were considered not reliable (i.e. not detectable = n.d.). Measurement errors were in range of 0.9–3.2% for  $^{137}\text{Cs}$  and 9–45% for  $^7\text{Be}$ .

### 2.3. Bioturbation potential (BP) and irrigation potential (IP)

The bioturbation potential (BP) introduced by Solan et al. (2004a) was calculated as follows:

$$BP_i = (B_i/A_i)^{0.5} * A_i * M_i * R_i, \text{ whereas } BP_c = \sum_{i=1}^n BP_i$$



where  $A_i$  is the number of individuals per  $m^2$ ;  $B_i$  is the biomass (i.e. AFDW) of individuals in grams per  $m^2$ ;  $M_i$  and  $R_i$  are species categorical scores that reflect increasing mobility ( $M_i$ ) and increasing sediment reworking ( $R_i$ ). Calculations were conducted for individual taxa ( $BP_i$ ) and for the whole community ( $BP_c$ ). In this study, information on mobility and reworking mode was coded following Queirós et al. (2013) and Gogina et al. (2017). For a few species it was not possible to retrieve this information from the literature, therefore scores were assigned based on expert knowledge (see Table S1 for species categorical scores of  $M_i$  and  $R_i$ ). Sedentary epifauna species not involved in sediment transport (those scored with  $M_i$  and  $R_i$  both equal to 1) were excluded from the calculation.

The irrigation potential (IP) was introduced for the first time by Wrede et al. (2018). The taxa ( $IP_i$ ) and the community ( $IP_c$ ) irrigation potential were calculated as follows:

$$IP_i = (B_i/A_i)^{0.75} * A_i * BT_i * FT_i * ID_i \text{ and } IP_c = \sum_{i=1}^n IP_i$$

where  $B_i$  is the body weight ( $g\ m^{-2}$ ) while  $A_i$  its abundance ( $m^{-2}$ ). The chosen traits that characterize the irrigation behaviour of macrozoobenthic organisms and their effects on the ecosystem functioning were: burrow type ( $BT_i$ ), feeding type ( $FT_i$ ), and injection pocket depth ( $ID_i$ ). These traits were subdivided into categories that describe the species-specific occurrence of the respective trait. To each category, a descriptive numerical score was assigned (Wrede et al., 2018). Since in the study area the sub-suspension/funnel habit was lacking, whereas the deposit feeding one was predominant, we introduced a little adjustment. The calculation was modified by dividing deposit feeder habit in surface and subsurface deposit feeder modalities, with different categorical scores (see Table S1 for taxa categorical scores of  $BT_i$ ,  $FT_i$ ,  $ID_i$ ). Information on  $BT_i$  modes were obtained from the literature (Kristensen et al., 2012; Renz et al., 2018; de-la-Ossa-Carretero et al., 2012). For  $FT_i$  type we follow the authors Jumars et al. (2015) and the databases: [www.marlin.ac.uk/biotic](http://www.marlin.ac.uk/biotic); [www.polytraits.lifewatchgreece.eu](http://www.polytraits.lifewatchgreece.eu). In particular, information on  $ID_i$  the following references were used: Morys et al. (2017); Atkinson et al. (1998); Levin et al. (1997).

## 2.4. Functional traits (BTA)

To investigate the effect of terrigenous/riverine OM on macrofaunal bioturbation activities in the Po River Delta lagoonal system, we integrated the Bioturbation potential ( $BP_c$ ) and Irrigation potential ( $IP_c$ ) community index with the functional identity information on macrofaunal community, derived from the biological traits analysis (BTA). By combining the BP and IP calculations with the BTA analysis, it was possible to gain more information on macrofaunal bioturbation attributes. The biological traits considered in this study (4 traits, 16 categories) were based on (i) the invertebrates' potential relationship with different OM inputs and loads, and (ii) their role in bioturbation (i.e. mobility and sediment reworking) and bioirrigation (feeding habits and sediment ventilation) processes (Table 1).

The macrofaunal taxa were coded for categories of each trait using a "fuzzy-coding" procedure (see Table S2 for taxa scores). The latter 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. The fuzzy coding procedure (applied in this BTA), allows taxa to exhibit trait-categories to different degrees and to consider intraspecific variations in traits occurrences (Bremner et al., 2003; Chevenet et al., 1994). This can give more insight into information on bioturbation attributes that may be considered from different points of view. In fact, the sole use of categorical scores of  $BP_c$  and  $IP_c$  indices could lead to possible misjudgements and might not include the potential effects of species' behavioural changes along environmental gradients. For this reason, we considered the variation in traits-occurrences among taxa and the different degree

**Table 1**

Biological traits with their categories characterizing multiple aspects of bioturbation in relation to terrigenous/riverine input of organic matter for the investigated taxa. In the text abbreviations are used.

Traits	Categories	Code
Feeding strategy	Suspension feeder	Susp
	Surface deposit feeder	Sdep
	Subsurface deposit feeder	Ssdep
	Herbivore	Herb
	Carnivore	Car
Mobility	Sessile	Sess
	Semi-motile	Smot
	Motile	Mot
Reworking	No-reworking	Non
	Superficial modifier	Smod
	Biodiffuser	Bdif
	Conveyor	Cnvy
	Regenerator	Regr
Ventilation	No-burrow	Nbrw
	Blind-ended burrow	Blnb
	Open-ended burrow	Opnb

of affinity for certain traits, integrating the  $BP_c$  and  $IP_c$  calculations with the functional identity analysis.

Traits for each taxon were obtained from literature sources (i.e. Ruppert et al., 2004; Queirós et al., 2013; Jumars et al., 2015; Gogina et al., 2017) and databases ([www.marlin.ac.uk/biotic](http://www.marlin.ac.uk/biotic); [www.polytraits.lifewatchgreece.eu](http://www.polytraits.lifewatchgreece.eu)). When we were not able to find information about traits of a particular species, data on the genera or families were used. Further, we were unable to obtain information on a higher taxonomic level, the species was given a zero score for each category and for all traits. In addition, to determine the functional identity, i.e. which modalities of each trait occurred by the taxa in a sample, the community level weighted means (CWM) was calculated (Weigel et al., 2016; Nasi et al., 2018). CWM values represent the occurrence of a trait by species of a given community, weighted by the abundance of species expressing that specific trait. For the computation of CWM in our study, the species assemblage (average of three replicates at each station) was considered. CWM was calculated for each sampling station using the FD library (R program ver 3.1.3) (Laliberté et al., 2014).

## 2.5. Data analysis

In this study, we applied the RLQ analysis (Dolédec et al., 1996) to assess the influence of different physical features on macrofaunal community bioturbation attributes in the sampling area. The RLQ analysis requires the generation of three different data tables: the R table, gathers information on the environmental variables (i.e. grain-size fractions; C: N ratio;  $^{137}\text{Cs}$  and  $^7\text{Be}$  radioisotopes;  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotopes) 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 sixteen different traits. Before running the RLQ 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. RLQ ordination was carried out with ade4 package in the R program (ver. 3.1.3) (Dray et al., 2007).

Based on RLQ analysis results, three groups of stations (i.e. outer; inner-finer grain-size and inner coarser grain-size stations) were identified considering the lagoonal confinement gradient and the grain-size distribution pattern. A one-way PERMANOVA test was applied to check for significant differences in physical-chemical parameters among

groups of stations, where factor 'confinement/grain-size' was selected as fixed factor. When significant differences were observed, PERMANOVA pairwise tests were performed. Unrestricted permutation of raw data and 9999 permutations were applied. Prior to analyses the abiotic parameters were normalized and the Euclidean distance was applied.

Univariate diversity analysis was applied to macrofaunal community composition considering the total species number and diversity ( $H'$ , Shannon and Weaver, 1949).

The differences in total species numbers, diversity,  $BP_c$ ,  $IP_c$  and traits occurrences (functional identity-CWM values) among lagoons and groups of stations ('confinement/grain-size' factor) were computed by Kruskal-Wallis H and Mann-Whitney U tests, respectively.

To highlight any variations in macrofaunal bioturbation attributes (taxa bioturbation potential- $BP_i$ ; taxa irrigation potential- $IP_i$  and CWM values) linked to inter-lagoonal (among different lagoons), intra-lagoonal (among stations within the same lagoon) and grain-size distribution patterns a PERMANOVA test was used. The factor 'confinement/grain-size' was applied as fixed factor in a one-way analysis. When significant differences were observed, PERMANOVA pairwise tests were performed. Unrestricted permutation of raw data and 9999 permutations were applied.

In order to observe any spatial patterns of individual bioturbation and bioirrigation values in the study area, a non-metric multidimensional scaling analysis (nMDS) was performed for two matrices (i.e.  $BP_i$  and  $IP_i$ , separately). To detect which taxa were mainly responsible for bioturbation and irrigation activities ( $BP_i$  and  $IP_i$  data, respectively) at stations gathered in the RLQ diagram, SIMPER (SIMilarityPERcentage) analysis was used and factor 'confinement/grain-size' was assigned. A cut-off at 60% was applied.

A BIO-ENV analysis was used as a first exploratory approach, to assess which environmental variables best correlated with the bioturbation and irrigation potential ( $BP_i$  and  $IP_i$ ), and CWM values. Data were normalized before entering the analysis and the BIO-ENV routine was calculated using the Spearman's coefficient. The main environmental parameters correlated with species composition and trait modalities occurrences were used to perform Distance-based Linear Models (DistLM). The latter was carried out to assess which variables explained differences ( $p < 0.05$ ) in species and traits composition. The environmental parameters were normalized and a selection based on  $R^2$  was applied.

For each univariate and multivariate analyses (i.e. PERMANOVA, SIMPER, DistLM and nMDS) the two matrices based on  $BP_i$ ,  $IP_i$  values were Log ( $x+1$ ) transformed and the Bray-Curtis similarity was applied; whereas for CWM data matrix a Log ( $x+1$ ) transformation and Euclidean distance were used. These analyses were performed using PRIMER 7 (PRIMER-E Ltd. Plymouth, UK) (Clarke et al., 2014).

To highlight the spatial relationship between predictor variables (the considered abiotic parameters) and bioturbation attributes, a Spearman's correlation and linear regression were computed. These analyses were tested for response variables as: (i)  $BP_c$  and  $IP_c$ ; (ii) functional occurrences (CWM values); (iii) bioturbation and individual irrigation potential, selected by SIMPER analysis. By doing so, the predictive power of environmental parameters for each bioturbation attribute was discriminated via the coefficient of determination  $r_s$  (Spearman's correlation) and  $R^2$  (linear regression).

The Mann-Whitney and Kruskal-Wallis tests were computed using STATISTICA 7 software and only significant data ( $p < 0.05$ ,  $< 0.01$  and  $< 0.001$ ) were reported.

### 3. Results

#### 3.1. Environmental variables

The measured environmental factors, thoroughly described in Cibic et al. (2019), are briefly summarized in Table 2. We observed  $^7Be$  activity at all the investigated lagoons with a different pattern: in Caleri it

was detected only at station CL2 (74.4 Bq  $kg^{-1}$ ); in Marinetta-Vallona and Canarin at two stations, but with lower activities (i.e. MV6 = 22.0 Bq  $kg^{-1}$ , MV5 = 58.2 Bq  $kg^{-1}$ , CN12 = 31.8, Bq  $kg^{-1}$  and CN10 = 66.0 Bq  $kg^{-1}$ ); at Scardovari at all stations, with an average value around  $48 \pm 15$  Bq  $kg^{-1}$ .  $^{137}Cs$  displayed the following activities: 6.4–10.9 Bq  $kg^{-1}$  in Marinetta-Vallona; 4.3–12.3 Bq  $kg^{-1}$  in Caleri (except for CL1 where it was not detected); 7.7–12.8 Bq  $kg^{-1}$  in Scardovari; 4.6–13.9 Bq  $kg^{-1}$  in Canarin.

We did not observe any pattern of  $^7Be$  with grain-size (Fig. 2a). On the contrary, we noticed, even though not consistently, increasing activities of radioisotope  $^{137}Cs$  at stations characterized by higher percentages of clay and silt and fresher OM (Fig. 2b and d). Conversely, at sandier stations we observed C-depleted values of OM and low  $^{137}Cs$  activities (Fig. 2d). CL1 and CL2 seemed to represent an exception, displaying relatively high  $\delta^{13}C$  values typical of marine origin (Bongiorni et al., 2018). The OM coming from the surrounding land and river, marked by  $^7Be$  (Fig. 2c), varied in relation to  $\delta^{13}C$  values and again was not strictly correlated with the grain-size.

#### 3.2. RLQ combined analysis

The first RLQ axis accounted for 63.6%, whereas the second axis represented 32.0% of the total variance (Table 3). This analysis showed a strong association between sand, in the positive part of RLQ1 and *no-burrow*, *sessile* and *suspension feeder* modalities. The corresponding species, associated with higher percentage of sand, were the polychaetes *Laonice cirrata* and *Polydora ciliata*, the crustacean, *Upogebia pusilla* and the decapod *Carcinus aestuarii*. Clay, silt and  $^{137}Cs$  were the predominant elements of the negative part of RLQ1 axis related with *bio-diffuser*, *open-ended burrow* and *subsurface deposit feeder* as trait-categories. These associations largely matched the characteristics of the polychaete *Alitta succinea* and oligochaetes. The positive part of RLQ2 revealed that *conveyor* modality, mainly expressed by the polychaetes *Heteromastus filiformis* and *Streblospio shurubsolii*, was associated to C:N ratio. The negative part of RLQ2 showed that  $\delta^{15}N$  and  $\delta^{13}C$  were associated with *motile* and *no-reworking* trait categories. The main corresponding taxa were the bivalve *Haminoe navicula* and chironomids. On the RLQ plot, stations were separated into three groups: those nearby the lagoonal mouths ('outer': CN11, CL3, SC15 and MV5) were placed on the right and upper side of the plot, in correspondence to sand and high value of C:N ratio. The inner stations of Marinetta-Vallona and Caleri ('inner-coarser' grain-size: MV7, CL1 and CL2), characterized by coarser sediments with shell fragments and increasing values of  $\delta^{13}C$ , were gathered on the right and low side of the diagram. The last group, located on the left side of the plot ('inner-finer' grain-size: SC17, CN10, CN11, MV6), compromised stations with fine grain-size and high activities of  $^{137}Cs$  (Fig. 3). The PERMANOVA one-way main-test performed on environmental data revealed that the considered abiotic variables significantly differed (Pseudo-F = 7.65;  $p < 0.001$ ) among the three groups. All pairwise comparisons between these groups were significantly different, varying from  $t = 2.30$ ;  $p < 0.05$  for 'outer' vs 'inner-coarser' to  $t = 3.60$ ;  $p < 0.01$  for 'outer' vs 'inner-finer'.

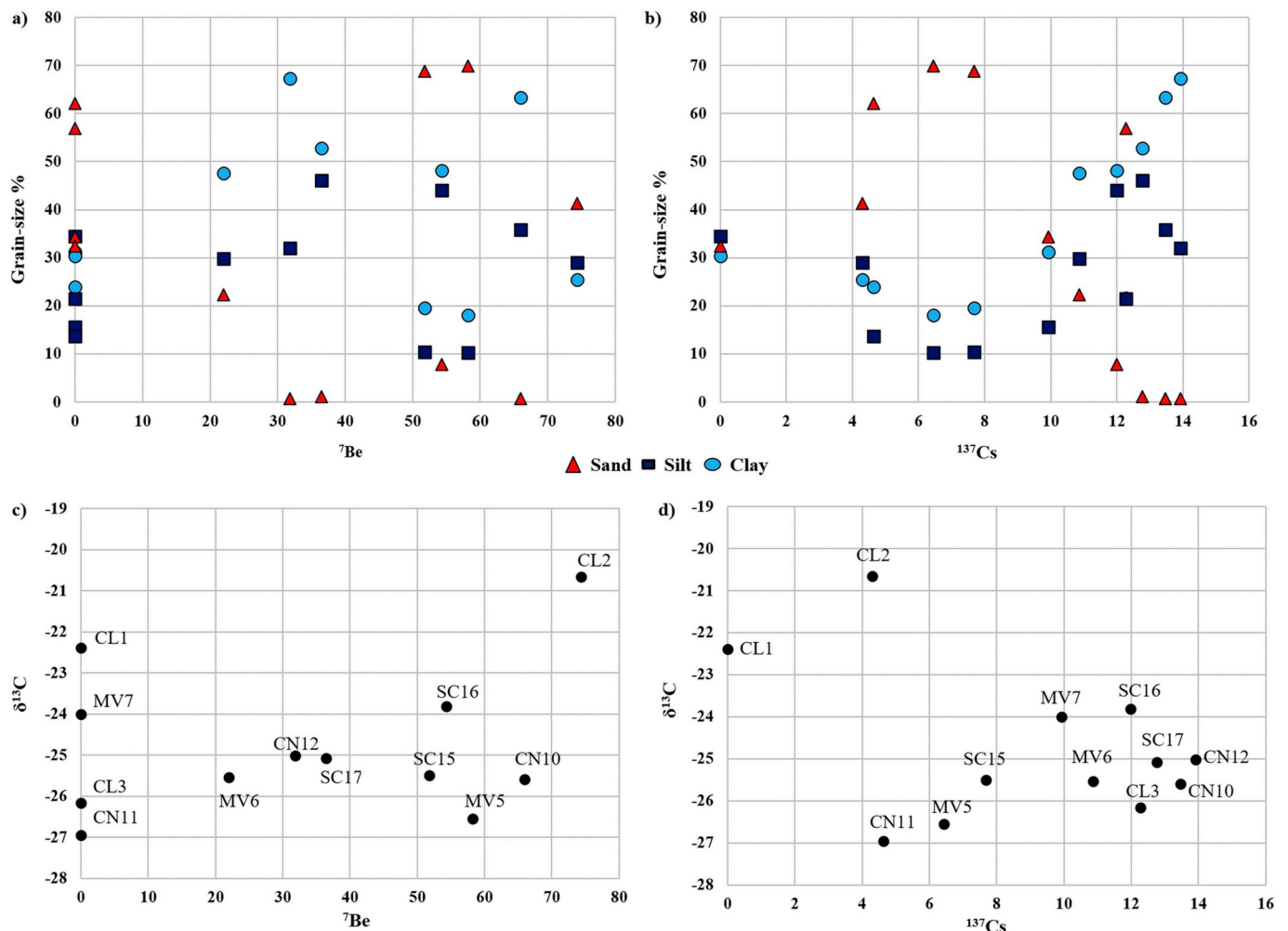
#### 3.3. Macrofaunal bioturbation attributes

Considering all sampling stations of the Po Delta lagoons, the macrofaunal abundance varied from 1081.0 ind.  $m^{-2}$  at CL1 to 14236.0 ind.  $m^{-2}$  at MV7. At the same stations, the lowest (13.2 g  $m^{-2}$ ) and highest (3386.0 g  $m^{-2}$ ) values of biomass were noticed, respectively. Polychaetes and oligochaetes were the dominant taxa (48.6% and 24.1% of the total abundance) followed by crustaceans (18.3%) and molluscs (5.6%) and other groups (anthozoans, nemertea, oligochaetes and chironomids, together <5%). The latter are highly abundant at CL2 (2237.0 ind.  $m^{-2}$ ). In the whole sampling area, a total of 97 taxa were found. The number of taxa varied from a minimum of 11 to a maximum of 39 at SC17 and MV5, respectively. Diversity values ( $H' \log_2$ ) ranged from

**Table 2**

Coordinates, grain-size, expressed as percentage of dry mass and sediment organic matter features at the sampling sites: Cs and Be radioisotopes ( $^{137}\text{Cs}$  and  $^7\text{Be}$ ), C and N stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and carbon and nitrogen ratio (C:N) (Cibic et al., 2019). n.d.: non detectable.

	Latitude	Longitude	shells	sand			clay	$^7\text{Be}$	error	$^{137}\text{Cs}$	error	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N
				%										
CL1	45° 06' 58.7"	12° 18' 32.2"	2.8	32.4	34.5	30.3	n.d.	<32.8	n.d.	<3.3	-22.4	3.9	8.6	
CL2	45° 05' 29.5"	12° 18' 02.2"	4.3	41.3	29.0	25.4	74.4	±29.6	4.3	±1.6	-20.7	4.3	7.8	
CL3	45° 05' 08.1"	12° 19' 35.2"	0.0	56.9	21.4	21.7	n.d.	<44.9	12.3	±2.5	-26.2	2.7	12.8	
MV5	45° 03' 35.3"	12° 22' 11.3"	1.8	69.8	10.3	18.1	58.2	±17.8	6.4	±2.3	-26.6	2.3	12.6	
MV6	45° 02' 48.9"	12° 22' 55.2"	0.3	22.3	29.8	47.6	22.0	n.d.	10.9	±2.0	-25.5	4.1	10.4	
MV7	45° 01' 13.2"	12° 22' 48.5"	19.0	34.3	15.6	31.2	n.d.	<41.2	9.9	±2.3	-24.0	4.1	8.0	
CN10	44° 55' 57.9"	12° 29' 54.8"	0.0	0.7	35.9	63.4	66.0	±20.0	13.5	±2.4	-25.6	2.0	9.1	
CN11	44° 55' 17.3"	12° 30' 01.3"	0.2	62.1	13.8	23.9	n.d.	<26.2	4.6	±0.9	-27.0	1.6	14.7	
CN12	44° 54' 14.9"	12° 29' 11.8"	0.0	0.7	32.0	67.3	31.8	±9.0	13.9	±1.2	-25.0	4.3	7.8	
SC15	44° 50' 36.7"	12° 26' 45.8"	1.2	68.8	10.4	19.6	51.8	±16.0	7.7	±2.5	-25.5	0.2	10.0	
SC16	44° 51' 22.7"	12° 24' 50.1"	0.0	7.9	44.0	48.1	54.3	±16.7	12.0	±2.7	-23.8	2.3	7.9	
SC17	44° 50' 21.6"	12° 24' 21.2"	0.0	1.1	46.2	52.8	36.5	±11.0	12.8	±2.6	-25.1	1.2	9.3	



**Fig. 2.** Relationship between  $\delta^{13}\text{C}$  and values radioisotopes signals,  $^{137}\text{Cs}$  (a) and  $^7\text{Be}$  (b) observed at sampling areas.

1.3 at SC17 to 3.2 at MV7.

The community bioturbation potential ( $\text{BP}_c$ ) and irrigation potential ( $\text{IP}_c$ ) values were lower at SC16 (168.0 and 42.5 respectively) whereas the highest values of  $\text{BP}_c$  (2341.4) and  $\text{IP}_c$  (1053.7) were calculated for MV5 and SC15, respectively (Fig. 4). The Kruskal-Wallis H test did not reveal any variation in taxa numbers, biodiversity, and bioturbation

indices (i.e.  $\text{BP}_c$  and  $\text{IP}_c$ ) in relation to inter-, intra-lagoonal and sediment grain-size. However, higher values of total number of taxa and  $\text{IP}_c$  were observed at outer stations rather than inner ones, characterized by fine sediments (Fig. 5). On the contrary, the PERMANOVA main-test performed on individual bioturbation potential ( $\text{BP}_i$ ) highlighted significant differences among groups of stations that emerged from RLQ

**Table 3**  
RLQ results. Individual R-L-Q separate analysis and RLQ analysis.

Cumulative projected inertia (%):					
Ax1	Ax1:2				
63.7	95.7				
Projected inertia (%):					
Ax1	Ax2				
63.7	32.0				
Eigenvalues decomposition:					
eig1	eig	covar	sdR	sdQ	corr
eig2					
3.71	1.93	1.92	1.96	1.96	0.51
1.87	1.37	1.6	1.44	1.44	0.60
Inertia & coinertia R (env):					
	inertia	max	ratio		
eig1	3.71	3.9	0.94		
eig1+2	6.27	6.36	0.99		
Inertia & coinertia Q (traits):					
	inertia	max	ratio		
eig1	3.84	4.25	0.90		
eig1+2	5.90	6.75	0.87		
Correlation L (CA on abundance):					
	corr	max	ratio		
eig1	0.51	0.86	0.59		
eig2	0.60	0.70	0.85		

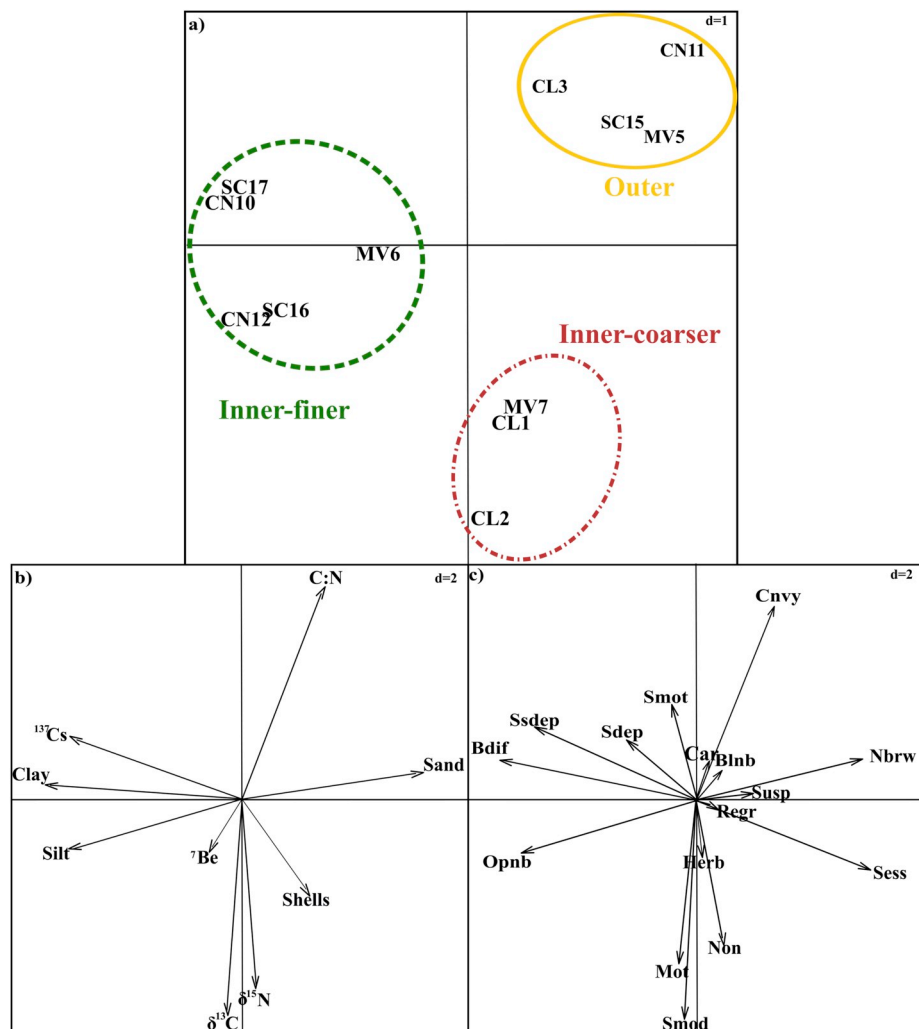
analysis ('confinement/grain-size' as factor) (Pseudo-F = 1.94;  $p < 0.05$ ). The pairwise test confirmed a highly significant difference between 'inner-coarse' vs 'inner-finer' groups ( $t = 1.58$ ;  $p < 0.05$ ). Similar results were obtained by PERMANOVA main-test performed on

individual irrigation potential (IP<sub>i</sub>) (Pseudo-F = 1.74;  $p < 0.05$ ). A significant difference was highlighted between 'inner-coarser' and 'inner-finer' groups by pairwise test ( $t = 1.47$ ;  $p < 0.05$ ).

The nMDS performed on BP<sub>i</sub> and IP<sub>i</sub> values further confirmed the PERMANOVA results (Fig. 6). The two ordination plots clearly separated inner stations characterized by coarse sediments from those where major percentages of silt and clay were observed. This was particularly evident for SC16 and SC17 that were positioned on the right side of the plot at the maximum distance (Bray-Curtis maximum dissimilarity) from CL2, indicating a tight link between bioirrigation macrofaunal activities and grain-size (Fig. 6a and b).

Further, SIMPER analysis performed on BP<sub>i</sub> and IP<sub>i</sub> values showed differences among the three groups of sites ('confinement/grain-size' as factor), corroborated by the RLQ results (Table S3).

Considering the macrofaunal community of the study area, CWM values showed higher occurrences for categories: *semi-motile*, *biodiffuser* and *surface deposit feeder*. On the contrary, less represented categories (CWM values  $< 0.5$ ) of traits were: *no movement*, *regenerator* and *herbivore*. Some trait categories were differently expressed among the three groups of sites ('confinement/grain-size' as factor) (Fig. 7). At outer stations *conveyor* and *blind-ended burrow* invertebrates were highly expressed compared to inner stations. In particular, the *conveyor* modality significantly differed for the three groups of stations ( $H = 7.26$ ;  $p < 0.01$ ), specifically between 'outer' and 'inner-coarser', ( $Z = 2.6$ ;  $p < 0.05$ ). A prevalence of *carnivore*, *sessile*, *semi-motile*, *motile*, *no-reworking*, *superficial modifier*, *regenerator*, *no burrow* as trait categories



**Fig. 3.** RLQ diagram as defined by two first axes with a projection of sampling sites a), environmental variables b) and different trait categories c). The d value in the upper right of the corner is the scale of each graph. The circles gather significant group of stations (by PERMANOVA tests). Yellow full circle encloses the outer stations; scarlet dashed and dotted circle encloses the inner coarser grain-size stations; Green dashed circle encloses the inner finer grain-size stations. (For the interpretation of the reference to colour in this figure legend, the reader is referred to the Web version of this article.)

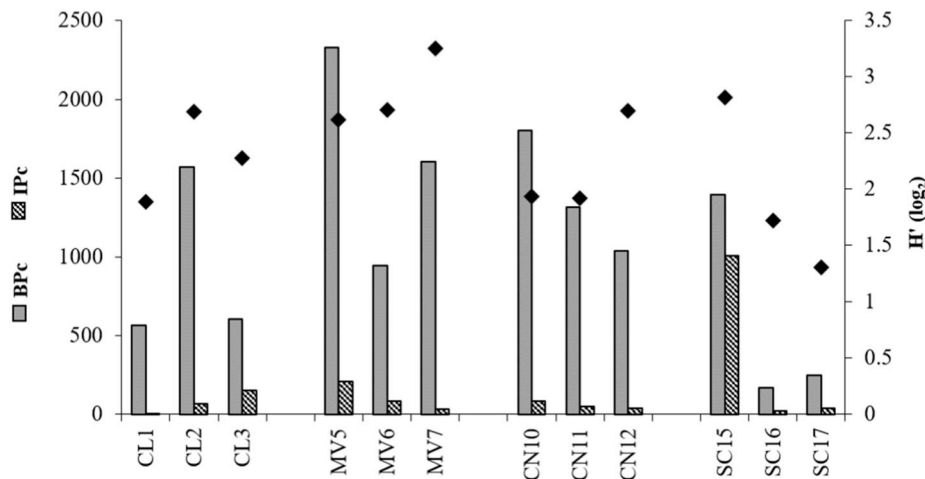


Fig. 4. Bioturbation potential (BP<sub>c</sub>) and irrigation potential (IP<sub>c</sub>) of community and species diversity (H').

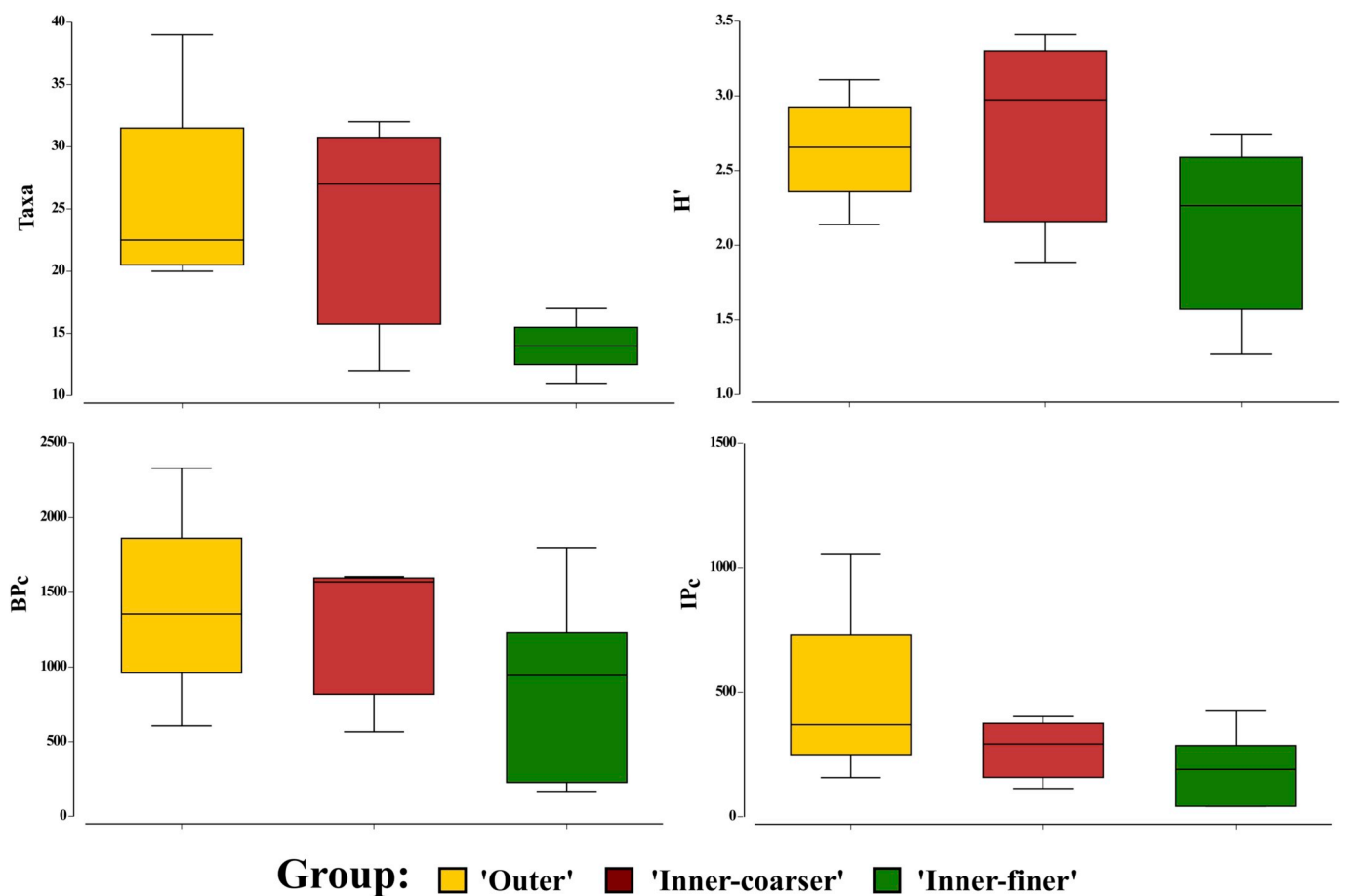


Fig. 5. Boxplots showing the variability of number of taxa, species diversity, bioturbation potential (BP<sub>c</sub>) and irrigation potential (IP<sub>c</sub>) of community at stations gathered by RLQ analysis. The different groups are indicated with different colours. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

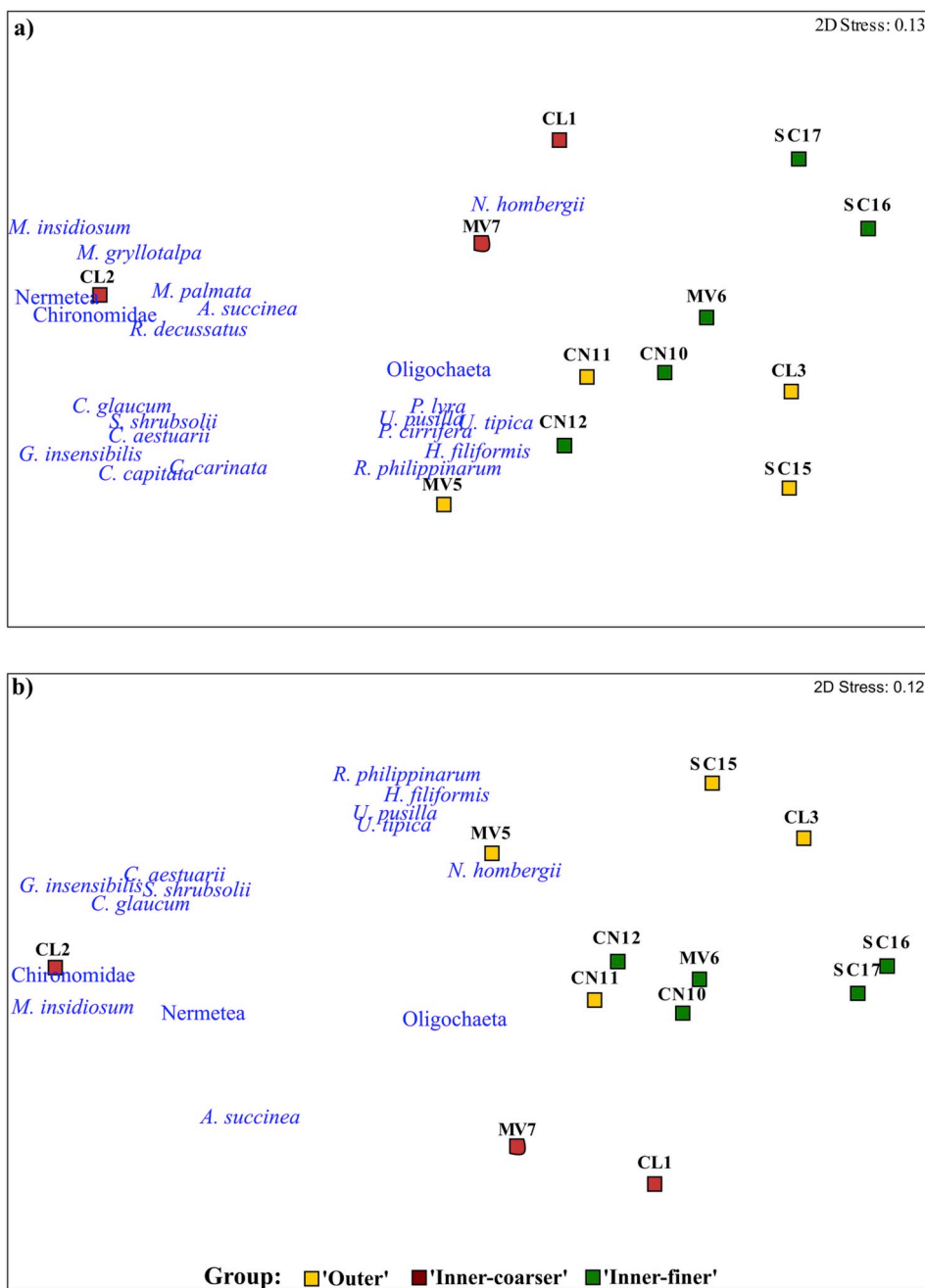
were observed at station gathered in the 'inner-coarser' group. In particular, significantly higher values of *regenerator* and *superficial-modifier* invertebrates were observed at 'inner coarser' stations rather than at 'inner-finer' (H = 7.22 and Z = 2.40; p < 0.05) and 'outer' ones (H = 6.54 and Z = 2.42; p < 0.05). Further, the *biодiffuser* and *opened burrow* as modalities were highly expressed at inner stations characterized by fine sediments. This difference was statistically significant for *biодiffuser* invertebrates (H = 6.54; p < 0.05), which

dominated at 'inner-finer' stations compared to 'outer' ones (Z = 2.42; p < 0.05).

### 3.4. Relation between bioturbation indices or traits, and environmental variables

The BIO-ENV analysis was performed on bioturbation and irrigation potential taxa and traits occurrences, separately, and the considered





**Fig. 6.** nMDS ordination plot based on BP<sub>i</sub> (a) and IP<sub>i</sub> (b) values at sampling stations gathered by RLQ analysis. The most relevant taxa (according to SIMPER analyses) are represented. See Table S1 for taxa name.

environmental variables. BP<sub>i</sub> and IP<sub>i</sub> values were best correlated with silt fraction, <sup>137</sup>Cs, <sup>7</sup>Be, δ<sup>13</sup>C and δ<sup>15</sup>N (r<sub>s</sub> = 0.51, 0.50), whereas the trait-categories occurrences were related with clay fraction, <sup>137</sup>Cs, <sup>7</sup>Be, δ<sup>13</sup>C and δ<sup>15</sup>N (r<sub>s</sub> = 0.42). The significant variables obtained from the BIO-ENV analyses performed on BP<sub>i</sub> and IP<sub>i</sub> data (i.e. silt, <sup>137</sup>Cs, <sup>7</sup>Be, δ<sup>13</sup>C, and δ<sup>15</sup>N) were used to perform a Distance-based Linear Model (DistLM), from which it emerged that δ<sup>13</sup>C was the significant driver of bioturbation and irrigation macrofaunal attributes. In addition, the significant environmental variables obtained from the BIO-ENV analysis performed on CWM values (i.e. clay, <sup>137</sup>Cs, <sup>7</sup>Be, δ<sup>13</sup>C, and δ<sup>15</sup>N) were included in the DistLM. According to the model output, clay and δ<sup>15</sup>N were the significant drivers of the trait-categories occurrences. The outputs of DistLM analyses are summarized in Table 4.

The linear regression was computed using trait-categories occurrences and bioturbation and irrigation potentials of taxa, that emerged

from the SIMPER analysis (as response variables), and abiotic variables (as predictors). Categories belonging to ‘feeding strategy’, ‘Mobility’, and ‘Reworking’ traits and BP<sub>i</sub> and IP<sub>i</sub> of chironomids, the amphipod *Monocorophium insidiosum* and polychaetes *Capitella capitata* and *S. shurubsolii*, were well predicted by grain-size fractions, stables isotopes and C:N, as reported in Table S4.

Moreover, a significant relationship was found between *M. insidiosum*, superficial modifier, and δ<sup>13</sup>C. At ‘inner-coarser’ stations we observed the highest abundance of the superficial modifier *M. insidiosum* in relation to fresh OM (Fig. 8a). In addition, oligochaetes and the *biodiffuser* modality were well predicted by the radioisotope <sup>137</sup>Cs (Fig. 8b). On the contrary, the decapod *C. aestuarii* and *regenerator* modality were significantly related to lower values of <sup>137</sup>Cs (Fig. 8c). Moreover, *S. shurubsolii* and *conveyor* modality were well predicted by C: N ratio (Fig. 8d).

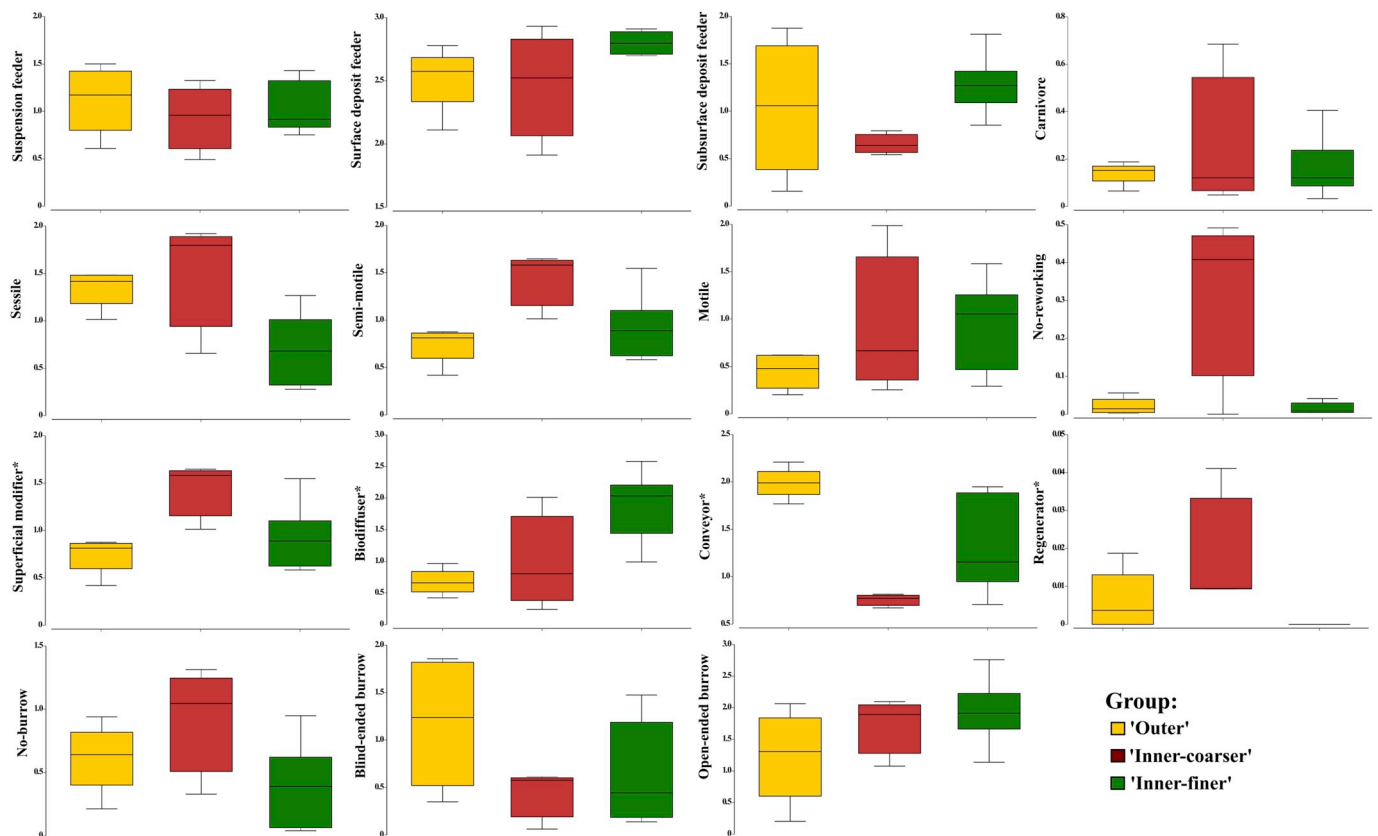


Fig. 7. Boxplots showing the variability of trait modalities occurrences (community-weighted means-CWM) at sampling stations gathered by RLQ analysis. Variables with an asterisk explain significant difference between two groups of sites (K-W test). Herbivore as trait modality is not represented.

Table 4

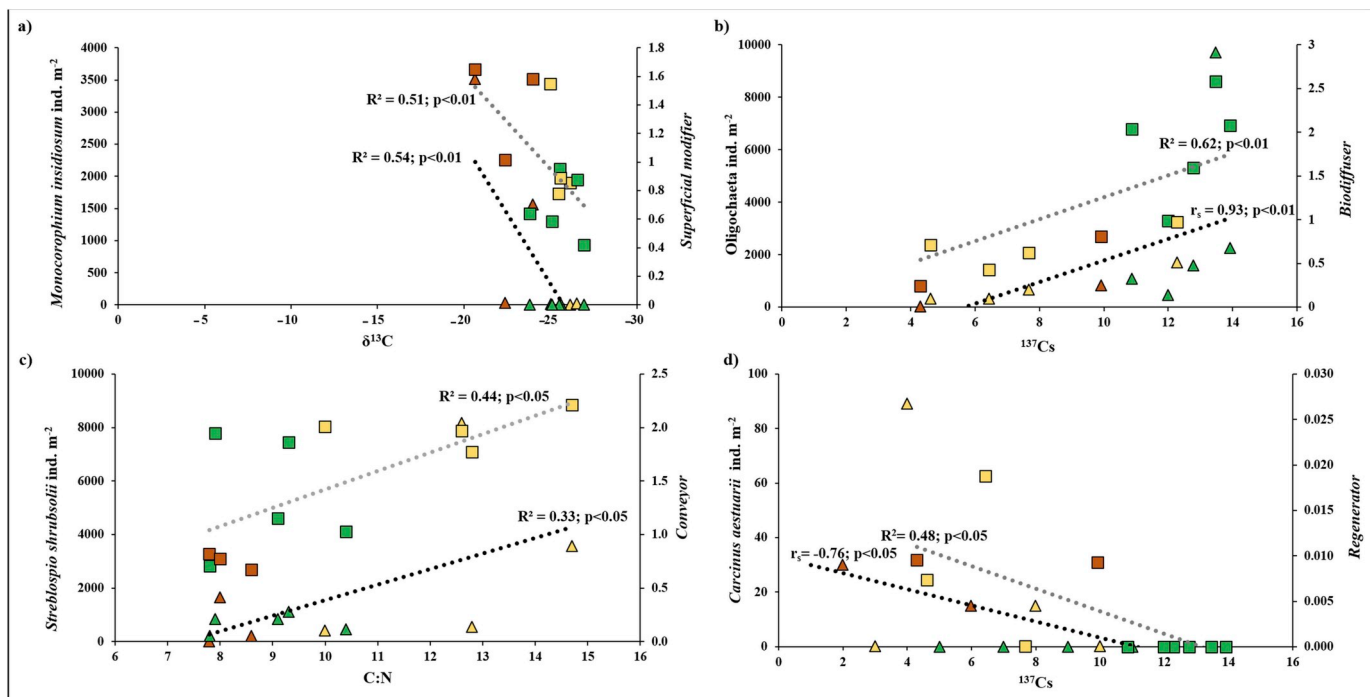
DistLM (Marginal test) results for species bioturbation potential, species irrigation potential and trait-categories occurrences in relation to environmental variables. SS: Sum of square. Prop: proportion of variance explained by each variable. p values having statistical significance are highlighted in bold.

Response variables	R <sup>2</sup> (DistLM)	Selected predictors	SS(trace)	Pseudo-F	P	Prop.
BP <sub>1</sub>	0.61	Silt	3350.70	1.65	0.09	0.14
		<sup>137</sup> Cs	3454.40	1.70	0.07	0.14
		<sup>7</sup> Be	1924.60	0.88	0.56	0.08
		δ <sup>13</sup> C	3966.20	2.01	<b>0.03</b>	0.17
		δ <sup>15</sup> N	3220.10	1.57	0.10	0.13
IP <sub>1</sub>	0.60	Silt	3255.90	1.52	0.12	0.13
		<sup>137</sup> Cs	2923.8	1.34	0.38	0.12
		<sup>7</sup> Be	2420.50	1.08	0.20	0.09
		δ <sup>13</sup> C	4135.9	2.01	<b>0.03</b>	0.17
		δ <sup>15</sup> N	3465.6	1.63	0.08	0.14
Trait-categories	0.66	Clay	2.26	3.21	<b>0.02</b>	0.24
		<sup>137</sup> Cs	1.01	1.22	0.31	0.10
		<sup>7</sup> Be	0.43	0.48	0.750	0.46
		δ <sup>13</sup> C	1.11	1.36	0.27	0.12
		δ <sup>15</sup> N	2.74	3.40	<b>0.04</b>	0.21

#### 4. Discussion

Our results show that a reduction of bioturbation and bioturbation potential indexes was linked to a decrease of biodiversity. This was in line with our first hypothesis that zoobenthic community structure would influence bioturbation processes. Our second hypothesis was also not rejected, since differences in macrofaunal bioturbation attributes displayed both an inter-lagoonal (among lagoons) and an intra-lagoonal (among stations within the same lagoon) pattern. The different renewal time of marine water inside lagoons (confinement) may have influenced the survivorship of certain species and therefore some bioturbation attributes were not expressed. Differences in macrofaunal bioturbation attributes among lagoons were likely due to the overall diverse grain-

size composition, as well as to the degree of freshness and origin of OM (estimated by <sup>7</sup>Be and <sup>137</sup>Cs). Indeed, grain-size and the presence of riverine-derived OM could be considered the main drivers of different bioturbation attribute patterns. In particular, our results show that invertebrates with high burrowing depths and blind-ended burrows, abundant at sandier stations, enhanced the bioturbation. In contrast, the reworking activities (i.e. categories belonging to ‘mobility’ and ‘reworking’ traits) of macrofaunal invertebrates seemed to be linked to allochthonous OM deposition rather than sediment grain-size. In particular, the high density of conveyors increases the bioturbation activities, enhancing the overall ecosystem functioning in sites influenced by river-derived OM matter.



**Fig. 8.** Relationship between *Superficial modifier*, *Bdiffuser*, *Regenerator*, and *Conveyor* (CWM values), significant environmental variables (Spearman correlation- $r_s$  and linear regression test- $R^2$ ):  $\delta^{13}C$  (a),  $^{137}Cs$  (b, c) and C:N (d); and increase abundance values (ind. m<sup>-2</sup>) of taxa: *Monocorophium insidiosum* (a), *Oligochaeta* (b), *Careinus aestuarii* (c) and *Streblospio shrubsolii* (d). The grey and black fitted lines indicate significant relationships between modalities, taxa abundance and abiotic parameters, respectively.

#### 4.1. Macrofaunal bioturbation attributes in the estuarine coastal area

The macrofaunal community in Po River Delta lagoons can be considered as a typical community of brackish coastal lagoons (e.g. Koutsoubas et al., 2000; Nicolaidou et al., 2006; Basset et al., 2012) with euryhaline and eurythermic species. At sites near the inlets, more influenced by the seawater, a dominance of marine/estuarine species was observed, such as the polychaete *Heteromastus filiformis* and the bivalve *Abra prismatica*. On the other hand, the innermost parts of the lagoons were dominated by lagoonal/paralic taxa (e.g. the polychaete *Alitta succinea* and the crustacean *Monocorophium insidiosum*).

On a large scale, our results revealed that the intra-lagoonal position of stations is highly related to the bioturbation attributes of the macrofaunal community inhabiting the sediments of the Po Delta lagoons. Several key factors, including salinity, affect biodiversity and functional taxa identity in the lagoonal system. Species richness in coastal lagoons is not dependent on salinity alone, but it is a result of the so-called “confinement”, i.e. the time required for the renewal of marine elements in a basin (Tagliapietra et al., 2009). In fact, in our study area, the macrofaunal diversity appeared to be highly influenced by the renewal time of marine water, showing low biodiversity and strong dominance of few paralic species (Guelorget and Perthuisot, 1983) in areas where the renewal time was high. Maicu and co-authors (2018), by applying an unstructured 3D numerical model, revealed that the northern part of Scardovari remains very confined, with an estimated average time of water renewal of up to 20 days. In addition, the innermost part of Scardovari is frequently subjected to hypoxia/anoxia events (ARPAV, 2016). The low water exchange may further magnify the negative effects of oxygen deficiency on benthic organisms. Indeed, we observed the lowest  $H'$  values in the Scardovari lagoon, particularly at the two inner stations SC16 and SC17. This low diversity, in turn, likely influenced the bioturbation activities within the sediments since at these stations the lowest values of  $BP_c$  and  $IP_c$  indices were obtained (see Fig. 4). Similarly, benthic communities at lower trophic levels were also poorly structured (i.e. low biodiversity and presence of tolerant species) in the innermost

part of Scardovari (Cibic et al., 2019; Franzo et al., 2019).

On a small scale, physical environmental features, mainly different grain-size fractions, determine the broad pattern of benthic organism distribution. Our findings are in line with previous studies that have reported grain-size as the main driver of the spatial distribution pattern of the bioturbation attributes (Gogina et al., 2017; Morys et al., 2017). These authors highlighted differences in bioturbation potential, both considering the entire community ( $BP_c$ ) and the individual taxa ( $BP_i$ ), between stations characterized by coarse and fine sediments. They also observed an increase in the number of species, abundance, biomass and consequently  $BP_c$  values, in correspondence with a higher sand fraction, as we did in our study area. The latter was observed in Marinetta-Vallona lagoon, where the sandier station located nearby the lagoonal mouth was characterized by the highest  $BP_c$  values; whereas the highest value of  $H'$  was noticed at the inner station influenced by sandy-mud sediments with a high amount of shell fragments. In fact, the high grain-size variability in unconsolidated bottoms (i.e. mixed grain-size particles) contributes to the development of high species richness, particularly in soft sediments (Gray, 2002). Overall, in our study, a higher percentage of sand was found at the lagoonal mouths where marine and euryhaline/lagoonal species inhabit the same sediments, increasing the biodiversity at those stations and, consequently, leading to the highest values of  $BP_c$ . Similarly, the difference in bioirrigation potential ( $IP_c$ ) observed between coarse and fine sediments was also likely ascribable to high species richness, abundance, and biomass at sandier stations. The bivalve *Ruditapes philippinarum* and polychaete *H. filiformis* majorly contributed to this difference, due to high individual biomass and abundance, respectively. However, the significant difference obtained for  $IP_c$  values (PERMANOVA test) was mostly due to irrigation functional features of certain species: the crustaceans *Upogebia tipica* and *Upogebia pusilla*, the polychaetes *Capitella capitata*, *Pseudoleiocapitella fauveli*, and Maldanidae. Following the categorical scores for IP calculation promoted by Wrede et al. (2018), these taxa (in particular *Upogebia* spp. and Maldanidae, see Table S1) were characterized by high burrowing depth and type of burrowing

structure that increase the water ventilation within the sediment layers. The burrowing depth is important for the pore water exchange and local input of oxygen into anoxic sediments. In particular, the effects of deep burrowing organisms are enhanced in oxic or suboxic sediments compared to those of shallow burrowers (Aller, 1988). Furthermore, the burrow morphology plays a key role in determining the modality of pore water exchange within sediments (Kristensen and Kostka, 2005). In more permeable sandy sediments, the burrow irrigation is characterized by advection of pore water, leading the building of blind-ending burrows (Kristensen et al., 2012). Conversely, in finer sediments, the permeability is low and therefore the open ending burrows with two or more openings to the sediment surface are common and amplify the pore water exchange with radial diffusion mode. This difference underline that the two pore water exchange systems, i.e. advection and diffusion, are fundamental in bioirrigation processes (e.g. biogeochemical cycling, microbial respiration). In fact, there is experimental evidence (Mermillod-Blondin and Rosenberg, 2006) that the advection-dominated system enhances microbial respiration (over 20-fold) at the burrow wall - sediment interface, compared to diffusion-dominated systems. This was also confirmed by our findings. Among modalities belonging to 'ventilation' traits, the *blind-ended burrow* was more expressed (CWM-values) at sandy stations whereas the *open-ended burrow* was dominant at muddy ones (see Fig. 7) indicating that the sediment ventilation (in an advection-dominated system) was more intense at stations characterized by coarse sediments.

#### 4.2. Feeding strategy, sediment reworking, and relation with environmental variables

The feeding strategy of macrofaunal invertebrates is fundamental to determine the type of burrows and the modes of locomotion and defecation (Jumars et al., 2015; Kristensen and Kostka, 2005; Kristensen et al., 2012). As in Wrede et al. (2018) and Renz et al. (2018), in the IP<sub>c</sub> calculation, we added the trait 'feeding habit' in the BTA analysis. Our results indicated that surface and subsurface deposit feeders mostly contributed to BP<sub>c</sub> and IP<sub>c</sub> of the lagoon system, followed by suspension feeders, herbivores, predators and scavengers, as previously found in shallow marine environments (Gogina et al., 2017; Breine et al., 2018) and other lagoon systems (Marchini et al., 2008). The Kruskal-Wallis test did not highlight any pattern in functional habits across the study area, although from the RLQ analysis differences in suspension feeders and deposit feeders, in particular subsurface, were observed from 'inner-coarser' stations to 'inner-finer' ones. Our results are in accordance with Dauwe et al. (1998), who observed that the input of relatively fresh organic matter promotes the growth of suspension feeders. Stations in Caleri lagoon (in particular CL1 and CL2) are influenced by organic matter of marine origin, (i.e. slighter positive values of δ<sup>13</sup>C). However, we noticed a dominance of suspension and sessile invertebrates also at all stations located in front of the lagoonal mouth, deeply influenced by OM of riverine origin, in particular in Marinetta-Vallona and Scardovari lagoons (at MV5, and SC15, respectively) (see Fig. 6) due to the high density of bivalves *R. philippinarum*. It has been experimentally observed that suspension feeders are able to capture large amounts of water for feeding and respiration, leading to well-flushed burrows (Kristensen and Kostka, 2005). Conversely, subsurface deposit feeders were more abundant at 'inner-finer' stations favoured by slow and continuous deposition of relatively fresh organic matter. This feeding habit was, in fact, found to be dominant in muddy sites, where a high proportion of detrital food, very palatable for these invertebrates, is usually associated with silt-clay sediment particles (Donald and Larry, 1982; Thrush et al., 2004; Hermand et al., 2008).

In our lagoonal system, we mostly ascribed bioirrigation activities to deposit feeders, in particular to subsurface deposit ones. This was primarily due to either their abundance or bioirrigation features (the main subsurface deposit feeders are classified as biodiffusers). The burrows built by deposit feeders are principally used for respiration, even though

they actively feed at the sediment-water interface or below the sediments (i.e. surface and subsurface deposit feeders, respectively) (Lee and Swartz, 1980). Sediment reworking deposit feeders are involved in sediment particles mixing, due to sediment ingestion and defecation together with their partial and/or active mobility through sediment layers (Lopez and Levinton, 1987; Kristensen et al., 2012; Queirós et al., 2015).

In the Po Delta lagoonal system, the reworking activities (i.e. categories belonging to 'mobility' and reworking' traits) of macrofaunal invertebrates seemed to be linked to allochthonous OM deposition rather than sediment grain-size. Among deposit feeders, semi-motile and conveyor invertebrates were dominant at stations located nearby the lagoonal mouths (i.e. CN11, MV5, CL3, SC15) which were characterized by riverine OM inputs (C:N > 10 and δ<sup>13</sup>C > -25.00) (Table S3 and Fig. 8c). The high occurrence of *conveyor* modality were principally due to the highest abundance of three polychaetes: the surface deposit feeder *Streblospio shrubsolii* and the subsurface deposit ones *Capitella capitata* and *Heteromastus filiformis* (see Figs. 6 and 8c). These invertebrates are usually dominant in sandy-mud of marine/brackish environments and, in particular, where high deposition of OM occurs (Hermand et al., 2008; Marchini et al., 2008; Bongiorno et al., 2018), generally displaying an opportunistic behaviour (Méndez et al., 2000). The increase in the food supply could rapidly stimulate the growth, reproduction rate and survivorship of individuals. These animals are able to modify their behaviour and choose to feed on fresh organic matter at very small spatial scales (Cruz-Rivera and Hay, 2000). In addition, *H. filiformis* and *C. capitata* are classified as head-down conveyor-belt feeders that transport sediments from the sulfidic environment surrounding their heads to the oxic environment surrounding their tails, as faecal pellets on surface sediments (Neira and Höpner, 1994; Tsutsumi et al., 2005). In general, *conveyors* move sediment particles through their gut by ingestion and secretion, transferring particles from deeper to superficial layers and vice-versa thus enhancing the organic matter remineralization (Chareonpanich et al., 1993; Belley and Snelgrove, 2016). This activity may contribute to modify sediment properties (in particular the sediment porosity; Wild and Huettel, 2005) and promote microbial population resulting in accelerated degradation of organic matter (Kinoshita et al., 2008). In addition, the most common burrow type of these polychaetes is the blind-ended one; therefore, they are also able to intensify the pore water advection across sediment layers (Kristensen et al., 2012). We infer that despite the riverine OM enrichment, the high density of conveyors increases the bioturbation activities, enhancing the overall ecosystem functioning in sites nearby the lagoonal mouths.

Conversely, at 'inner-coarser' stations characterized by organic matter of marine origin (both low C:N ratio and <sup>137</sup>Cs activities, and higher δ<sup>13</sup>C values), in particular at MV7 and CL2, high occurrences of *superficial modifier* and *regenerator* modalities were obtained. Superficial modifiers are considered weak bioturbators since these animals are able to rework only the most superficial sediments and thus have a low impact on bioturbation processes compared to the other reworking modalities (i.e. *conveyors*, *biodiffusers*, and *regenerators*) (Queirós et al., 2013). However, when abundant, these weak bioturbators may strongly contribute to sediment reworking (Belley and Snelgrove, 2016). This likely occurred in the Caleri lagoon, in particular at CL2, where the high dominance of the superficial modifier, the euryhaline crustacean *Monocorophium insidiosum* (3511 ind. m<sup>-2</sup>), may have increased the overall bioturbation process. The latter was enhanced also by the presence of regenerator invertebrates, in particular the species *Carcinus aestuarii*. Regenerators are considered excavators that dig and maintain burrows in the sediments and by doing so transfer sediments from deep layers to the surface (Kristensen et al., 2012) (see Fig. 8 a and d).

We noticed high occurrences of *motile* and *biodiffuser* modalities at stations characterized by higher percentages of silt and clay, and <sup>137</sup>Cs radioisotope signals (classified as 'inner-finer') (see Figs. 6 and 8b). *Biodiffuser* modality includes organisms whose activities produce constant and random (i.e. horizontal and vertical) local sediment biomixing



over a short distance resulting in particle transport. Among them, gallery biodiffusers often occur in finer sediments in which they are promoters of diffusive local biomixing primarily due to burrowing activities within the upper 10–30 cm of sediments (Kristensen et al., 2012). We observed typical gallery biodiffusers, the taxon oligochaeta and the polychaete *Alitta succinea*, in high individual numbers, in particular at muddy stations. Both taxa are commonly considered euryhaline omnivorous species that have been principally classified as deposit feeders (Casellato, 1994; Jumars et al., 2015). In particular, *A. succinea* lives in relatively permanent U-shaped or branching burrows and often reaches very high densities in intertidal and lagoonal areas (Kristensen, 1989; Nizzoli et al., 2007). *A. succinea* was one of the most abundant taxa also in our lagoonal system that highly contributed to BP<sub>c</sub> and IP<sub>i</sub> values at most of the stations but in particular at MV6, CN10, and CN12. At the latter station, the highest value of <sup>137</sup>Cs activity was detected, tracing the mixing of older and deeper sediments with the uppermost, freshly deposited layers. Experimental studies highlighted the implication of *A. succinea* also in oxygen exchanges and nutrient fluxes within the sediment layers and at the water-sediment interface (Nizzoli et al., 2007). According to these authors, the high bioturbation activity of *A. succinea*, by intensifying the oxygen fluxes into the sediments, plays a major role in the re-oxidation and detoxification of highly reduced sediments generated during the annual dystrophic crisis in the nearby Sacca di Goro.

## 5. Conclusion

In the present study, the BP<sub>c</sub> and IP<sub>c</sub> were integrated with the functional identity information to assess the influence of terrigenous/freshwater OM on invertebrates' bioturbation attributes in a lagoonal system. The confinement gradient (i.e. intra-lagoonal variation) deeply influenced the biodiversity and, consequently, the bioturbation processes that were mirrored in the lowest BP<sub>c</sub> and IP<sub>c</sub> values. Grain-size was the main driver of the differences in BP<sub>c</sub> and IP<sub>c</sub>, in fact the major number of taxa in coarse sediments contributed to the highest values of bioturbation indices. Moreover, the irrigation functional features of the collected species determined the high IP<sub>c</sub> value. Invertebrates with high burrowing depths and blind-ended burrows enhanced the biorrigation at sandy stations.

Furthermore, sediments differently influenced by allochthonous OM seemed to modify the spatial pattern of functional trait occurrence concerning the sediment reworking modalities. *Semi-motile* and *conveyors* invertebrates were dominant nearby the lagoonal mouths where freshwater OM enrichment was observed. Conversely, at stations characterized by aged OM, high occurrences of *motile* and *biodiffuser* modalities were detected. Overall, the presence of invertebrates able to mix and ventilate sediments increased the bioturbation activities, enhancing the overall ecosystem functioning of the area.

The present study represents a contribution to the growing body of bioturbation research, especially in the scantily investigated coastal river lagoons. However, since these lagoonal systems are spatially variable ecosystems, future studies should consider a wider sampling grid, particularly in lagoons subjected to hypoxia/anoxia events (e.g. Scardovari). In the latter, the use of BP<sub>c</sub> and IP<sub>c</sub> could be matched with experimental data in order to corroborate our findings and help introduce the application of functional traits in the assessment of the benthic ecosystem functioning. The deepening of the knowledge on macrofaunal bioturbation attributes in relation to their sediment reworking and ventilation abilities, after anoxic and dystrophic events, is of paramount importance in the framework of an efficient management and sustainable use of coastal resources, especially lagoons that area strongly exploited for aquaculture.

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## Appendix A. Supplementary data

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