Common patterns of functional and biotic indices in response to multiple stressors in marine harbours ecosystems☆

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ABSTRACT

Evaluating the effects of anthropogenic pressure on the marine environment is one of the focal objectives in identifying strategies for its use, conservation and restoration. In this paper, we assessed the effects of chemical pollutants, grain size and plastic litter on functional traits, biodiversity and biotic indices. The study was conducted on the benthic communities of three harbours in the central Mediterranean Sea: Malta, Augusta and Syracuse, subjected to different levels of anthropogenic stress (high, medium and low, respectively). Six traits were considered, subdivided into 22 categories: reproductive frequency, environmental position, mobility, life habit, feeding habit and bioturbation. Functional diversity indices analysed were: Functional Divergence, Quadratic Entropy, Functional Evenness and Functional Richness. To assess the trait responses to environmental gradients, we applied RLQ analysis, which considers simultaneously the relationship between three components: environmental data (R), species abundances (L) and species traits (Q). From our analyses, significant relationships (P-value = 0.0018 for permutation of samples, and P-value = 0.00027 for permutation of species) between functional traits and environmental data were highlighted. The trait categories significantly influenced by environmental variables were those representing feeding habits and mobility. In particular, the first category was influenced by chemical pollutants (organotin compounds and polycyclic aromatic hydrocarbons) and grain size (silt and sand), while the latter category was influenced only by chemical pollutants.

Pearson correlations performed for functional *vs* biotic and diversity indices confirmed the validity of the chosen conceptual framework for harbour environments. Finally, linear models assessing the influence of stressors on functional parameters underlined the link between environmental data *vs* benthic and functional indices. Our results highlight the fact that functional trait analysis provides a useful and fast method for detecting in greater depth the effects of multiple stressors on functional diversity in marine ecosystems.

1. Introduction

The recent expansion of human activities in the marine domain has benefited societies and increased economic activity but has led concurrently to a loss of ecosystem services, biodiversity, and functional diversity (Halpern et al., 2008; Korpinen and Andersen,

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2016; Lotze et al., 2018). Studying the effects of anthropogenic pressure on marine ecosystems is essential to conduct appropriate economic and environmental management. In view of this, we chose as study areas marine harbour environments, which, being generally characterised by heavily impacting activities, represent some of the best examples of stressed ecosystems in which to conduct research concerning environmental management (Iannelli et al., 2012; Romeo et al., 2015). Indeed, the broad literature reveals that these areas are afflicted by pronounced biological (*e.g.*, invasion by non-indigenous species) and chemical (*e.g.*, heavy metals, persistent organic pollutants, polycyclic aromatic hydrocarbons) contamination in all compartments, especially concerning sediments and biota (Deidun et al., 2016; D'Alessandro et al., 2016a; Servello et al., 2019). In particular, the seabed, which acts both as a sink and as a source of environmental contaminants, directly affects the communities that live in close contact, such as macrozoobenthic organisms (Tamburrino et al., 2019). Benthic communities, due to their ability to adapt their composition and structure in response to different sources of disturbance, are considered perfect indicators of environmental pressure (Pearson and Rosenberg, 1978; Consoli et al., 2008, Kim et al., 2019). In this context, the study of functional traits could supply important information in the understanding in greater depth of the responses of communities to environmental alterations. In recent years, mainly as an attempt to address the objectives of a number of European Directives (*e.g.*, Marine Strategy Framework Directive – MSFD – 2008/56/EC, EU Biodiversity Strategy to 2020 and Water Framework Directive – WFD – 2000/60/EC), a wide array of valid biotic indices relating to diverse aspects of the structure of macrobenthic communities have been proposed; *e.g.*, AMBI (AZTI's Marine Biotic Index; Borja et al., 2000), M-AMBI (multivariate AMBI; Muxika et al., 2007) and Bentix (Simboura and Zenetos, 2002). All these indices have relied on the taxonomic identification of species. However, the application of functional traits of species, rather than taxonomic identity, as a method to reveal salient descriptors of community responses to environmental change, has increased over the years (Bremner et al., 2006; Song et al., 2014; Beauchard et al., 2017). Functional traits, consisting of all those morphological, behavioural, phenological and structural features that can explain the interaction between species and their environment (Dıaz and Cabido, 2001; Beauchard et al., 2017), supply the tools to investigate the functional diversity in benthic communities. This methodological approach is based on the underlying concept that taxonomically unrelated species can develop the same functional adaptations (Winemiller et al., 2015). Estimating how functional traits are related to specific environmental conditions can offer important insights into the mechanisms that determine species distributions and is an essential step in assessing environmental status (Peng et al., 2013; Culhane et al., 2014; Vinagre et al., 2017; Bianchelli et al., 2018). Indeed, numerous studies focusing on the links between marine benthic system functional traits, such as modes of feeding, bioturbation, mobility, reproduction and anthropogenic stressors, have been conducted in recent years (*e.g.*, Gusmao et al., 2016; Nasi et al., 2018). Functional diversity, defined as: '*the value and the range of those species and organism traits that influence ecosystem functioning*' (Tilman et al., 2001), represents an alternative classification to measure the hierarchical ecological importance of species in a community. In general, within ecosystems, a higher functional diversity is also indicative of a higher resilience to anthropogenic impacts (Cardinale et al., 2012).

The most integrated method to investigate the relationship between the functional structure of benthic communities and the degree of environmental stress is represented by the multivariate ordination method – RLQ and fourth-corner analysis (Dolédec et al., 1996; Leaver et al., 2019). This method directly correlates habitat and environmental variability to modifications in functional diversity (Rachello-Dolmen and Cleary, 2007), allowing the identification of both those environmental quality and those functional traits affected by disturbance, as well as the relationships between them (Dolédec et al., 1996; Ribera et al., 2001; Hausner et al., 2003; Gámez-Virués et al., 2015). RLQ analysis considers the relationship between the environmental characteristics of samples (R-table) and species trait data (Q-table), driven by the relative abundance of the species (L-table), while the fourth-corner approach evaluates the bivariate associations, one at a time, between one environmental variable and one trait. As demonstrated by Dray et al. (2014), the integration of RLQ analysis and the fourth-corner approach improves the analysis of ecological data.

This study aimed to evaluate the responses of the functional traits of the benthic community subjected to different levels of anthropogenic stress as represented by different exposure levels to chemical (trace metals, organotin compounds and polycyclic aromatic hydrocarbons, PHAs) and plastic pollutants. The study area comprised three busy harbours of the Central Mediterranean Sea, sited along the Strait of Sicily: la Valletta (Malta), Augusta and Syracuse (Sicily).

In this study, for the first time, the integration of RLQ analysis and the fourth-corner approach has been applied to harbour environments. To assess the applicability of this method, correlations between benthic and diversity indices were investigated. Therefore, an integrated functional approach evaluating the response of the marine ecosystem to multiple stressors was considered. More specifically, the goals of the study were: *i*) to analyse patterns of variability in functional and benthic index values in the areas of study in relation to different levels of anthropogenic impact; *ii*) to evaluate which functional traits were mainly subject to specific stressors; and *iii*) to verify the applicability of functional diversity indices in marine monitoring and assessment.

2. Material and methods

2.1. Study areas

Sampling was carried out in three harbours of the central Mediterranean Sea exposed to different levels of anthropogenic pressure: Valletta's Grand Harbour (high level), Augusta (medium level) and Syracuse (low level). Of these, the first site lies in Malta, while the latter two are placed in the south-western Ionian Sea (Fig. 1). All the study areas are located along the Malta–Sicily channel, a crucial marine traffic passageway, linking the south-to-north and east-to-west sectors of the Mediterranean Sea. The Maltese harbour is exposed to the highest number of ongoing anthropogenic activities, ranging from ship repair industries, fisheries, transport, cruises, and fuel and cargo management (Deidun et al., 2016). This area is characterised by low hydrodynamism and fine-grain sediments (Romeo et al., 2015).

The harbour of Augusta is affected by marine pollution from industrial and petrochemical plants, but also from agriculture and urban waste, navigation and past dredging of polluted sediments (Di Leonardo et al., 2014). Its sediments are characterised by high levels of trace elements. Indeed, Augusta harbour is considered an important contributor of Hg to the whole Mediterranean Sea (Sprovieri et al., 2011).

Syracuse, bordering the adjacent Plemmirio Marine Protected Area, is a semi-enclosed area mostly characterised by recreational and fishing-related maritime traffic and is the least anthropised area (Copat et al., 2012).

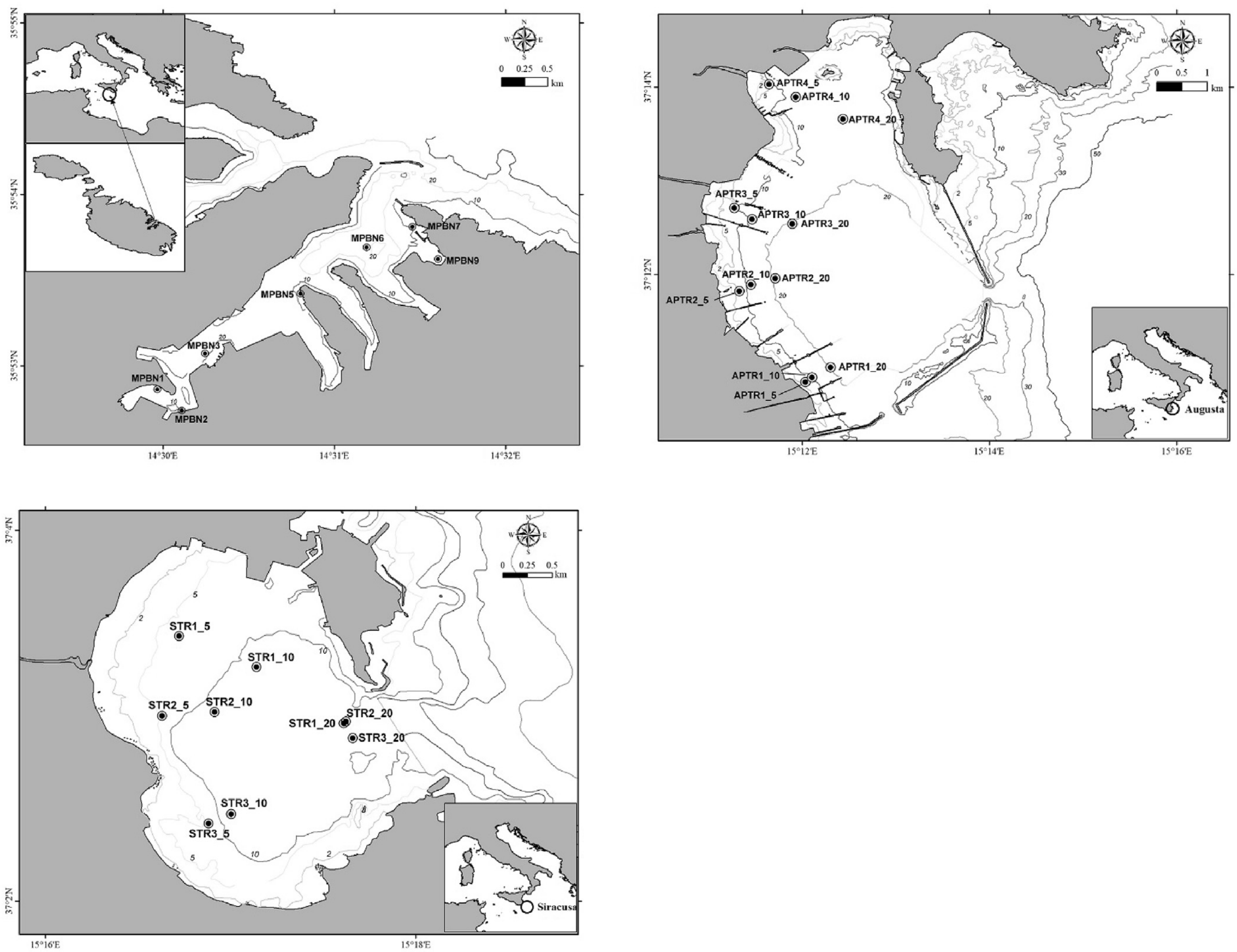

Fig. 1. Sampling maps: Malta on top left, Augusta on top right and Syracuse on the bottom left.

2.2. Sampling and analytical methods

A total of 28 sampling stations were studied during summer 2013. In Augusta and Syracuse harbours, samples were collected along transects perpendicular to the coastline. This sampling design could not be replicated within the Maltese harbour due to its peculiar morphology (Fig. 1). In each area, samples were collected at three different depths: 5, 10 and 20 m, with four replicates being collected at each depth, by means of a 0.1 m^2 Van Veen grab. Among these, three were used for faunistic analysis (macrozoobenthic communities) and one for the study of abiotic parameters (grain size and chemical and plastic analyses) (Supplement 1).

All the methodologies of sampling, storage and processing were performed according to Romeo et al. (2015) and D'Alessandro et al. (2018). Faunistic samples were sieved onboard by means of a 0.5 mm mesh and preserved in 90% ethanol (Supplement 2). In the laboratory, sorting and taxonomic identification were conducted by means of optical (Optika Vision Lite 2.1) and stereomicroscopes (Zeiss Discovery V8). Grain size samples were refrigerated at 4 °C and analysed in the laboratory using the column dispersion method (Buchanan and Kain, 1971) computing the percentages of the granulometric fractions applying the Wentworth scale (Wentworth, 1922). Samples for plastic litter were refrigerated at 4 °C and treated in the laboratory according to D'Alessandro et al. (2018), paying particular attention to contamination (Woodall et al., 2015). Classification of plastic items was conducted according to the MSFD (Galgani et al., 2013) and expressed as dry sediment. Chemical samples were frozen at −20 °C and the presence of trace metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn), PAHs (anthracene, benzo(b)-fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, benzo(k)fluoranthene, fluoranthene, naphthalene, indenopyrene) and organotin compounds (tributyltin [TBT], dibutyltin [DBT], and monobutyltin [MBT]) was studied.

2.3. Functional analysis

As a first step, the structure of the benthic community within each harbour was analysed by calculating the biodiversity indices of Shannon (H') and Pielou (J), and the frequency (%F) and the abundance (%N) percentages of each collected species (Supplement 3). Then, to investigate how macrofaunal functional traits change in relation to environmental parameters, a fuzzy-code method was applied (Chevene et al., 1994; Nasi et al., 2018). Six functional traits were considered: reproductive frequency, adult environmental position, adult mobility, adult life habit, adult feeding habit and bioturbation type (Table 1). These traits were subdivided into 22

Table 1
Functional traits and their categories and abbreviations.

Functional traits	Categories	Abbreviations
Reproductive frequency	Semelparous	Sem
	Iteroparous	Iter
	Semi-continuous	Scon
Adult environmental position	Endofauna	Endo
	Epifauna	Epi
	Epibiont	Epib
Adult mobility	Sessile	Sess
	Semi-motile	Smot
	Motile	Mot
Adult life habit	No mov.	Nmo
	Swimmer	Swim
	Crawler	Craw
	Tube-builder	Tubl
	Burrower	Bur
Adult feeding habit	Suspension feeder	Susp
	Deposit feeder	Df
	Herbivore	Herb
	Carnivorous	Pred
Bioturbation	Superficial modifiers	Sumo
	Biodiffuser	Bdif
	Epifauna	Epi
	Conveyors	Cnvy

different categories according to Gusmao et al. (2016) and a fuzzy code was formulated (Supplement 4), using a score ranging from 0 (no affinity for traits) to 3 (complete affinity). Information regarding functional traits was collected from online databases (MarLIN, 2006; Biotic; Polytraits Team, 2019) and the literature (*e.g.*, Fauvel, 1923; Dauvin and Ibanez, 1987; Jumars et al., 2015). To analyse changes in trait composition and expression, the functional identity as Community-level Weighted Mean (CWM) of trait category expression was calculated, where a community was defined as the species assemblage in each replicate sample (Nasi et al., 2018). CWM gives an indication of the trait strategies of a species in response to environmental data and represents the expression of a trait by species in a given community, weighted by the abundance of species expressing that specific trait (Muscarella and Uriarte, 2016; Nasi et al., 2018).

The values of the main functional diversity indices, Functional Richness (FRic; Villéger et al., 2008), Functional Evenness (FEve; Villéger et al., 2008), Functional Divergence (FDiv; Villéger et al., 2008) and Rao's Quadratic Entropy (RaoQ; Botta-Dukát, 2005), were calculated on the basis of the computed fuzzy matrix and of the abundance of species, by means of the R package 'FD' (Laliberté and Legendre, 2010). Of these indices, FRic, FEve and FDiv are multiple-trait indices, while Rao's is a single-trait index. FRic represents the volume occupied by the community within the functional space, FEve describes the evenness of abundance distribution in a functional trait space, FDiv represents how abundance is distributed along a functional trait axis (Mason et al., 2005), while RaoQ uses species traits to calculate dissimilarity among species (Botta-Dukát, 2005).

Data from all sampling were transformed following the Hellinger method in order to standardise biotic abundance across the gradients represented by the three sampled harbours (Legendre and Gallagher, 2001). 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). For the abundance table, we applied Correspondence Analysis (CA), while for the traits and environmental tables, we applied Principal Component Analysis (PCA). Afterwards, we carried out RLQ analysis, considering simultaneously the three components R, L and Q. This analysis estimates the correlation between functional traits and environmental components through the computation of a crossed array (cross-covariance matrix weighted by abundances). The functional groups were built *post hoc*, applying the Calinsky-Harabasz stopping criterion, after exploring the relationships between traits (*i.e.*, functional identity) and the environmental components (Kleyer et al., 2012). The fourth-corner methods were applied to measure, one at a time, the associations between the species traits and the environmental variables. Moreover, considering that this method considers different variables in different statistical units, to avoid Type I error we applied the methodology proposed by Dray and Legendre (2008). We combined two permutation models, permutation of samples and permutation of species (for further details see Dray et al., 2014), carrying out 49,999 permutations and applying the adjustment method of P-values for multiple testing (False Discovery Rate [FDR] method; Benjamini and Hochberg, 1995). The correlations between traits and environmental variables were considered significant if the largest P-values of the permutation models (i.e.: samples and species permutation models) were lower than α.

The relationships between each functional and diversity index, each benthic index and the recorded contaminants were tested by fitting linear models. Akaike's Information Criterion (AIC; Akaike, 1974) was applied for the model selection process between all classes of competing models, after applying backwards and forwards stepwise variable elimination (Famoye and Rothe, 2001; Czado et al., 2007). Finally, in order to evaluate the analysis assumptions (*i.e.*, normality and homogeneity of variances) we used the gvlma package in R (Pena and Slate, 2014). The analyses were carried out using the software program R, version 3.5.2, R packages: car, ade4, gvlma, Performance Analytics, vegan (R Development Core Team, 2018).

3. Results

3.1. Abiotic and biotic results

Plastics were found in the highest abundance in Malta (59 particles kg^{-1}), followed by Augusta (38 particles kg^{-1}) and Syracuse (9 particles kg^{-1}). Microplastic was the most abundant plastic category, representing 58.2% of total plastic debris. In general, all plastic size categories were recorded within sediment samples, except for Syracuse harbour, where mega-plastic debris was not recorded (Supplement 1).

The comparison between the three sampled harbours indicated Zn to be the most abundant trace element (342.6 mg kg^{-1}), while Cd showed the lowest average values. Investigations of PAHs revealed fluoranthene, benzo(b)fluoranthene and benzo(a)pyrene to be the most abundant compounds within this category, with highest abundances being recorded in the Maltese harbour (Supplement 1). Among the organotin compounds, TBT was the most abundant, with the highest abundances once again being recorded in the Maltese harbour (Supplement 1).

Faunistic analysis recorded a total of 4489 specimens (Supplement 2). Shannon (H') and Pielou (J) indices showed the highest average values in Syracuse harbour (2.11 ± 0.67 S.D. and 0.83 ± 0.09 S.D., respectively), while the highest mean number of species was recorded in the Maltese harbour (27.33 ± 11.94 S.D.) (Supplement 3). In total, the most abundant and common species was the Polychaeta *Aricidea (Aricidea) pseudoarticulata* (N% = 15.3; F% = 82.8), which dominated the community in Augusta harbour, followed by the mollusc *Corbula gibba* (N% = 13.6%; F% = 75.9%), which was the dominant species in the Maltese harbour. Within Syracuse harbour, the most abundant species was *Branchiostoma lanceolatum* (N% = 2.3; F% = 3.5) (Supplement 2). Regarding to benthic indices, AMBI showed the highest average value in Malta (2.87 ± 0.85 S.D.), while the highest Bentix and M-AMBI mean index values were recorded in Syracuse and in Augusta (3.99 ± 1.88 S.D. and 0.80 ± 0.09 S.D., respectively) (Supplement 3).

3.2. Functional analysis

FRic indices showed the highest average value in Augusta (56.98 ± 20.34 S.D.), while all the other indices (FDiv, RaoQ and FEve) showed their highest mean value in Syracuse (0.83 ± 0.07 S.D.; 17.74 ± 5.58 S.D.; 0.63 ± 0.11 S.D., respectively). The PCA (Fig. 2a) performed on species traits generated first and second axes that explained 21.1% and 15.2% of the total variability, respectively. The main contribution to the first axis was related to the 'endofauna' and 'conveyors', while 'motile' and 'superficial modifiers' were the main representative traits of the second axis. The PCA (Fig. 2b) performed on environmental variables produced two axes that explained 40.6% and 15% of the total variability. Only granulometric parameters (sand, silt and clay) were related to the first axis; all the other environmental variables were correlated to the second axis. The main contribution to the latter was given by PHAs (fluoranthene, anthracene, benzo(a)pyrene; benzo(b)fluoranthene; benzo(k)fluoranthene), Cu and microplastics.

Fourth-corner analysis revealed significant positive associations (Fig. 3) between 'semi-motile' and organin compounds (TBT, DBT and MBT); 'semi-motile' and indenopyrene; 'suspension feeder' and indenopyrene and 'deposit feeder' and silt. Negative correlations were found between 'deposit feeder' and sand; motile and benzo(g,h,i)perylene and 'motile' and indenopyrene. The first two axes of the RLQ multivariate analysis explained 91.5% of the total inertia of the three tables, although variability was better explained for environmental variables than for traits (Table 2). Significant correlation between the functional trait composition and environmental variables ($P = 0.00184$ and $P = 0.00027$ respectively). This correlation was best summarised by the first RLQ axis, which explained 68.1% of the total cross-variance between the functional traits and environmental variables, whereas the second axis contributed only 23.4%.

Fig. 4 underlines significant relationships between the RLQ environmental axes and individual traits (Left), and between the RLQ trait axes and individual environmental variables (Right). Significant positive correlations were recorded between 'semi-motile', 'suspension feeder', 'superficial modifiers' and AxcR1 (on the right of the diagram). Negative correlations were found for the same axis *vs* the 'motile', 'biodiffuser' and 'predator' traits (on the left of the diagram). Regarding the second axis (AxcR2), positive correlations were recorded for the 'epifauna' and 'herbivore' traits (on the top of the diagram), while negative correlations were recorded for the 'deposit feeder' trait (on the bottom of the diagram). Concerning environmental variables, only positive correlations were recorded between AxcQ1 and TBT, DBT, MBT, benzo(a) pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i) perylene, indenopyrene, anthracene and fluoranthene. The second AxcQ2 was correlated positively with gravel and sand and negatively with silt and clay. After the application of the FDR adjustment method for multiple comparisons (Monte Carlo test), significant high correlations were recorded ($p = 0.0018$). Cluster analysis (Fig. 5) revealed the subdivision of the global set of environmental variables and functional traits into four groups. Clear influences of 'silt', 'clay' and 'Cr tot' within group A and of 'PHAs', 'Cu', 'Pb' and 'organin compounds' within group B emerged. As evident in Fig. 6, significant correlations were also found between M-AMBI and FRic (0.77); between Bentix and FEve and between Bentix and RaoQ, both with a value of 0.51; between H′ and FRic (0.58); and between S and FRic (0.48).

The best-fitted linear models for combinations of biotic indices and environmental variables are reported in Table 3. The most important environmental variables within FDiv models were silt, Hg, TBT, Total Crome, Cd, and 'port' factor. RaoQ was mainly related to silt, Cd and benzo(g,h,i)perylene. The variables considered in the S model were pebble, clay, MBT and Cd. The most important variables for J were silt, plastic, Hg, fluoranthene and 'depth' factor. The Bentix index was related to three variables: silt, naphthalene and 'port' factor.

4. Discussion

Analyses of functional diversity are increasingly included in the studies of community responses to environmental stress (*e.g.*, van der Linden et al., 2016; Kokarev et al., 2017; Leaver et al., 2019).

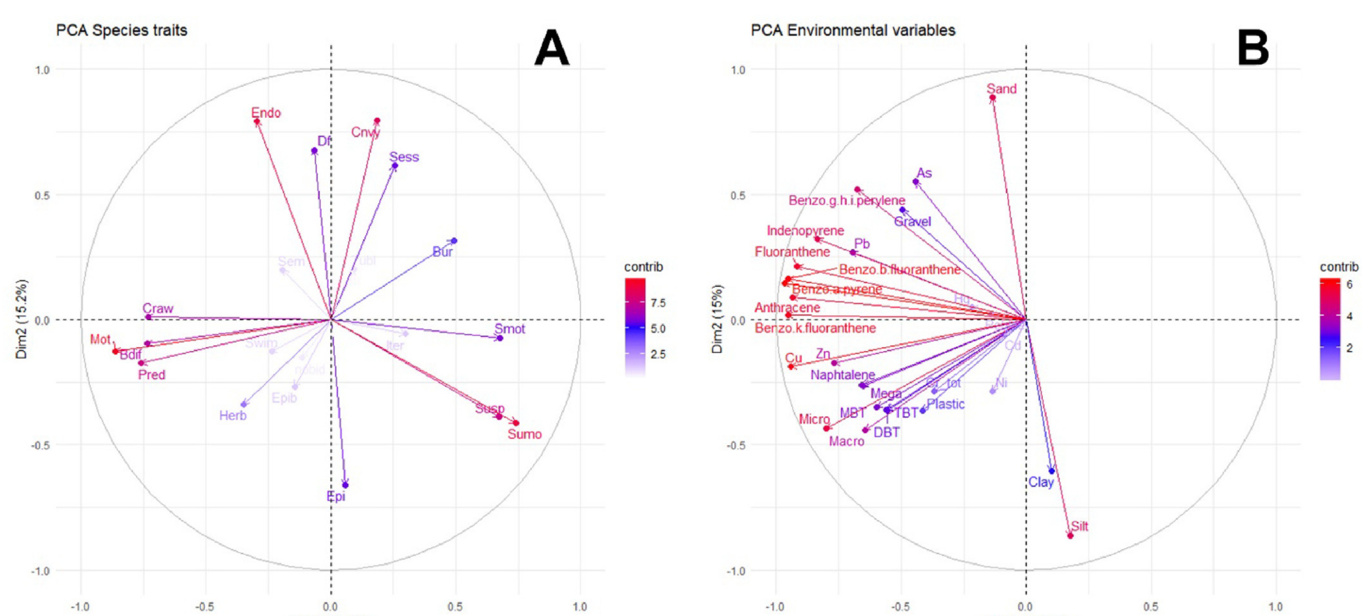

Fig. 2. PCA graphics performed on species traits (left) and environmental variables (right).

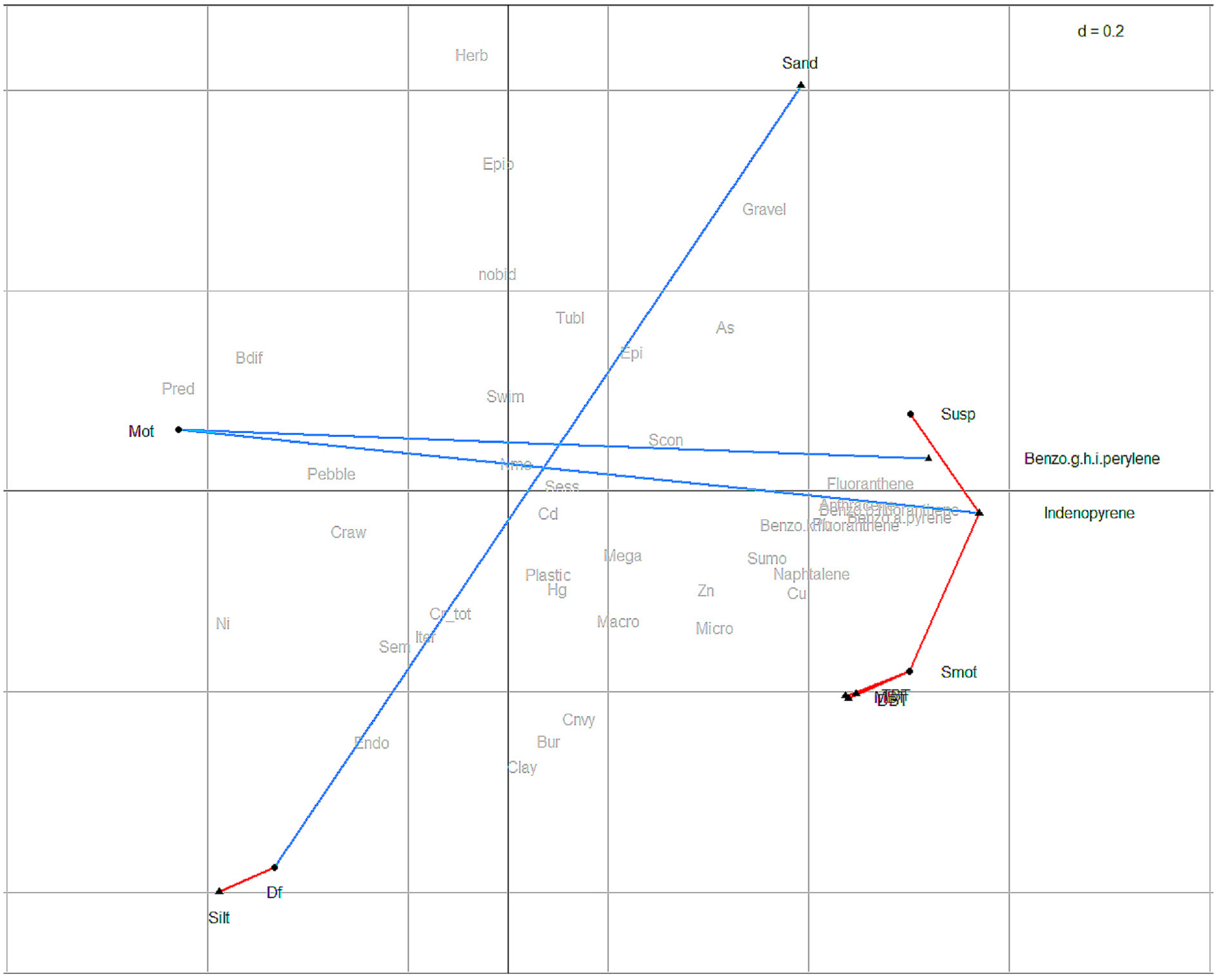

Fig. 3. Representation of associations carried out by fourth-corner on the factorial map of RLQ analysis. Significant positive (red lines) and negative (blue lines) associations between functional traits and environmental variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Summary of RLQ analysis.

Cumulative projected inertia(%):					
Ax1	Ax1:2				
68.09	91.53				
Projected inertia (%):					
Ax1	Ax2				
68.09	23.44				
Eigenvalues decomposition:					
	eig	covar	sdR	sdQ	corr
eig1	2.184	1.478	3.062	1.974	0.2445
eig2	0.7517	0.8670	2.057	1.686	0.2500
Inertia & coinertia R (env):					
	inertia	max	ratio		
eig1	9.379	11.37	0.825		
eig1+2	13.61	15.59	0.873		
Inertia & coinertia Q (traits):					
	inertia	max	ratio		
eig1	3.895	4.632	0.8408		
eig1+2	6.736	7.975	0.8447		
Correlation L (CA on abundance):					
	corr	max	ratio		
eig1	0.2445	0.8065	0.3032		
eig2	0.2500	0.7488	0.3339		

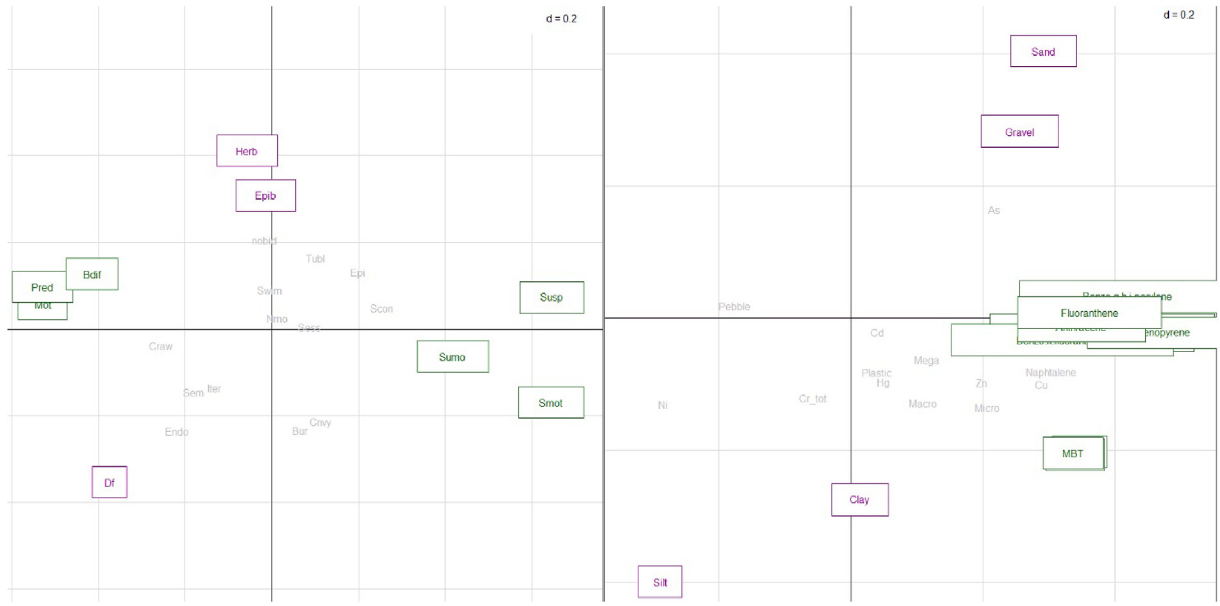

Fig. 4. Relationships between the RLQ environmental axes and individual traits (left) and between the RLQ trait axes and individual environmental variables (right). Significant associations with the first axis are represented in green, with the second axis in violet, while variables with no significant association are in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

In the present paper, in order to understand the functioning of the marine ecosystem of interest, we tested the applicability of functional diversity in environmental harbours that represented good examples of heavily stressed marine ecosystems (Romeo et al., 2015; D'Alessandro et al., 2016b).

4.1. Abiotic and biotic

In our study, the Maltese harbour was identified as the most affected area. Pollutants that mainly affect this area are microplastics, Zn, organotin compounds, fluoranthene and pyrene. The high abundance of microplastics could be due both to direct introduction into the environment or to a combination of multiple processes (physical, biological and chemical) that reduce the structural integrity of larger plastics, generating smaller ones (Cole et al., 2011).

Trace elements within the marine environment arise from the natural weathering of geological material and anthropogenic sources, such as water runoff contaminated with fertilisers or industrial products. A potentially significant source of Zn in the marine environment is the deployment of sacrificial anodes as an anti-corrosion measure. As highlighted in a previous study (Romeo et al., 2015), the majority of the PAH congeners reached their maximum values in the Maltese harbour; this could potentially be related to extensive hydrocarbon use in the area (*e.g.*, fuels, engine oils, maritime traffic, berths for recreational vessels, shipyards, etc.) that might result in spills into the marine environment. The island's main sewage outfall is located less than 10 km to the south-east of the harbour mouth, and this could also potentially impinge on the PAH content (Romeo et al., 2015).

The high concentrations of organotin compounds (TBT, MBT and DBT) in the Maltese harbour, related to activities within the harbour and extensive historical employment of tin-based biocides in antifouling paints, underlines the persistence and toxicity of these substances (De Mora, 1996; Yebra et al., 2004). The predominance of opportunistic (*e.g., C. gibba*) and tolerant (*e.g., A. pseudoarticolata*) species in Valetta and Augusta harbours, also highlights the degree of stress to which the sampled areas are subjected. In fact, *C. gibba* is considered to be an excellent indicator species of organic enrichment, typical of unstable bottoms and a 'pioneer' species in the recolonisation of soft-bottomed de-faunated areas (Nicoletti et al., 2004). The relatively undisturbed condition of the Syracuse harbour was highlighted by the prevalence of *B. lanceolatum*, which is usually recorded in relatively undisturbed benthic areas (Rota et al., 2009).

4.2. Patterns of functional and benthic index values

Spatial patterns in the diversity and functional traits indices (FEve, RaoQ, FDiv), showing highest values in Syracuse harbour, are consistent with values of pollutants and could be related to the high stability of the area. The high values for the FEve index, commonly associated with high ecosystem resilience values, reveal an effective utilisation of the entire range of available resources by the species present, by virtue of their niche space utilisation (Mouchet et al., 2010; Mouillot et al., 2013). The lower FEve index values recorded in Malta suggest a highly disturbed community, by virtue of a heterogeneously occupied functional space (Mason et al., 2005). The only two indices that did not follow such a spatial variability trend were species richness number, which was highest in the Maltese harbour, and functional richness (FRic), the highest in Augusta harbour. FRic and species numbers are assumed to be sensitive predictors of disturbance, since they decrease at high disturbance levels. However, the *prima facie* anomalous results obtained for FRic and FEve indices could be explained by the 'intermediate disturbance' hypothesis (*sensu* Connell, 1978). Indeed, according to this hypothesis, taxonomic and functional diversity can potentially increase in heterogenous habitats where there are more niches available to provide new opportunities for resource partitioning and for species assemblages (Schoener, 1974; Kisel et al., 2011). The high correlations recorded in this study between the most commonly used benthic indices and functional diversity

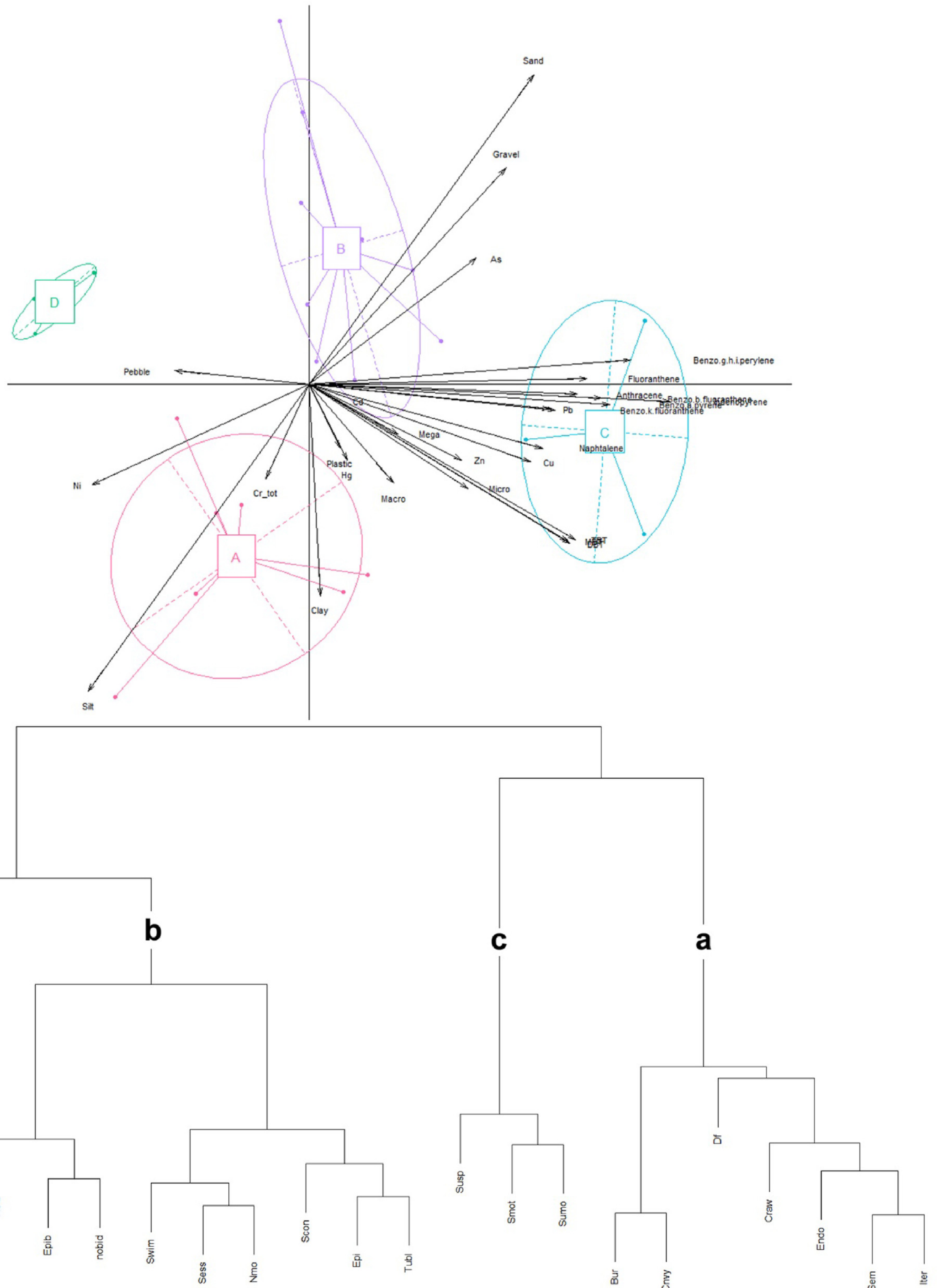

Fig. 5. Functional groups (upper panel) obtained from cluster analysis (lower panel) along the first two RLQ axes traits response to environmental variables.

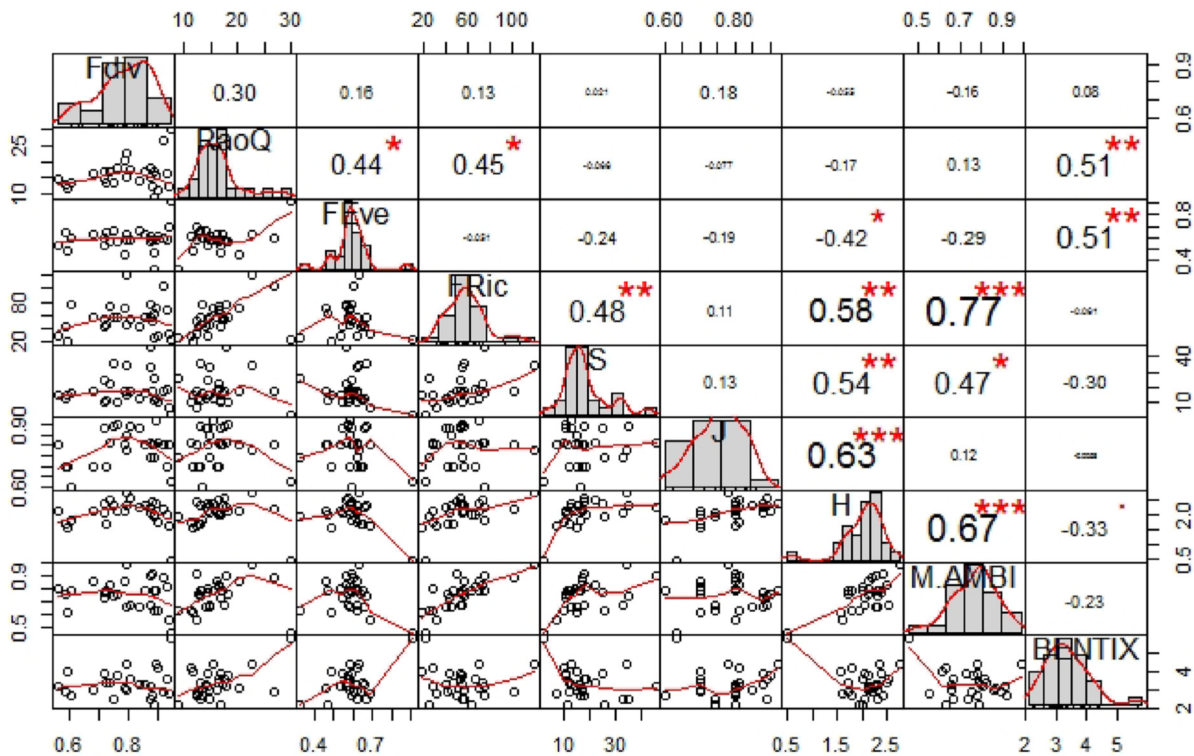

Fig. 6. Pearson's correlations between functional, biodiversity and benthic indices.

(M-AMBI vs FRic; Bentix vs FEve and RaoQ), support the validity of the latter indices in the study of environmental health.

4.3. Functional traits and monitoring assessment

The results of the RLQ analysis underlined the relationships between benthic community functional traits and the degree of environmental pollution. As an example, benthic species characterised by limited or no mobility were highly influenced by seafloor abiotic features. The high correlation values recorded in this study, *i.e.,* between suspension-feeders and silt and PHA, could be explained in terms of the behavioural habits of these species, which normally prevail in low-energy areas, where smaller-sized sediment particles normally accumulate at the surface. The predominance of these fine sediments, in turn, highlighted the availability of food and the presence of high levels of trace elements (Lopez and Levinton, 1987). As evident from the linear model results, the main factors influencing functional diversity were granulometric features and pollutant concentrations; this is supported by results from other studies (Paganelli et al., 2012; Bolam et al., 2017; D'Alessandro et al., 2018).

The highest significant negative correlation recorded between plastic content and evenness indices might be due both to the physical damage exerted by this pollutant to marine benthos and also due to the co-occurrence of plastic with other contaminants added during the plastic production process, or adsorbed onto the plastic from seawater (Auta et al., 2017; D'Alessandro et al., 2018). Although different studies have underlined the effects of plastics on marine organisms, additional studies are required to comprehend the effects of plastics on biodiversity (Avio et al., 2016; Green, 2016). Increasingly, within the domains of conservation and management there is a need to identify areas and habitats that may be especially at risk from multiple stressors (*e.g.,* Vulnerable Marine Ecosystems [VMEs] according to Auster et al., 2010; Ecologically or Biologically Significant Areas [EBSAs] according to Clark et al., 2014). The results of the present study, integrating different indicators and models, underline the importance of functional diversity as a tool to assess the sensitivity of marine ecosystems to several human stressors.

Ecosystem-based management needs to balance the expected outcomes due to various anthropogenic activities and requires metrics, indices and systematic methods able to assess ecosystem sensitivity (Mangano et al., 2017; Sarà et al., 2018). An accurate characterisation of the association between traits and their sensitivity to environmental stressors could be useful in developing an early warning system to predict and anticipate presently unforeseeable habitat depletion.

5. Conclusions

Data from taxonomic (*i.e.*, diversity, richness and composition) and functional (*i.e.*, biological trait analysis and functional diversity indices) approaches showed similar patterns in the composition and variability of benthic communities along a pollution gradient, in which a healthier ecological status was recorded in the less disturbed sites. Our results highlight that the analysis of functional traits provides a useful and rapid method to detect ecosystem disturbance. The functional-based approach, using the application of both biological trait analyses and functional diversity indices, as adopted in the present study, demonstrate its potential applicability within future monitoring programmes.

Table 3
Results of linear models performed on functional diversity indices, biodiversity indices and environmental variables. Significant codes: 0 '***' 0.001 '**' 0.01 '*'.

	Estimate	Std.	Error	P value
FDiv = Silt + TBT + Cr_tot + Cd + Hg + factor (Port)				$R^2 = 0.58$
(Intercept)	0.843	0.0410	20.6	<0.0001 ***
Silt	−0.002172	0.0005520	−3.935	<0.0001 ***
TBT	0.0008092	0.000237	3.414	0.0028 **
Cr_tot	0.007282	0.001601	4.550	<0.0001 ***
Cd	0.9770	0.2983	3.275	0.0038**
Hg	−0.02594	0.008417	−3.082	0.0059**
factor (Port)Malta	−0.2	0.1	−3	0.0045**
factor (Port)Syracuse	0.0	0.0	0.3	0.78
RaoQ = Silt + Cd + Benzo.g.h.i.perylene				$\mathbf{R^2 = 0.44}$
(Intercept)	24.25	1.819	13.34	<0.0001 ***
Silt	−0.1158	0.02497	−4.637	<0.0001 ***
Cd	15.80	11.70	1.350	0.19
Benzo(g,h,i)perylene	−0.01625	0.004639	−3.503	0.0018 **
S = Pebble + Clay + MBT + Cd				$\mathbf{R^2 = 0.52}$
(Intercept)	20.23	2.722	7.432	<0.0001 ***
Pebble	−0.4732	0.3581	−1.321	0.20
Clay	−1.064	0.5346	−1.990	0.059
MBT	0.1367	0.04377	3.123	0.0048**
Cd	95.27	22.32	4.267	<0.0001 ***
J = Silt + Plastic + Hg + Fluoranthene + factor (Depth)				$\mathbf{R^2 = 0.42}$
(Intercept)	0.771	0.0348	22.20	<0.0001 ***
Silt	0.001111	5.283e-04	2.103	0.048*
Plastic	−0.069	0.015	−4.6	<0.0001 ***
Hg	−0.01399	0.006405	−2.184	0.040
Fluoranthene	3.108e-05	1.185e-05	2.622	0.016*
factor (Depth)10	−0.061	0.031	−1.97	0.062
factor (Depth)20	0.014	0.035	0.41	0.69
Bentix = Silt + Naphtalene + factor (Port)				$\mathbf{R^2 = 0.51}$
(Intercept)	3.89	0.285	13.6	<0.0001 ***
Silt	−0.0111	0.003586	−3.085	0.0052 **
Naphtalene	−0.01020	0.005338	−1.910	0.069
factor (Port)Malta	−0.2	0.3	−0.8	0.43
factor (Port)Syracuse	0.8	0.2	3.2	0.0042 **

CRediT authorship contribution statement

Michela D'Alessandro: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Erika M.D. Porporato:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Valentina Esposito:** Formal analysis, Investigation, Writing - original draft. **Salvatore Giacobbe:** Investigation, Supervision. **Alain Deidun:** Investigation, Funding acquisition, Writing - review & editing. **Federica Nasi:** Conceptualization, Methodology, Formal analysis. **Larissa Ferrante:** Formal analysis. **Rocco Auriemma:** Formal analysis. **Daniela Berto:** Investigation, Writing - original draft. **Monia Renzi:** Investigation, Writing - original draft. **Gianfranco Scotti:** Formal analysis, Investigation. **Pierpaolo Consoli:** Investigation. **Paola Del Negro:** Resources, Supervision. **Franco Andaloro:** Resources, Funding acquisition, Project administration, Supervision. **Teresa Romeo:** Investigation, Resources, Funding acquisition, Project administration, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.113959.

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