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Functional diversity of macrobenthic assemblages decreases in response to sewage discharges

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ABSTRACT

We analyzed the effects of sewage discharge on a subtropical estuary by comparing the functional diversity of intertidal macroinvertebrate assemblages in contaminated with non-contaminated reference areas. Functional structure was assessed using biological traits analysis (BTA) and four multivariate indices (FRic, FEve, FDis and Rao's Q) of functional diversity. Our results showed clear and temporally consistent changes in macrobenthic functional structure in contaminated areas. However, these results depended on whether abundance- or biomass-based measurements were used, with abundance-based analyses distinguishing most clearly between sewage contamination conditions. Differences between contaminated and non-contaminated conditions were also displayed by BTA for all the functional trait categories. FDis (functional divergence) and Rao's Q (functional dispersion) were higher in the non-contaminated condition and increased with higher benthic environmental health, as measured by the AMBI index. These patterns of higher functional divergence and dispersion were driven by the numerical dominance of opportunistic annelids in the contaminated condition. We suggest that abundance-based BTA, and the FDis and/or Rao's Q indices are reliable approaches to detect changes in functional structure with respect to sewage pollution. They have a great potential for environmental assessment and monitoring of subtropical estuarine ecosystems.

1. Introduction

Functional diversity is the component of biodiversity that describes the variety of functions developed by organisms in an assemblage, community or ecosystem (Tilman, 2001). It can be quantified by estimating the extent, dispersion and relative abundance of species functional traits (Mason et al., 2005) and provides an informative complement to studies addressing the interrelationship between community structure, environmental heterogeneity and ecosystem functioning (Gagic et al., 2015). Functional traits are defined as any organismal characteristics related to individual performance that can directly or indirectly affect one or more ecosystem properties and processes (Mlambo, 2014; Violle et al., 2007). Studies focusing on functional diversity usually assess how organisms affect ecosystem properties/processes (Gagic et al., 2015) and which environmental factors and disturbances shape assemblage functional trait diversity and distribution along space and time (Gerisch et al., 2012; Bremner et al., 2006a).

In the past two decades, many methods and techniques have been proposed to assess the functional diversity of assemblages. The most widely used are analysis of functional or trophic groups (Gusmao-Junior and Lana, 2015), biological traits analysis (BTA; Bremner, 2008) and calculation of functional diversity indices (Laliberté and Legendre, 2010; Villéger et al., 2008). BTA is based on multivariate ordinations to describe variation patterns of functional traits along spatial or temporal gradients (Chevenet et al., 1994; Dolédec and Chessel, 1994). It has been used in marine benthic systems to assess human impacts like dredging (Wan Hussin et al., 2012) and nutrient input (Paganelli et al., 2012), or spatial and temporal variability of benthic ecosystem functioning (Bremner et al., 2006a,b; Darr et al., 2014; Pacheco et al., 2011). On the other hand, functional diversity indices have varying abilities to reflect the different aspects of assemblage functional trait structure. Functional Richness (FRic), Functional Evenness (FEve), Functional Divergence (FDiv), and Rao's Quadratic Entropy (Rao's Q) are multi-trait measurements that encompass the multivariate aspects of the trait functional structure (Botta-Dukát, 2005; Laliberté and Legendre, 2010; Villéger et al., 2008). FRic, FEve and

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FDiv are interpreted as analogous to taxonomic diversity metrics. Raos'Q estimates functional dispersion by measuring how species differ regarding functional traits, also weighting their abundances. Except FEve that describes abundance distribution, higher values of these functional diversity indices are expected when species in an assemblage differ greatly in their functional traits. These indices have been used to assess the functional diversity of birds (Luck et al., 2013), fishes (Villéger et al., 2010), zooplankton (Massicotte et al., 2014), arthropods (Gerisch et al., 2012), as well as the ecology (Darr et al., 2014), paleontology (Villéger et al., 2011) and biogeography (Berke et al., 2014) of marine benthos.

Despite their high potential applicability in assessing human-driven impacts (Mouillot et al., 2013), functional diversity approaches are scant in marine benthic ecosystems. Metrics such as species diversity (Shannon-Index H'), species richness, environmental quality indices for coastal ecosystems (e.g. AMBI and M-AMBI; Borja et al., 2004; Muxika et al., 2007) and distance-based measures such as Bray–Curtis are the most widely used approaches to assess the effects of anthropogenic disturbance on benthic structure. These metrics, however, do not consider changes in functional trait structure, which are central to understand the effects of human disturbances on ecosystem functioning (Darr et al., 2014). Thus, it is necessary to assess how functional parameters relate to different environmental drivers and disturbances in the marine benthos to test the usefulness of these analytical tools as indicators of ecological status.

One of these disturbances in coastal marine systems is sewage input that is a stress source that may eliminate sensitive in favor of tolerant or opportunistic species (Borja et al., 2000). Since tolerance levels and the ability to explore environmental resources are both related to species functional traits, a contamination gradient may affect the functional structure of macroinvertebrate assemblages. Based on these assumptions we predict: (1) the functional traits composition of macrofaunal assemblages will change depending on the sewage contamination condition; (2) lower functional diversity values are expected in contaminated areas; and (3) functional diversity indices will be negatively related to indicators of bad environmental quality. To test these hypotheses, we analyzed the effects of distinct levels of sewage discharges on the functional diversity of macroinvertebrate assemblages inhabiting tidal flats of a subtropical estuary. Biological trait analysis and the calculation of four functional diversity indices were used to test our predictions.

2. Material and methods

2.1. Study area

This study was carried out in the Cotinga sub-estuary (25°31′47″S, 48°28′03″W) of Paranaguá Bay, Brazil. Paranaguá Bay, covering 612 km^2, is one of the largest and best preserved estuarine systems of southern Brazil. The climate is strongly influenced by the semi-permanent South Atlantic anti-cyclone and by the passage of polar fronts during the winter (Lana et al., 2001). The water balance of the region differs significantly between the rainy season (late spring and summer) and the dry season (late fall and winter), and it depends on freshwater input.

The Cotinga sub-estuary extends for nearly 15 km and is located in the polyhaline sector of the Bay, near the mouth of the estuary (Fig. 1). The tidal regime is semi-diurnal with mean neap and spring tidal heights of 1.3 and 1.7 m, respectively, and mean water depth of 5.4 m (Lana et al., 2001; Marone and Jamiyanaa, 1997). Intertidal habitats cover about 34% of its surface area and include mangroves, marshes and tidal flats (Brauko et al., 2015; Noernberg et al., 2006).

The Cotinga sub-estuary is the main dilution path for the sewage discharged from Paranaguá city (Barboza et al., 2013; Souza et al., 2013). Only 60% of the sewage output of about 150,000 inhabitants undergoes treatment, while the rest is released *in natura* to the sub-estuary (CAB, 2015). From the inner area to the outer part of the sub-estuary, there is a compressed, sharp gradient of domestic sewage contamination indicated by decreasing levels of *Escherichia coli* activity, and concentrations of fecal steroids, which are highly stable organic markers (Barboza et al., 2013; Martins et al., 2010). As in most coastal cities in developing countries, it is very difficult to estimate safely the amount of discarded sewage, either by lack of governmental transparency or clandestine disposal. Thus, the degree of environmental integrity can be evaluated only from reliable biological indicators. The strongest signal for sewage contamination is the coprostanol level, a fecal steroid confined to sites close to Paranaguá city (Martins et al., 2010). These sites have average coprostanol concentrations of up to 1.69 $\mu g\, g^{-1}$, which are above the threshold limits of heavy contamination (>0.5 $\mu g\, g^{-1}$). As the distance from the sewage source increases, these concentrations decrease (Abreu-Mota et al., 2014; Brauko et al., 2015).

2.2. Sampling

Sampling surveys were carried out over two years (2011 and 2012) using a hierarchical sampling design to evaluate the spatial and temporal variability of intertidal macrobenthic assemblages in response to distinct levels of sewage contamination. The three spatial scales included: kilometers (two conditions of sewage contamination: contaminated and non-contaminated), hundreds of meters (two different tidal flats within the contaminated and non-contaminated conditions) and tens of meters (four sites per tidal flat with three replicates each). The three temporal scales sampled included years (2011 and 2012), seasons (summer and winter), and fortnights (three replicates per season). Tidal flats did not exhibit significant differences in salinity, sediment texture, exposure to tides and slope (Noernberg et al., 2006; Souza et al., 2013).

Replicated samples of macrofauna were collected using PVC corers (10 cm diameter, 10 cm deep), and all sampling points were taken in parallel to the water line, at similar tidal levels. Samples were sieved through a 0.5 mm mesh, fixed in 6% formaldehyde and preserved in 70% alcohol. In the laboratory, organisms were counted and identified to the lowest possible taxonomic level. A sediment sample was taken at each site to determine total phosphorus (P), total nitrogen (N) and total organic carbon (C) contents. Additional sediment samples were taken at each tidal flat to determine grain size, sediment sorting coefficient, mud content, coprostanol concentration, salinity, carbonate content ($CaCO_3$), and depth of the redox layer. Analyses of sediment parameters followed Suguio (1973). Organic matter content was determined by change in weight after burning 5 g of sediment at 550 °C for 60 min. Carbonate content was estimated by change in weight after acidification of 10 g of sediment in 20 ml of 10% HCl. The sampling, extraction, fractioning and analysis of the organic marker coprostanol were made following the methods described by Kawakami and Montone (2002). Concentrations of N and P followed Grasshoff et al. (1983), and the concentrations of C were measured with the oxidation method (Strickland and Parsons, 1972). The variation trends of environmental variables in each tidal flat were analyzed using principal component analysis (PCA). Samples from different fortnights and sites (for P, N and C) were averaged together within the same season and tidal flat.

2.3. Biological traits analysis

To analyze how the macrofaunal functional trait composition varies among different tidal flats, conditions and seasons, we used Biological Traits Analysis (BTA). Although including as many traits as possible for the BTAs does increase the general information about

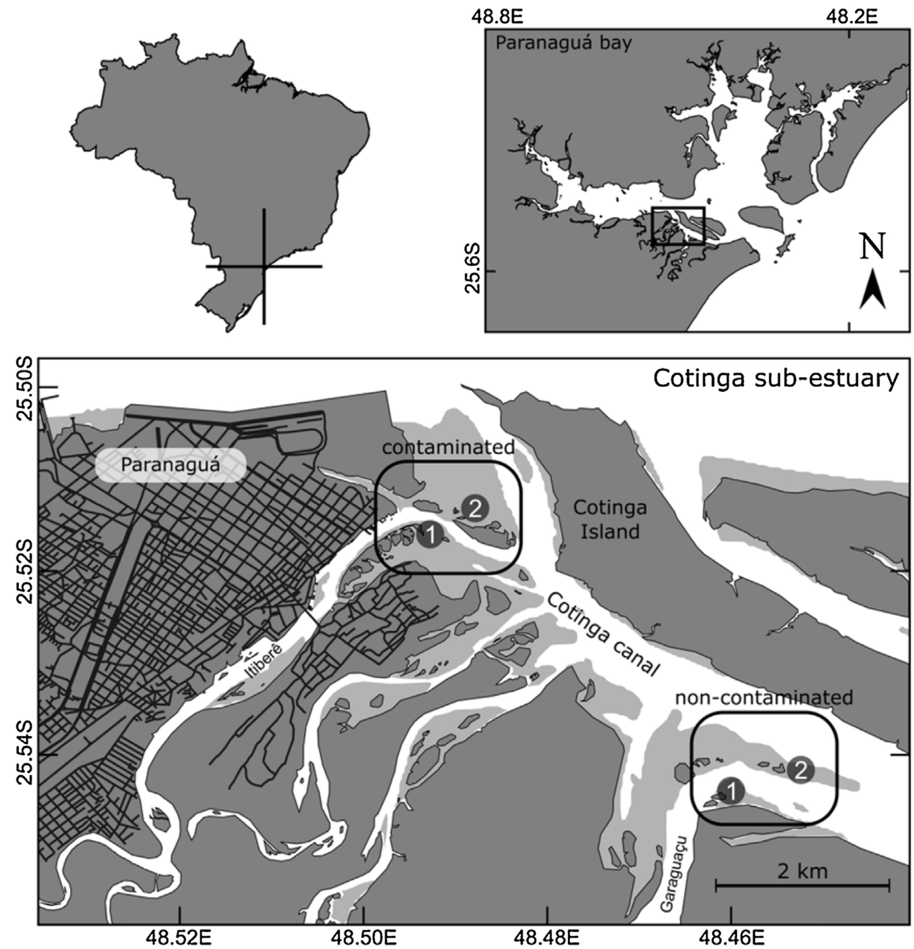

Fig. 1. Location of the Cotinga sub-estuary in Paranaguá Bay. The distribution of tidal flats are depicted in light gray. The contaminated and non-contaminated conditions and the sampled tidal flats (1 and 2) in each condition are highlighted.

changes in functional trait distribution along gradients (Bremner et al., 2006a), we chose to use only five functional traits related to bioturbation. Focusing on traits directly related to sediment bioturbation would give a better picture of animal-mediated sediment processes such as nutrient exchange through the sediment-water interface. Moreover, the use of BTAs in this study aimed to complement the information given by the functional diversity indices which values can be biased when unnecessary trait information are included (Lefcheck et al., 2015). The five traits are bioturbation type, depth penetration in the sediment in cm, body size in mm, feeding mode, and relative adult mobility. Based on Jones and Frid (2009), the five traits were further sub-divided into several modalities (Appendix 1) to represent the range of variation for each functional trait.

Fuzzy coding (Chevenet et al., 1994), with a scoring ranging from 0 to 3, was used to classify each taxon according to its association with different modalities of functional traits. No affinity for a trait was coded as 0 and complete affinity as 3. Information regarding the functional traits for each species was gathered from online data bases (Faulwetter et al., 2014; MarLIN, 2006), literature (Arruda et al., 2003; Jumars et al., 2015; Queirós et al., 2013; Rios, 2009) and ad hoc information from local specialists. Trait scores of each taxon were multiplied by its abundance for every sample and subsequently summed to provide a matrix with the overall frequency of each trait modality per sample. This matrix was square root transformed and analyzed with PCA in the statistical software R (R Development Core Team, 2009) using the packages "vegan" (Oksanen et al., 2009) and "ade4" (Dray and Dufour, 2007).

2.4. Functional indices

Four multivariate functional indices were calculated to assess different components of the functional diversity: FRic, FEve, FDiv and Rao's Q. These indices are considered adequate and complementary measures of functional diversity components for different environments and assemblages (Laliberté and Legendre, 2010; Mouchet et al., 2010; Schleuter et al., 2010; Schuldt et al., 2014). They were calculated on the basis of the fuzzy matrix of functional traits with the R package "FD" (Laliberté and Legendre, 2010). Each index measures different aspects of the multivariate functional space constructed by a principal coordinates analysis (PCoA) based on the Euclidian dissimilarity matrix of species traits. FRic estimates the amount of functional space occupied by a given species assemblage by calculating the convex hull volume that comprises the entire trait space filled by all species of this assemblage (Villéger et al., 2008). It can thus be used as a proxy of the range of functional traits represented in an assemblage, but does not take into account differences in species abundance (Schuldt et al., 2014). FRic is affected by the addition or removal of species with unique trait combinations. FEve index estimates the regularity of the abundance distribution in trait space by summing the branch lengths of the minimum spanning tree that is required to connect all species in an assemblage weighted by the species abundances (Villéger et al., 2008). FDiv measures the distribution of the species abundances in relation to the gravity center of the functional trait space (Villéger et al., 2008). The Rao's quadratic entropy index (Rao's Q) is calculated as the product of the distance between pairs of species in the trait space, based on the differences of their traits values and weighted by the relative abundance of the species (Botta-Dukát, 2005). We did not include the index Functional Dispersion (FDis) of Villéger et al. (2010) because of its high collinearity with the Rao's Q index (Laliberté and Legendre, 2010). We chose Rao's Q rather FDis to quantify functional dispersion since it is more commonly used in ecological studies.

The AMBI index (AZTI Marine Biotic Index), a macrobenthic assemblage based index for environmental health assessment, was also calculated to compare the sensitivity of the four functional diversity indices to detect structural changes in assemblages inhabiting areas subjected to distinct levels of organic contamination. AMBI was calculated using the software available on AZTI's web page (http://ambi.azti.es). The index is based on the percentage of each of five ecological groups with different sensitivity/tolerance levels to organic pollution, which are listed in the software (Borja et al., 2000, 2003). The AMBI values vary from 0 to 7, where each numerical interval represents an environmental ecological status as follows: 0–1.2 corresponds to *High*; 1.2–3.3 for *Good*; 3.3–4.3 for *Moderate*; 4.3–5.5 for *Poor* and 5.5–7 for *Bad* status. Since some species or taxa present at Paranaguá Bay are not as yet assigned into the AMBI list, we followed the protocol of Brauko et al. (2015) to classify the species into each ecological group. AMBI has been recently indicated as a suitable index to assess the environmental health of Cotinga sub-estuary (Brauko et al., 2015).

2.5. Data analysis

Mixed model ANOVAs were used to compare the differences of the functional diversity indices values between contamination conditions and seasons. As we did not have a priori predictions addressing different scales of variability and considering the complexity in analyzing a six-factor mixed ANOVA, we chose to simplify the linear model to a three factor design: season (fixed, two levels: winter and summer), condition (fixed, two levels: contaminated and non-contaminated) and tidal flat (random, two levels, nested in condition). As temporal variability can be confounded by spatial variability over short scales, samples from different fortnights were averaged together within the same season. Events (years 2011 and 2012) were pooled together as replicates of seasons. Variance heterogeneity was analyzed using Cochran test and transformations were applied when necessary. ANOVAs and Cochran's test were run in R software using the package "GAD" (Sandrini-Neto and Camargo, 2010).

The relationship between each functional diversity index and AMBI was tested by fitting linear models between the AMBI index and each of the four functional diversity indices. Different models were generated using linear and polynomial regressions and generalized linear models. The best models were chosen according to

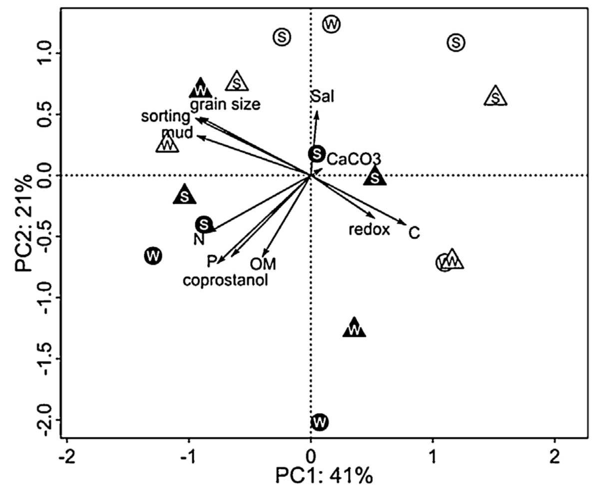

Fig. 2. Principal Component Analysis (PCA) depicting the environmental variables for each season, condition and tidal flat. W = winter, S = summer, black circles = contaminated, white circles = non-contaminated, circles = tidal flat 1, triangles = tidal flat 2, OM = organic matter content, Sal = salinity, sorting = sediment sorting coefficient, C = total organic carbon, N = total nitrogen, P = total phosphorus.

the Akaike information criterion (i.e. AIC smallest value). We also applied this methodology to test the dependence of each functional index in relation to species richness.

3. Results

3.1. Environmental variables

The PCA ordination of the 10 environmental variables revealed gradients within the first two axes (Fig. 2). The fist axis explained 41% of the total variability and was associated to changes in sediment grain size, mud content, sorting coefficient, and C, P and N contents. The second axis explained 21% of the total variability and was associated to changes in salinity, coprostanol, P, and organic matter content. The second axis distinguished tidal flats in the contaminated condition from those in the non-contaminated condition. This difference was related to increasing organic matter content, coprostanol and total phosphorus concentrations and salinity decrease in the contaminated condition (Table 1). There was no clear separation between different seasons.

3.2. Species composition, species richness and AMBI

Numerically dominant taxa in the contaminated condition were one unidentified oligochaete species (Tubificinae sp1) and the polychaete *Laeonereis culveri* Webster, 1879, while the

Table 1
Mean values (±SD) of environmental variables by season and condition.

Environmental variables	Winter		Summer	
	Contaminated	Non-contaminated	Contaminated	Non-contaminated
Coprostanol ($\mu g\, g^{-1}$)	4.02 ± 3.16	0.11 ± 0.13	1.11 ± 0.24	0.01 ± 0.01
Total organic C ($mg\, g^{-1}$)	15.71 ± 7	16.19 ± 8.14	18.64 ± 8.62	18.07 ± 10.88
Total P ($mg\, g^{-1}$)	2.14 ± 1.3	1.32 ± 0.96	1.55 ± 0.8	0.67 ± 0.43
Total N ($mg\, g^{-1}$)	1.88 ± 1.97	1.4 ± 1.54	2.12 ± 1.6	0.92 ± 1.14
Mud (%)	5.18 ± 3.00	3.91 ± 3.45	5.64 ± 1.31	4.09 ± 2.06
Grain size (ϕ)	1.74 ± 0.014	1.74 ± 0.023	1.75 ± 0.013	1.75 ± 0.022
Sorting coefficient	0.68 ± 0.20	0.55 ± 0.19	0.68 ± 0.12	0.65 ± 0.16
$CaCo_3$ (%)	1.47 ± 0.17	1.54 ± 0.14	1.45 ± 0.23	1.40 ± 0.22
Organic matter (%)	1.90 ± 0.12	1.71 ± 0.14	1.40 ± 0.10	1.33 ± 0.18
Redox depth (cm)	1.46 ± 0.98	2.00 ± 0.34	0.77 ± 0.15	1.62 ± 0.31
Salinity (ppm)	27.15 ± 1.98	28.56 ± 2.66	23.58 ± 1.52	27.37 ± 1.71

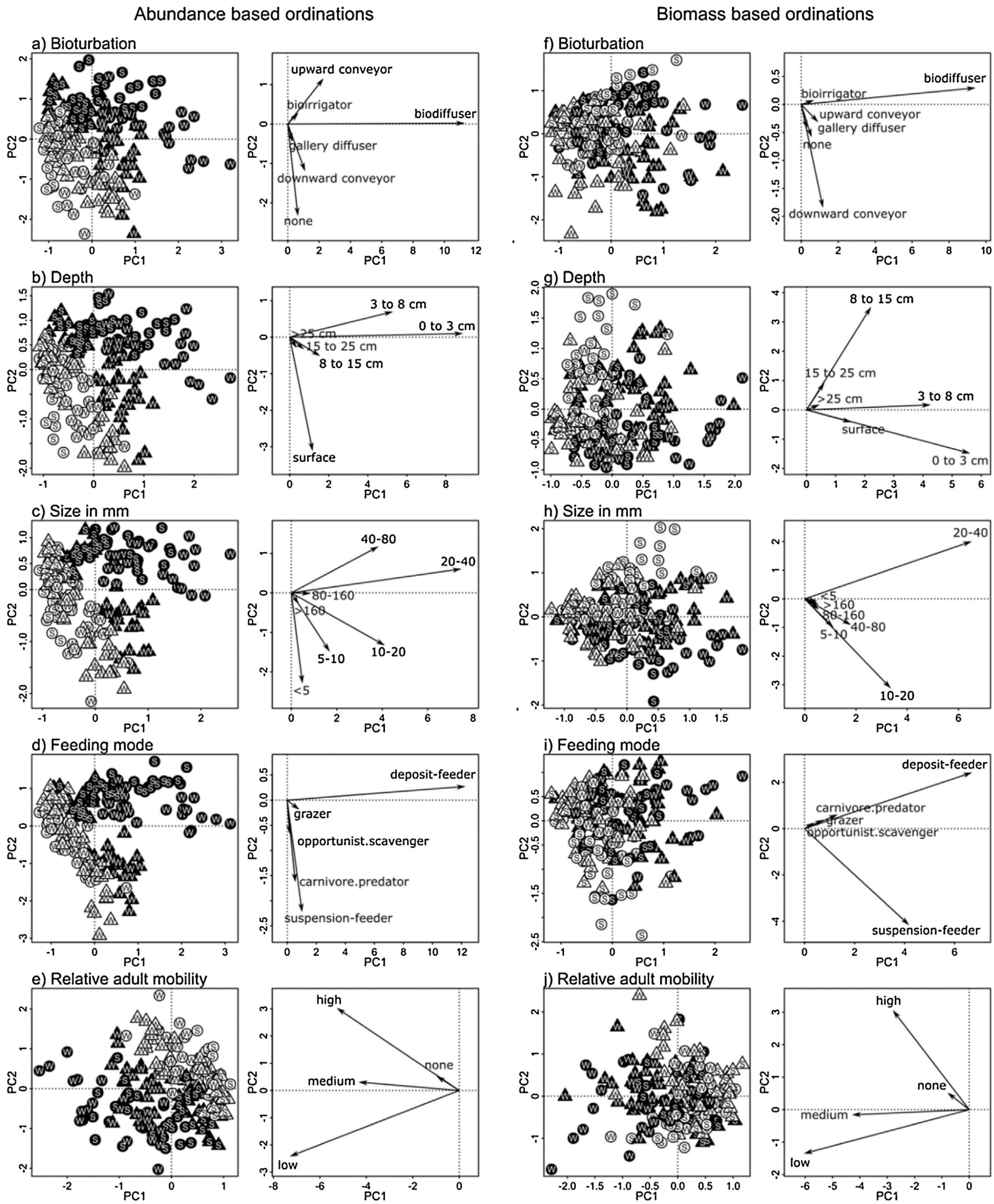

Fig. 3. PCA ordinations depicting the variability in assemblage trait composition across season and condition. W = winter, S = summer, black symbols = contaminated, white symbols = non-contaminated, circles = tidal flat 1, triangles = tidal flat 2.

non-contaminated flats were numerically dominated by the gastropod *Heleobia australis* d'Orbigny, 1835 and, again, Tubificinae sp 1. The bivalves *Anomalocardia flexuosa* Linnaeus, 1767 and *Tellina versicolor* De Kay, 1843 had the highest biomass values in both contaminated and non-contaminated condition. Species richness was significantly higher in the winter than in the summer (Table 3, Fig. 4a). The contaminated condition displayed slightly higher species richness than the non-contaminated condition in the winter. Also, differences between tidal flats within each condition were higher in the winter. The AMBI index values were significantly higher on the contaminated tidal flat, although there was also high variability across tidal flat and season (Fig. 4c).

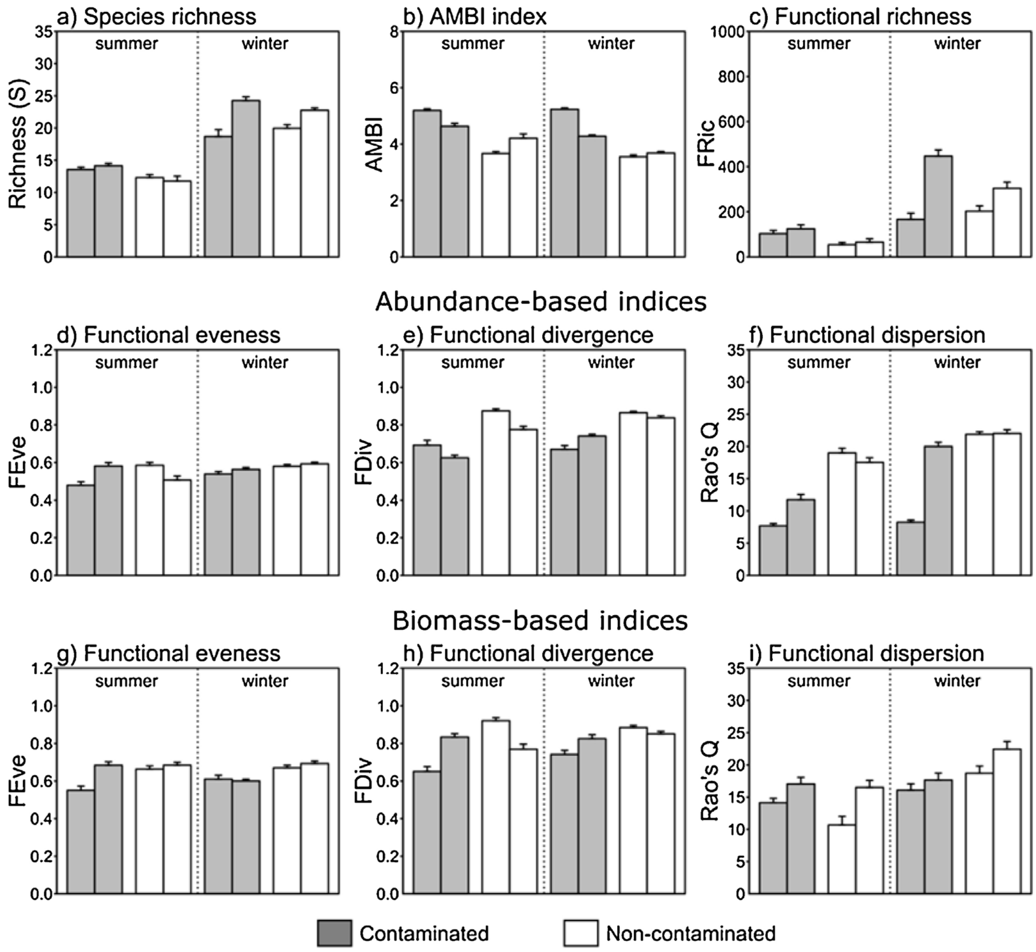

Fig. 4. Mean values (± SE) of species richness (a), AMBI index for environmental health (b), functional richness (c), the abundance (d–f) and biomass-based (g–i) functional evenness, functional divergence and functional dispersion for tidal flat, condition and season.

3.3. Biological traits analysis

All the BTAs based on species abundance showed a clear separation in assemblage functional trait composition between the contaminated and non-contaminated conditions (Fig. 3a–e). This separation was associated mainly with the first axis (Table 2). Assemblage trait composition in the contaminated condition were characterized by upward-conveyors and biodiffusers, limited penetration in the sediment (between 0 and 8 cm deep), medium size (between 20 and 80 mm), deposit feeders, and low to medium relative mobility. Traits composition in the non-contaminated condition was characterized by non-bioturbators and downward-conveyors, epifauna, small size (10 mm or less), carnivores and suspension feeders, and high relative motility. Gradients in trait composition between contaminated and non-contaminated condition were less differentiable in the BTAs based on species biomass (Fig. 3f–j). All the BTAs also showed clusters of assemblages sampled in the same tidal flat and season.

Table 2
Explained variation associated to the PCA-axis for the abundance and biomass-based BTAs.

	Bioturbation (%)	Depth (%)	Size (%)	Feed (%)	Mobility (%)
Abundance-based BTAs					
PCA1	90.8	89.7	83.9	93.9	83.3
PCA2	5.5	8.5	9.9	4.9	12.8
Biomass-based BTAs					
PCA1	91.8	73.0	72.3	70.0	75.2
PCA2	4.0	18.0	19.2	25.6	13.6

3.4. Functional diversity

Spatial and temporal variation in the functional diversity of the assemblages were driven by changes in the most numerous species (abundance based indices) and those with the higher biomass (biomass-based indices; see Appendices 2 and 3). The FRic (Table 3, Fig. 4c) followed the trends observed for species richness (Fig. 4a), showing significantly higher values during the winter, with high variability across tidal flats. Abundance and biomass-based FEve showed similar variation patterns (Table 3, Fig. 4d and g), which differences between conditions were dependent on the seasons and tidal flats. FDiv (Table 3, Fig. 4e and h) showed temporally consistent patterns, which the non-contaminated condition presented values significantly higher than the contaminated. Abundance and biomass-based Rao's Q showed contrasting variation patterns (Table 3, Fig. 4f and i). Abundance-based Rao's Q showed significantly higher values in the non-contaminated condition (Fig. 4f), while for the biomass-based index this differences were highly dependent on the season (Fig. 4i). Also, the variability within each condition (between tidal flats) showed different patterns in abundance and biomass-based analysis. For both functional divergence and functional dispersion, the largest contrasts between conditions were observed for the abundance-based indices. In general, whether there were significant differences between contaminated

Table 3
Analysis of variance comparing the mean values of species richness, AMBI index and the four functional diversity indices (based on abundance and biomass). The type of transformations used to correct variance heterogeneity are indicated (superscripted numbers).

Source	df	Species richness[1]		AMBI[2]		FRic[1]	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Season – Se	1	37.71	**<0.05**	3.02	0.22	17.29	0.05
Condition – Co	1	0.71	0.49	7.07	0.12	1.73	0.32
Se * Co	1	1.17	0.39	0.41	0.59	2.24	0.27
Flat – Fl(Co)	2	9.34	**<0.001**	44.18	**<0.0001**	8.90	**<0.001**
Se * Co * Fl(Co)	2	8.67	**<0.001**	5.15	**<0.01**	6.95	**<0.01**
Residual	184						
Source	**df**	**FEve**		**FDiv[1]**		**Rao's Q**	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Abundance-based indices							
Season – Se	1	1.06	0.41	0.91	0.44	4.26	0.18
Condition – Co	1	0.51	0.55	26.59	**<0.05**	4.25	0.18
Se * Co	1	0.10	0.78	0.09	0.80	0.03	0.87
Flat – Fl(Co)	2	11.00	**<0.0001**	7.36	**<0.001**	87.96	**<0.0001**
Se * Co * Fl(Co)	2	7.68	**<0.001**	12.60	**<0.0001**	21.52	**<0.0001**
Residual	184						
Source	**df**	**FEve**		**FDiv**		**Rao's Q[1]**	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Biomass-based indices							
Season – Se	1	0.0002	0.99	0.69	0.49	44.31	**<0.05**
Condition – Co	1	3.93	0.19	1.33	0.37	0.10	0.78
Se * Co	1	0.06	0.83	0.05	0.84	21.07	**<0.05**
Flat – Fl(Co)	2	8.15	**<0.001**	32.15	**<0.0001**	11.99	**<0.0001**
Se * Co * Fl(Co)	2	9.16	**<0.001**	7.23	**<0.001**	0.66	0.52
Residual	184						

[1] log transformed.
[2] Square root transformed.

and non-contaminated conditions depended on the season and tidal flat analyzed.

The relationship between the functional indices and the AMBI index depended on how the macrobenthos was quantified (Fig. 5). All the abundance based indices were negatively related with the AMBI (i.e. increased with increasing environmental health Fig. 5b, d and f). For the biomass-based indices, only FDiv was significantly related with AMBI, with a bell-shaped relationship (Fig. 5e). FRic showed no significant relationship with AMBI (Fig. 5a) but was the only functional index positively related with species richness ($p < 0.05$, $r^2 = 0.79$, Appendix 4).

4. Discussion

We have shown that macrobenthic functional structure can be successfully used as a complement to describe sewage contaminated areas. However, its usefulness depends on the selected metrics to quantify the macrobenthos. Gradients in functional trait composition across contamination conditions were evident for the abundance-based BTAs but not for the biomass-based analysis. Variation in functional diversity between contamination conditions were also variable depending on the index used but the indices FDiv (for functional divergence) and Rao's Q (for functional dispersion) showed temporally consistent patterns with higher values in the non-contaminated condition. In addition, there was a negative relationship between functional diversity indices and the AMBI environmental quality index for the abundance-based indices, but not for the biomass-based indices. Thus, abundance-based functional diversity indices and BTAs represent an useful and informative tool to describe the different aspects of organic pollution impacts in benthic assemblages, with high potential for application for environmental assessment and monitoring.

Gradients in functional trait composition across conditions were clearly observed in the abundance-based BTAs but not in the biomass-based analysis; therefore, our first prediction was only partially supported. Patterns in functional diversity variation between conditions varied greatly depending on the index. The indices FDiv (for functional divergence) and Rao's Q (for functional dispersion) showed temporally consistent patterns with higher values in the non-contaminated condition, which is in accordance with our second prediction. However, the most notable contrasts between conditions were observed for the abundance-based analyses. Also, our third prediction was only partially supported since negative relationships between functional diversity indices and the AMBI environmental quality index were only observed for abundance-based indices.

4.1. Biological traits analysis

The abundance-based BTAs showed clear patterns of functional turnover for all trait types across different conditions, depicting an effect of sewage contamination on macrobenthic functional composition. First, the predominance of deposit-feeders in the contaminated condition repeats already described patterns for the Cotinga sub-estuary (Souza et al., 2013) and other benthic systems (Borja et al., 2000; Pagliosa and Barbosa, 2006). The dominance of this functional group was driven by high numbers of Tubificinae spp. and *Laeonereis culveri*, presumed to be opportunistic species in eutrophic coastal waters (Brauko et al., 2015; Elliott and Quintino, 2007; Pagliosa and Barbosa, 2006). Second, the dominance of carnivore/predators and suspension feeders in the non-contaminated condition could be related to increased sediment quality, which

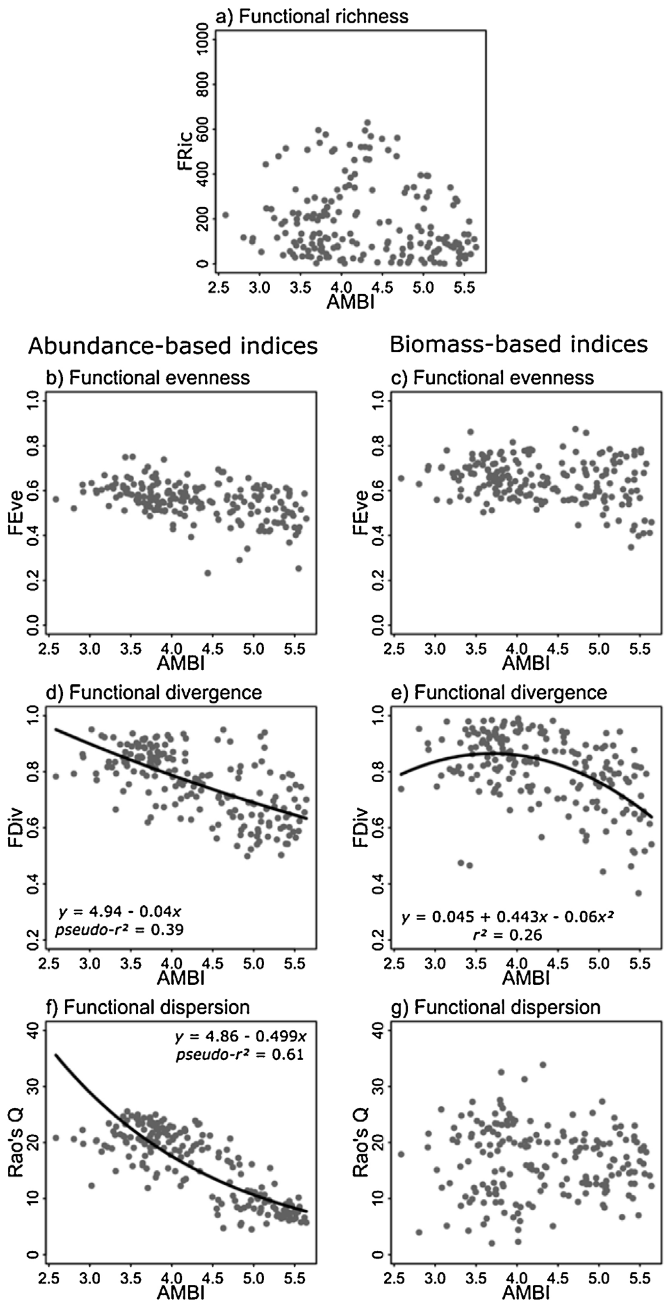

Fig. 5. Relationship between the functional and AMBI indices.

allows for the establishment of species with different feeding habits (Rosenberg, 2001). However, the predominance of medium-sized organisms (20 to 80 mm) with low motility and relatively high bioturbation capacity (3 to 8 cm) in the contaminated condition somewhat differs from predictions of conceptual models for macrofaunal responses to pollution gradients (Pearson and Rosenberg, 1978; Rosenberg, 2001). These patterns were driven by the dominance of annelids in the contaminated condition and the small gastropod *Heleobia australis* d'Orbigny, 1835 in the non-contaminated condition. These differences in trait composition together with their relative consistency over time highlight the potential effects of stress sources associated with the pollution gradient on benthic functional structure. Moreover, the high variability observed among tidal flats shows that small-scale habitat changes within each condition may also play a major structuring role in local tidal flats (Hewitt et al., 2008).

4.2. Functional diversity indices

The tested functional diversity indices showed clear differences in the abundance-based analyses. The use of functional evenness (FEve) and functional richness (FRic) did not reveal differences in macrobenthic functional structure between contamination conditions in our analyses. FEve did not show any consistent trend between the conditions, and FRic tended to be collinear with species richness. Also, the fact that FRic values only can be affected by adding or removing species with unique combinations (or extreme values) of functional traits categories (Villéger et al., 2008) decreases its power to detect moderate effects of disturbance. Considering that moderate levels of sewage contamination in marine systems could only affect species abundance rather than species presence, the estimation of other components of functional diversity such as functional divergence or functional dispersion could be more sensitive to this kind of impact (Mouillot et al., 2013). Indeed, the lower functional divergence and functional dispersion in the contaminated condition indicate assemblages with a lower relative abundance of species with extreme/unique categories of functional traits (Gerisch et al., 2012). Also, these two functional diversity indices showed a clear negative relationship with the AMBI environmental quality index, indicating that assemblages with lower functional divergence and dispersion are associated with low levels of biotic quality. However, since the nature of the relationship between these indices and the benthic environmental quality is not entirely clear, they are yet to be assessed as proxies for benthic environmental health.

4.3. Differences between abundance and biomass-based analysis

Despite the apparent inconsistencies between abundance- and biomass-based analyses, these responses corroborate the classical Pearson and Rosenberg model for organic contamination impacts on benthic assemblages. Their conceptual model predicts the responses of abundance, biomass and species richness to eutrophication gradients in benthic systems. Since these three descriptive components of benthic assemblage structure are not expected to be collinear along such gradients (Pearson and Rosenberg, 1978; Rosenberg, 2001), results could differ depending on the use of abundance or biomass as predictive measures. This effect is associated to the numerical dominance of small-sized opportunistic species at moderately enriched levels, which do not represent an expressive part of the biomass. Our results showed that the opportunistic annelids Tubificinae spp. and *L. culveri* were dominant in contaminated conditions and represented the main drivers of abundance-based results. In general, biomass-based analyses reflected the weight of bivalves, which seemed indifferent to the moderate local levels of sewage input. However, when considering a natural salinity gradient, Darr et al. (2014) detected functional changes only for biomass-based analysis. This discrepancy is a possible result of the use of a different set of traits and to the distinct nature of the environmental filter itself. Therefore, we suggest that abundance-based analysis in functional assessments are more suitable when either numerically dominant or heavier indifferent species are expected to thrive, as in moderate pollution situations. Biomass-based analysis, on the other hand, still seems an unpredictable surrogate for abundance, especially in response to natural environmental gradients.

4.4. Ecosystem functioning effects

Changes in the functional structure also indicate potential changes in ecosystem functioning across conditions. Since all the functional traits used in this study are related to bioturbation (Jones and Frid, 2009; Jumars et al., 2015; Kristensen et al., 2012), any reduction in functional diversity would be associated with a limitation of species modes in exploring and modifying the sediment. This change in the functional diversity of bioturbation could affect specific ecosystem processes, such as nutrient exchange across the sediment–water interface (Mermillod-Blondin, 2011). Such potential effects suggest that lower macrobenthic functional diversity in the contaminated condition could indicate lower rates of nutrient exchange through the sediment-water interface. However, assessments of the magnitude of biogeochemical losses in response to functional diversity shifts still require further investigations.

4.5. Cautions and limitations

The interpretation of the BTAs and the functional diversity indices depends on the selected functional traits and choices made during the analytical process (Bremner et al., 2006a; Lefcheck et al., 2015; Poos et al., 2009). Different traits can show distinct variation trends of their trait categories along gradients (Lavorel and Garnier, 2002). They also can be related in contrasting ways with specific ecosystem properties. For instance, functional traits related to dispersion, such as larval type and reproductive mode, are especially important to analyze colonization and recolonization after disturbances (e.g. Boström et al., 2010; Pacheco et al., 2013), but are not necessarily relevant for sediment-related processes. The use of multiple trait types would increase the amount of information regarding general aspects of ecosystem functioning (Bremner et al., 2006a) but does not represent a good choice when a specific ecosystem processes are focused (Lefcheck et al., 2015). Since we analyzed the potential implications of changes in functional trait structure driven by organic pollution, the selection of a specific set of traits related to bioturbation probably represent the most objective way to address this. Moreover, specific studies would be necessary to test how changes in trait type and number would affect analysis about the interrelationship among functional assemblage structure and specific ecosystem properties and processes.

Even though our results indicate a clear and temporally consistent effect of sewage condition on the functional structure of benthic assemblages, caution should be used in deriving any conclusions about this relationship. Although we have incorporated different spatial and temporal scales to detect the range of macrobenthic variability along space and time, the limited replication of environmental data constrained our capacity to build predictive models. Also, the sampling technique can also have an effect on the results since our sampling unit (corers: 10 cm diameter, 10 cm deep) could underestimate the abundance of big-sized animals with high sediment penetration capacity (e.g. polychaetes from the family Eunicidae and Onuphidae). Analytical procedures like adding, averaging and pooling samples together could attenuate the bias driven by the big animals (Beukema and Dekker, 2011), but not in the case of deep burrowers. Thus, for a complete picture of the functional structure of macroinvertebrate in the studied system, the incorporation of different sampling techniques (e.g. corers and counting of megafauna individuals in delimited areas) could be a useful approach. Moreover, the descriptive nature of our study does not allow the establishment of cause–effect relationships but only the detection of variation trends. Thus, the design of experimental studies incorporating the effect of different levels of sewage pollution on the macrobenthic functional diversity would represent a robust way to test the consistency of the herein described patterns in response to sewage contamination.

5. Conclusion

This work is the first spatially and temporally replicated study that describe variation patterns of functional diversity of estuarine macrobenthic assemblages in response to sewage contamination. Temporally consistent variations in macrobenthic functional structure across different sewage contamination conditions indicate evidence of sewage pollution in a subtropical estuarine habitat. Variation patterns derived from abundance-based metrics were more evident than those based on biomass-based metrics. The significant relationship between the abundance-based functional diversity indices (in particular, FDis and Rao's Q) and the AMBI index indicates that functional diversity is related to benthic environmental health. Therefore, we suggest BTA and functional diversity indices FDis and Rao's Q as a reliable approach to detect changes in functional assemblage structure along organically enriched gradients. This approach holds a high potential for application in real-world assessments and monitoring environmental quality.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2016.01.003.

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