

# Exploring ecosystem functioning in two Brazilian estuaries integrating fish diversity, species traits and food webs

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**ABSTRACT:** Ecosystem functioning is assessed through research that spans multiple biological organisational levels and disciplines, and it is quantified by measuring the magnitude and dynamics of several processes. This quantification is done using different tools, and the resulting information from each tool is often interpreted independently. Our main aim was to contribute to the understanding of the functioning of tropical estuarine ecosystems facing anthropogenic impact, using information on fish diversity, traits and food webs. We used biological and environmental data from 2 Brazilian estuaries with different levels and types of human disturbance: the heavily impacted Paraíba estuary and the environmentally protected Mamanguape estuary (IUCN, Protected Area Category V). Fish assemblages and their prey were collected along the salinity gradient in the dry and wet seasons, and we evaluated (1) species taxonomic diversity, (2) functional diversity based on effect traits and (3) food-web structure through analyses of the natural abundance of stable isotopes (carbon and nitrogen). Species turnover, i.e. the change in species identity, along each estuary was relatively high, and this dissimilarity was considerably greater than the trait turnover (i.e. low functional  $\beta$ -diversity). These results highlight that, although habitat selection was an important driver of the coexistence of fish species due to spatial environmental gradients, the competition for resources might be high because species tended to use the system in a similar way. The relatively narrow range of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among fish species also implies competition for the same resources. Moreover, the  $\delta^{15}\text{N}$  values indicated anthropogenic inputs of nitrogen in both estuaries, regardless of their protection framework, which highlights the need for more effective conservation measures.

**KEY WORDS:** Disturbance · Functional diversity · Habitat filtering · Redundancy · Stable isotopes · Tropical

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## INTRODUCTION

Marine ecosystems are complex and their study is a challenge. Our scientific background, the resources available and, most importantly, the research questions posed drive the way we look at marine ecosystems and the approaches we use to examining them.

Different structural elements, such as species taxonomic diversity, abundance and biomass, provide essential information on the system. These structural elements have even been used to describe and monitor the marine ecosystem and have furthermore been proposed as surrogates to evaluate the condition of the ecosystem (Gray & Elliott 2009, Lefcheck

et al. 2014, Strong et al. 2015). However, this sort of measurement is static, providing only a snapshot of the system at a determined space and time, and the patterns can be considerably different when functional or other more integrative approaches are used (Devictor et al. 2010, Cadotte et al. 2011, Dolbeth et al. 2012, Henriques et al. 2014). Crucial processes and functions occur, which are not visible using a structural approach (Mouillot et al. 2011, Solan et al. 2012). Examples of such processes and functions are nutrient cycling, the amount of material exported daily by the system, bioerosion or the key role of particular species in the flow of energy within the system (e.g. Strong et al. 2015).

Research into marine ecosystem functioning has intensified in the last decades focusing mostly on the role of biodiversity in maintaining functioning that ultimately affects the provision of ecosystem services essential to mankind (Solan et al. 2012). It is widely accepted that it is necessary to understand different facets of biodiversity within species assemblages in order to more accurately predict the impacts of global change on ecosystem functioning (Mouillot et al. 2013a, Lefcheck et al. 2015). Diversity is considered to be the natural capital for providing resilience to disturbance in the ecosystem and the ecosystem services that underpin human well-being, i.e. the outputs/benefits ecosystems provide to humanity (provisioning, regulation and maintenance and cultural services) (Cardinale et al. 2012, Lefcheck et al. 2015). However, the role of biodiversity as a predictive tool for environmental change and the mechanisms through which its relationship with functioning occurs are still subject to much debate (Naeem et al. 2012, Gamfeldt et al. 2015, Lefcheck et al. 2015). In this regard, functional diversity measured by species traits has been emerging as a priority research field, since it reflects multiple aspects of functioning (Mouillot et al. 2013a, Dolbeth et al. 2015, Gagic et al. 2015). For instance, environmental filtering and biotic interactions that shape biological communities are, at least partly, driven by species traits (e.g. Mouillot et al. 2013a, Dolbeth et al. 2013) which reflect how species use the resources available in the system. In fact, functional diversity may be used for a variety of purposes. For instance, trait identity may be important to predict ecological processes and functions, such as bioturbation (Gagic et al. 2015) or production (Dolbeth et al. 2015), and can thus aid in the evaluation of ecosystem services (Harrison et al. 2014). Other studies have partitioned taxonomic and functional diversity into  $\alpha$ ,  $\beta$  and  $\gamma$  components to depict the importance of processes that regulate community structure across envi-

ronmental gradients or spatial scales, such as habitat filtering or biotic interactions (De Bello et al. 2012, Villéger et al. 2012, 2013, Dolbeth et al. 2013). Habitat filtering may be a dominant process, particularly in estuarine systems, as it involves selecting traits which maximise the ability to acquire limited resources in demanding estuarine environmental conditions, which should lead to trait convergence (Dolbeth et al. 2013, Villéger et al. 2013). All of these are examples of how trait analyses can aid in the understanding of the functioning of an ecosystem. On the other hand, food web ecology is essential to infer how energy or mass flows within ecological communities and in this way provides information about ecosystem functioning (van Oevelen et al. 2010, Baeta et al. 2011). Progress in food web ecology has been made through stable isotope carbon and nitrogen analysis, which has been used to identify nitrogen sources (e.g. pollution), infer and estimate processes, and to detect the relative contributions of food sources to consumers in a time-integrated way, allowing us to track energy or mass flows and determine the trophic position of a consumer in the food web (Eggers & Jones 2000, Fry 2006, Baeta et al. 2009).

To quantify ecosystem functioning, we need to measure the magnitude and dynamics of several ecosystem processes, and this involves research that spans multiple biological organisational levels and disciplines (Naeem et al. 2009, Solan et al. 2012). As expected, ecosystem functioning is quantified using different tools; trait and food web analyses are examples that have been used, particularly in the last decade (e.g. Baeta et al. 2009, Gagic et al. 2015). Several studies have shown that different diversity measures, such as taxonomic and functional diversity, can elucidate different aspects of ecosystem functioning (e.g. Villéger et al. 2012, 2013, Dolbeth et al. 2013). Nonetheless, the information provided by functional diversity approaches and food web analyses is often interpreted independently and not in an integrative way. With this in mind, our goal was to understand what sort of information about ecosystem functioning we can obtain with different approaches (such as examination of taxonomic diversity, traits and food webs) for 2 hotspot tropical estuarine ecosystems which have been less studied from a functional perspective (but see Villéger et al. 2012).

To do this, we focused the study on the fish communities from 2 tropical Brazilian estuarine systems, which have different intensities of anthropogenic disturbance: one estuary is highly urbanised and lies between extensive sugarcane plantations and shrimp aquaculture, while the other estuary has been de-

clared a conservation unit for sustainable use (International Union for Conservation of Nature—IUCN, Protected Area Category V), although there are also sugarcane plantations and aquaculture facilities in the surrounding areas. Thus, we are also interested in understanding how disturbance impacts influence ecosystem functioning. Overall, we used a combined functional approach to achieve the following objectives: (1) to analyse the importance of processes that structure fish community assemblages (e.g. habitat filtering, biotic interactions using taxonomic, trait and stable isotope analyses); (2) to contribute to a better understanding of the food-web structure (using stable isotope analyses); (3) to assess the impact of anthropogenic disturbance in each system and (4) to better understand which functional characteristics of each system might confer higher resilience to the known disturbance (e.g. environmental data vs. all functional approaches). Our hypotheses are that (1) both habitat filtering and biotic interactions are important processes in both estuaries, although habitat filtering may be the dominant one; (2) basal food sources are important in the food web structure of the fish communities; (3) disturbance impacts will most

affect the functioning of the Paraíba estuary, the most human-impacted of the 2 systems; and (4) higher functional redundancy will be important in order to confer resilience to disturbance.

## MATERIALS AND METHODS

### Study area

The study was conducted in 2 tropical estuaries, Paraíba (3012 ha) and Mamanguape (690 ha), the largest estuaries of Paraíba state, on the northeast coast of Brazil (Fig. 1). According to the Köppen-Geiger climate classification, the climate type in the 2 estuaries is 'As', i.e. equatorial with a dry summer (Alvares et al. 2013). In both estuaries, the rainy season extends from February to August, with the highest rainfall occurring in June and the lowest in November. The Paraíba estuary has a wetter climate ( $1717 \text{ mm yr}^{-1}$ ) than the Mamanguape estuary ( $1392 \text{ mm yr}^{-1}$ ) (data from 1999–2014; Center for Weather Forecasting and Climate Research—CPTEC/INPE, [www.cptec.inpe.br](http://www.cptec.inpe.br), accessed in August 2015).

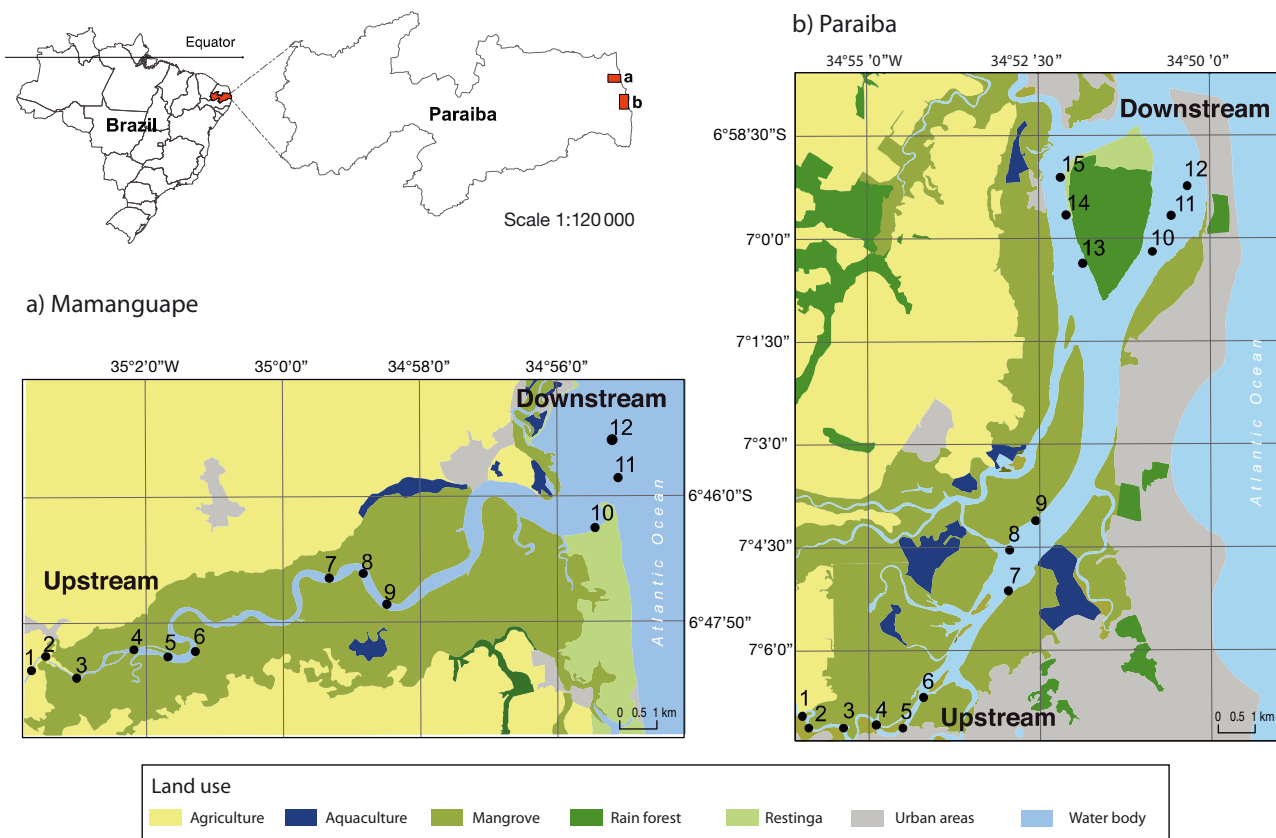


Fig. 1. (a) Mamanguape and (b) Paraíba estuaries in NE Brazil, their sampling sites (black dots) and main land uses

The Paraíba River basin drains the driest region of Brazil (the Borborema plateau). This means that most rainfall is retained in reservoirs, except in wetter years. During the study period, freshwater arriving at the Paraíba estuary originated on the humid coastal plains (Executive Agency for the Management of Waters in the State of Paraíba [AESAs], [www.aesa.pb.gov.br](http://www.aesa.pb.gov.br), accessed in August 2015). The watersheds that drain directly into the Paraíba River cover approximately 38 472 ha (Z. Teixeira pers. comm.). The Mamanguape River basin drains areas that are less dry, including a humid range, which leads to frequent water spills over the coastal plains (AESAs, [www.aesa.pb.gov.br](http://www.aesa.pb.gov.br), accessed in August 2015). The watersheds that drain directly into the Mamanguape River are approximately 25 055 ha in size (Z. Teixeira pers. comm.). In addition, Mamanguape has an 8.5 km long reef line perpendicular to the shoreline, which creates a protected region at the mouth of the estuary. Both estuaries have mangrove areas that grow around the main channel and the intertidal creeks, in addition to remnants of the Atlantic rainforest (Campos et al. 2015).

Both systems are subjected to different intensities of anthropogenic pressures. The Paraíba estuary lies between extensive sugarcane plantations, shrimp aquaculture areas and a metropolitan area with approximately 1 100 000 inhabitants, i.e. João Pessoa and 4 other contiguous cities ( $\pm 14\,618$  ha [38%],  $\pm 468$  ha [1.2%] and  $\pm 9418$  ha [24%] of land use, respectively; Z. Teixeira pers. comm.). The Mamanguape estuary has been declared a conservation unit for sustainable use (Barra de Mamanguape environmental protection area, IUCN Protected Area Category V); the goals of this status are the protection of coastal habitats and the marine manatee *Trichechus manatus* Linnaeus, 1758, which is classified as an Endangered Species ([www.iucnredlist.org/details/22103/0](http://www.iucnredlist.org/details/22103/0)). However, extensive sugarcane fields and shrimp aquaculture ( $\pm 14\,031$  ha [56%] and  $\pm 204$  ha [0.8%] of land use, respectively; Z. Teixeira pers. comm.) are also found beyond the mangrove area. The nearby cities have a total of 66 000 inhabitants ( $\pm 519$  ha [2%] land use).

### Sampling and laboratory procedures

Fish assemblages were sampled using a beach seine (10 × 1.5 m; mesh size: 8 mm) along the salinity gradient of the 2 Brazilian estuaries (15 sampling sites in the Paraíba and 12 sampling sites in the Mamanguape; Fig. 1). At each sampling site, three

30 m-long hauls were collected during the low tide in the wet (July 2014) and dry (November 2013) seasons. At each sampling site and season, physico-chemical parameters were measured *in situ* (surface water temperature, salinity, pH, turbidity [multiparameter probe] and transparency [Secchi depth]), and water samples were collected for nutrient content analyses and determination of chlorophyll *a*. In the laboratory, concentrations of ammonium ( $\text{NH}_3\text{-N}$ ,  $\mu\text{g l}^{-1}$ ), nitrite, nitrate ( $\text{NO}_x\text{-N}$ ,  $\mu\text{g l}^{-1}$ ) (APHA 2005) and total phosphorous (P,  $\mu\text{g l}^{-1}$ ) (Strickland & Parsons 1972) were measured. Chlorophyll *a* (chl *a*,  $\mu\text{g l}^{-1}$ ) was determined based on Lorenzen (1967).

All fish were identified, counted, weighted (g wet mass), and several morphological measurements were determined on a sub-sample to define and evaluate traits: total length, body height, body width, mouth width, mouth height, pectoral fin length, pectoral fin height, caudal fin height, caudal peduncle minimal height, distance between the centre of the eye to the bottom of the head, and head height along the vertical axis of the eye.

### Selection of traits

We selected traits that could quantify food acquisition and locomotion, both essential functions performed by fish, which have an impact on the ecosystem functioning and are known for this reason as effect traits (Violle et al. 2007). We defined 9 traits using fish morphological measurements, adapting the procedure by Villéger et al. (2012) and Pessanha et al. (2015): body mass, body transversal shape, relative head length, oral gape shape, eye position, aspect ratio of the pectoral fin, relative peduncle length, caudal peduncle compression and caudal peduncle throttling (details in Table S1 in the Supplement at [www.int-res.com/articles/suppl/m560p041\\_supp.pdf](http://www.int-res.com/articles/suppl/m560p041_supp.pdf)). All traits were initially checked for collinearity after inspection of their correlation and variation inflation factor ( $\text{VIF} > 3$ ; Zuur et al. 2009). The fish communities in our tropical systems were mainly composed of juveniles and sub-adults, and as such, we have not considered ontogenetic changes.

### Stable isotopes analyses

Fish and possible basal food sources were collected to determine  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the upstream oligohaline (Sites 1 to 3) and the downstream polyhaline and euhaline areas (Sites 7 to 9) of each estuary in both

seasons, to account for spatial and seasonal trophic dynamics within the water column. Particulate organic matter (POM) was obtained by filtering 1–1.5 l of estuarine water, from a depth of 0.5 m below the surface, onto pre-combusted (450°C, 4 h) Whatman GF/F filters with a low-pressure vacuum pump. Sediment samples from the upper 1 cm were collected with an acrylic corer (31 mm in diameter). Microphytobenthos (MPB) was collected by removing the biofilm on the top sediment layer. Mangroves leaves and macroalgae were collected by hand and gently cleaned of epiphytic material. Zooplankton was collected at each site by towing a Bongo net (0.3 m diameter, 68 µm mesh size) against the current for 5 min. The zooplankton samples for isotope analysis were composites of 20–200 individuals. All source samples were collected on the same sampling days as the fish community surveys and were stored in a freezer until processing. These potential food sources were selected after studies on the fish diet content analyses of some of the major fish species found in the communities studied (e.g. Claudino et al. 2015, Pessanha et al. 2015). Each sample was rinsed with Milli-Q water, placed in sterile Petri dishes and dried in an oven at 60°C for 48 h. Dried samples were ground (filters with POM were kept whole) into homogenous powder using a mortar and pestle, weighted, and loaded into tin capsules. Macroinvertebrates were also taken manually from each site. For the macrofauna, we removed the shell from molluscs and skeleton from crustaceans. For fish, we analysed the muscle of the dorsal region.

The carbon and nitrogen elemental and isotopic composition was determined using a Flash EA 1112 Series elemental analyser coupled online via Finnigan ConFlo III interface to a Thermo Delta V S mass spectrometer. The carbon and nitrogen isotope ratios are expressed in delta ( $\delta$ ) notation, defined as the parts per thousand (‰) deviations from a standard material (PDB limestone for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ ):  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . Precision in the overall preparation and analysis was better than 0.2‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

### Data analyses

Environmental variables were analysed with a principal components analysis (PCA) to clarify the differences between estuaries and seasonal patterns. Prior to the analyses, the variables turbidity and chlorophyll *a* were square-root transformed, and

total P,  $\text{NH}_3\text{-N}$  and  $\text{NO}_x\text{-N}$  were fourth-root transformed, after checking their variation patterns with a draftsman's plot. All variables were then normalised, and the correlation between variables was checked to remove the collinear environmental variables. These analyses were done using PRIMER + PERMANOVA software (Clarke et al. 2014).

The fish taxonomic diversity was evaluated with species number as a measure of species richness and the Simpson's diversity ( $D$ ) index. We evaluated the equivalent functional diversity indices, functional richness (FRic) and Rao's quadratic entropy (RaoQ). FRic expresses the amount of multidimensional trait space filled by the species in the community, measured here with the convex hull (Laliberté & Legendre 2010, Mouillot et al. 2013a). For this study, we measured FRic taking into account all traits for the whole estuary ('global' FRic, which includes all species in the estuary) and along each estuarine gradient (FRic for each sampling area). Neither species richness nor FRic accounted for abundance.

The abundance-weighted indices were Simpson's  $D$  and RaoQ. RaoQ is the average dissimilarity between species pairs in a community (Ricotta & Moretti 2011). Both Simpson's  $D$  and RaoQ were used for partitioning diversity into their  $\alpha$ ,  $\beta$  and  $\gamma$  components for each estuary, taking into account all sampling sites in each estuary and time period (wet or dry season). The partitioning of diversity assumed an additive relationship ( $\gamma = \alpha + \beta$ ; Lande 1996), where regional  $\gamma$ -diversity comprises  $\alpha$ -diversity (within-community), and  $\beta$ -diversity (among-communities, i.e. degree of change in species diversity along an environmental gradient) (Whittaker 1972). For the present case study,  $\gamma$  corresponds to the diversity of each estuary,  $\alpha$ -diversity to the diversity within communities of each sampling site and  $\beta$ -diversity to the diversity among those communities. The analyses were done with a Jost-correction based on the 'equivalent communities' concept (Jost 2007) to provide an independent  $\alpha$  and  $\beta$  (De Bello et al. 2010). This correction is recommended when comparing taxonomic and functional diversity; otherwise,  $\beta$  could be inappropriately low and biologically meaningless (De Bello et al. 2010). The upper limit for the Jost-corrected  $\alpha$ -Simpson is the total number of species for the region (De Bello et al. 2010), and the upper limit for the  $\alpha$ -Rao is the value of  $\alpha$ -Simpson. Functional redundancy was evaluated as the difference between the Simpson and Rao indices (De Bello et al. 2007). All the species are functionally identical when redundancy is zero, while maximum redundancy is achieved when it is equal to the Simpson index



(De Bello et al. 2007). All indices were computed within the R software package (R Core Team 2012), using the FD routines (Laliberté et al. 2015) and the 'Rao' function (De Bello et al. 2010) through which  $\alpha$ ,  $\beta$  and  $\gamma$  components are provided for taxonomic and functional diversity.

## RESULTS

### Characterisation of the study systems

The first 2 axes of PCA shown in the plot explained 61.4% of the total variability. Seasonal differences were evident in the environmental data, as samples from the dry season were clearly separated from the ones from the wet season. Differences were also evident when comparing the upstream and downstream areas of both estuaries (Fig. 2a). Salinity was generally higher in the downstream areas, particularly during the dry season but also in the upstream areas of Mamanguape. Turbidity, pH and temperature were generally highest during the dry season, while transparency was higher in the wet season (Fig. 2a). Higher nutrient concentrations (i.e. fourth root total P,  $\text{NH}_3\text{-N}$  and  $\text{NO}_x\text{-N}$ ) and chl *a* were associated with the Paraiba, for both seasons, reflecting the higher level of anthropogenic disturbance of the urban estuary. In fact, the analyses of the nutrient concentrations along the estuarine gradient of each estuary show all nutrient concentrations to be higher in Paraiba than in Mamanguape, particularly  $\text{NH}_4\text{-N}$ , on average 7 times higher (Fig. 2b). For Paraiba, nutrient concentrations were higher during the wet season and mostly in the upstream area. Nonetheless, nutrient loading also extended into areas further downstream, up to Site 11. For Mamanguape, nutrients concentrations were slightly higher during the dry season (Fig. 2b).

### Species and functional diversity

In the Mamanguape, the total number of species was higher in the dry season than in the wet season (47 species for the dry vs. 34 species for the wet season). Despite this lower number of species in the wet season, the functional space occupied by all species in the estuary (global FRic) was similar in both seasons (Fig. 3a). By functional space, we mean the space composed of functional traits (axes) along which species are placed according to their trait values (Mouillot et al. 2013a). Most of the functions

measured in the dry season overlapped those in the wet season. However, few species contributed with functions that were placed outside the overlapping functional space.

For the Paraiba, the total number of species was higher than those registered for the Mamanguape, and more species occurred during the wet season (48 species for the dry vs. 57 species for the wet season, Fig. 3a). Regarding the global FRic, the variations trends were similar to those in the Mamanguape: the functional volume occupied by the species was similar for both seasons, with overlapping functions between seasons and a few species contributing non-overlapped functions (Fig. 3a). If we compare the 2 systems, the total species number and functional space occupied by those species was higher in Paraiba than in Mamanguape.

Regarding the variation along each estuarine gradient and season, the species number varied within similar values among estuaries and seasons, with a tendency towards slightly higher values during the dry season in at least 50% of the sites. Nonetheless, the variation range in species number along the estuarine gradient was wide, i.e. some sampling sites had a low (<5) while others had a high (>15) number of species (Fig. 3b). For Mamanguape, the species number was lower in the upstream areas compared to the downstream ones, particularly at Sites 7 to 9, which had the highest values for both seasons (Fig. S1 in the Supplement). For Paraiba, the species number was generally higher from Sites 10 to 15, whereas the lowest values occurred from Sites 7 to 9 (Fig. S1). For the FRic along each estuarine gradient, we found generally higher values during the wet season in both estuaries (Fig. 3b). The spatial variation tendency of FRic was quite similar to the species number: lower FRic in upstream areas compared to those downstream for both estuaries, with highest values in Sites 7 to 9 in Mamanguape and from Sites 10 to 15 for Paraiba, particularly for the wet season (Fig. S1).

The abundance-weighted species diversity provided variation trends similar to species number along each estuarine gradient: Simpson's *D* was slightly higher during the dry season for both estuaries (Fig. 4). However, the difference among estuaries was higher with Simpson diversity compared to the species number:  $\gamma$ -Simpson was approximately 30% higher for Paraiba compared to Mamanguape. Despite these differences,  $\gamma$ -RaoQ was similar for both estuaries and seasons, being only slightly lower during the dry season for the Paraiba (1.1 for the dry vs. 1.2 for the wet season, Fig. 4). RaoQ trait diversity was always considerably lower than the Simpson

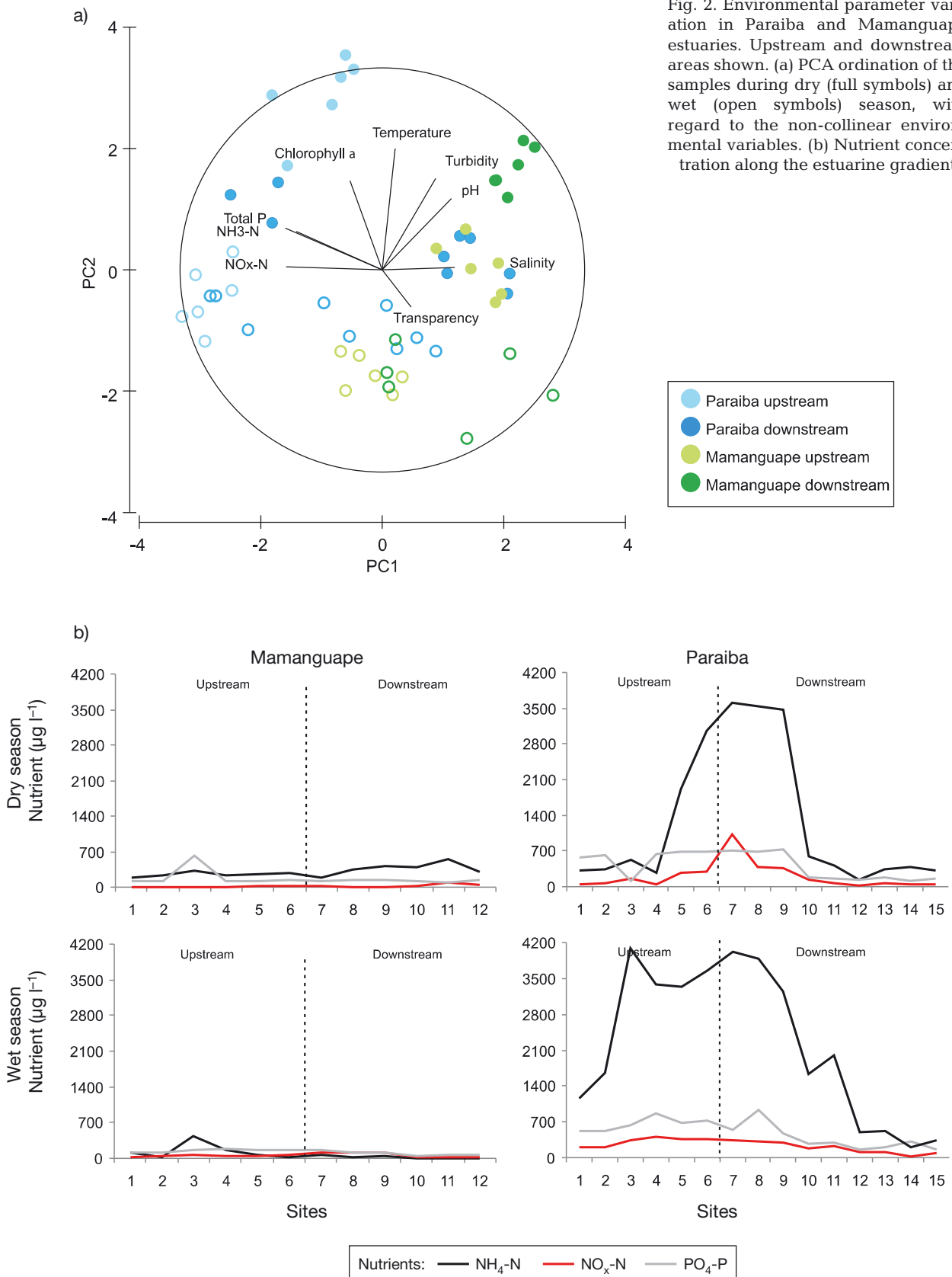


Fig. 2. Environmental parameter variation in Paraiba and Mamanguape estuaries. Upstream and downstream areas shown. (a) PCA ordination of the samples during dry (full symbols) and wet (open symbols) season, with regard to the non-collinear environmental variables. (b) Nutrient concentration along the estuarine gradient

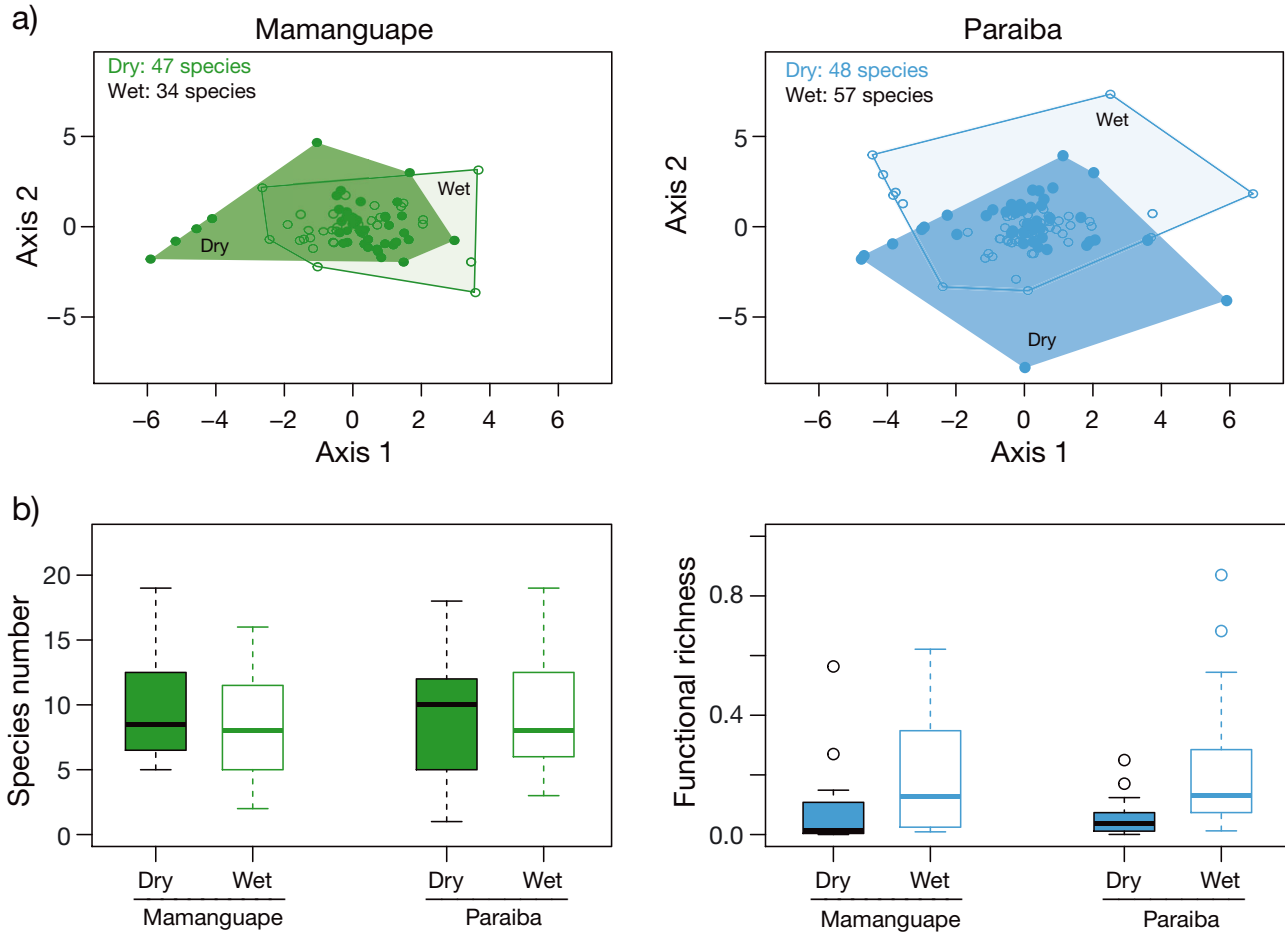


Fig. 3. Species number and functional richness (FRic) for Mamanguape and Paraiba estuaries during the dry and wet seasons including (a) all species for the whole estuary, measured as total species number and as the portion of the functional space in which all species were gathered, or (b) species along the estuarine gradient, measured with boxplots. Results of FRic were standardised by global FRic. Middle line indicates the 50th percentile; extremities of the box are the 25th and 75th percentiles; maximum length of each whisker represents the 5th and 95th percentiles; outliers (empty circles) shown individually

species diversity (i.e. the upper limit for RaoQ) within each estuary and taking into account all components of diversity (Fig. 4). Functional redundancy was highest for Paraiba and for the dry season: 6.6 and 6.2. for Paraiba during the dry and wet season, respectively, and 4.7 and 4.1 for Mamanguape.

When diversity was partitioned among its different components, mean  $\alpha$ -Simpson was lower than  $\beta$ -Simpson, and this was consistent across both estuaries and seasons (Fig. 4), reflecting a higher species turnover along each estuary for both seasons, i.e. species identity changed considerably among the sampling sites. For the wet season, the difference between mean  $\alpha$ - and  $\beta$ -Simpson was small for Mamanguape ( $\alpha$ : 2.5 and  $\beta$ : 2.8) but still expressed a higher species turnover along the estuarine gradient. The opposite trend was observed for the functional

diversity, as mean  $\alpha$ -RaoQ was considerably higher than  $\beta$ -RaoQ, indicating low turnover of functions, i.e. functions were similar along the spatial estuarine gradient. The variation and the ratio between  $\alpha$ -RaoQ and  $\beta$ -RaoQ were quite similar across seasons (Fig. 4), with a 0.01 difference among conditions. Along the estuarine gradient,  $\alpha$ -Simpson had an erratic spatial variation pattern for both estuaries and seasons, while  $\alpha$ -RaoQ varied within similar values for the whole gradient (Fig. S2 in the Supplement).

### Stable isotopes

In general, the dual-isotope plots showed a relatively narrow range of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among fish species (Fig. 5). Although the sample col-



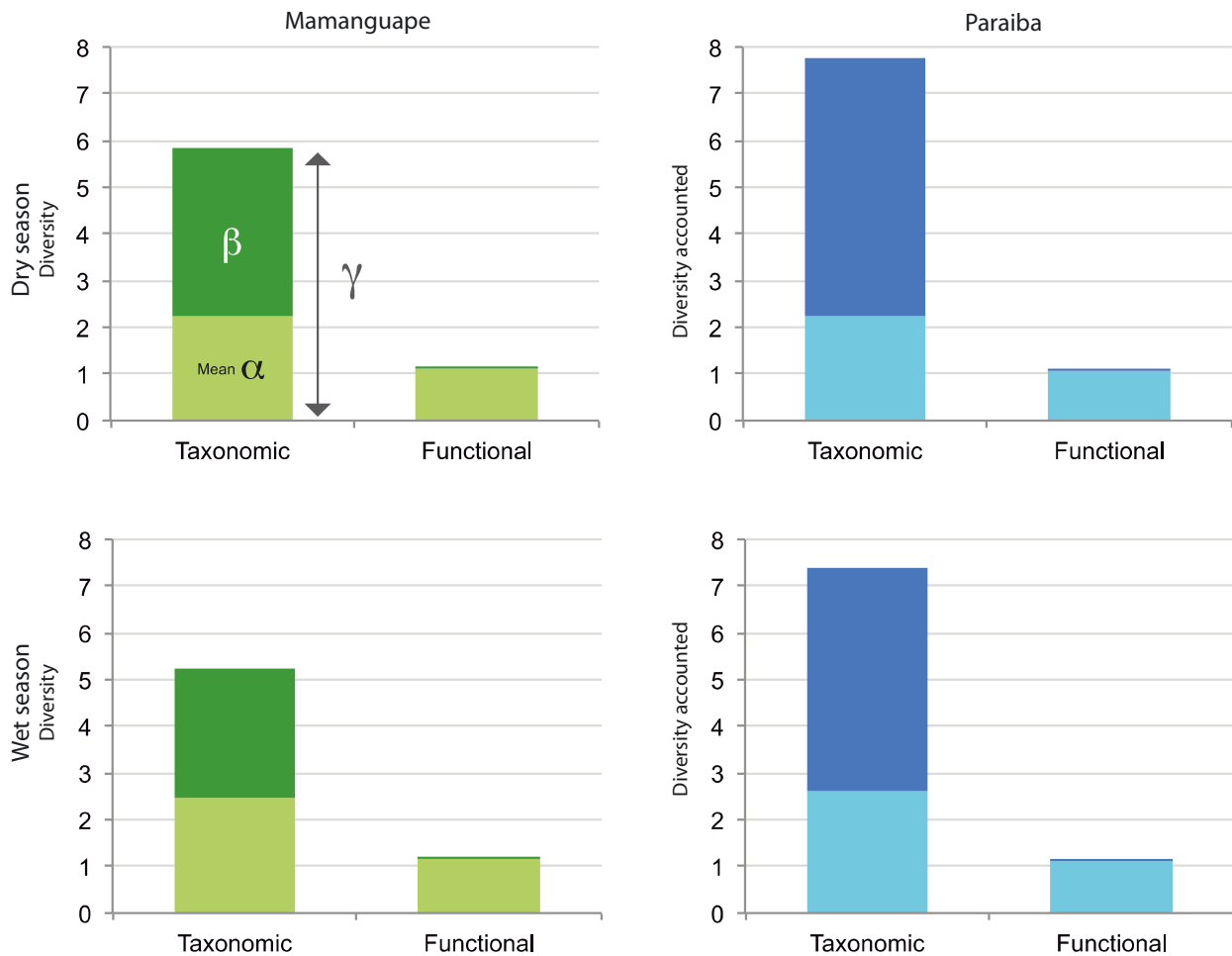


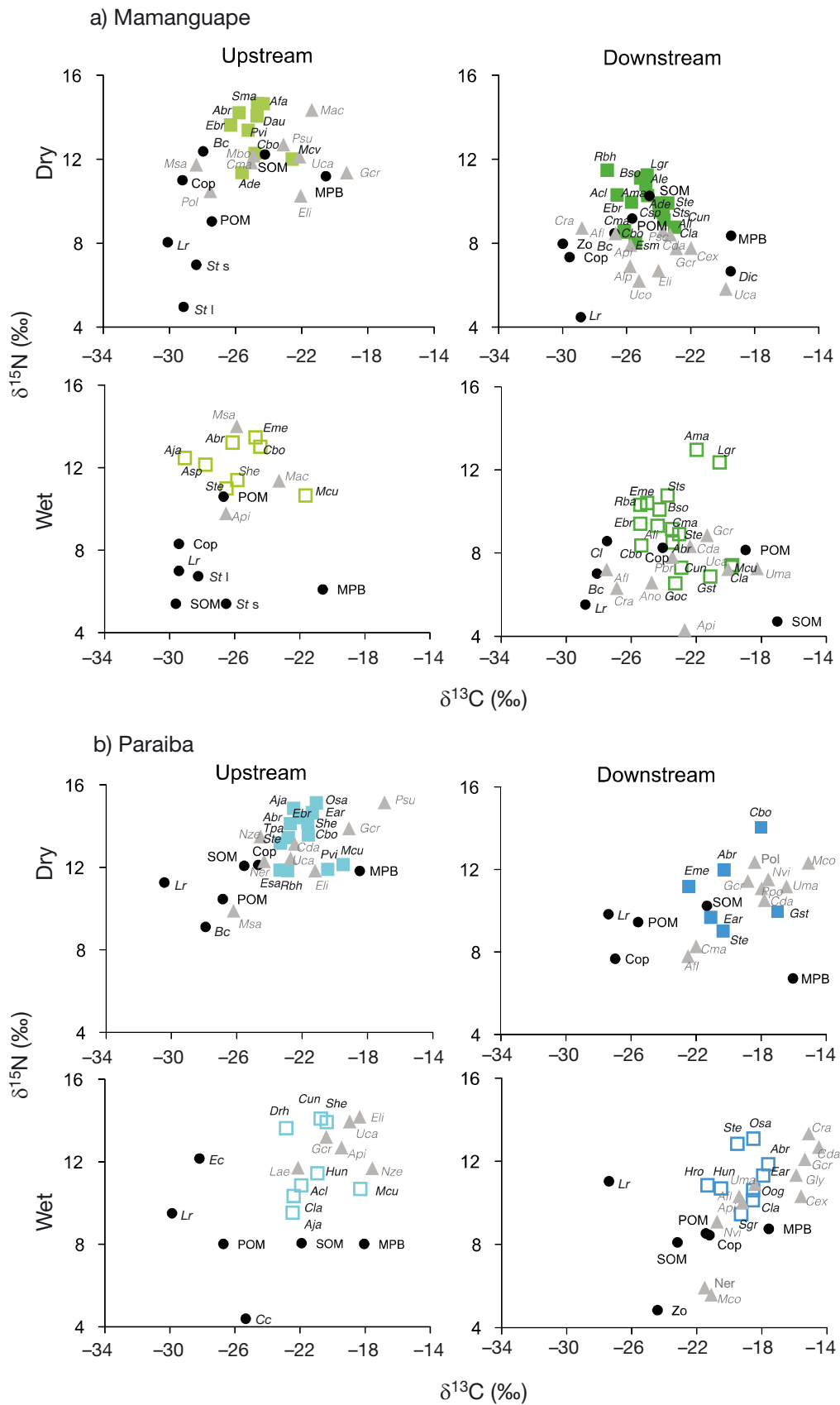
Fig. 4. Partitioned taxonomic diversity vs. functional diversity into  $\alpha$  (within community) and  $\beta$  (among communities) components of  $\gamma$  (regional diversity, bar length) over the 2 estuaries (12 and 15 sampling sites) for the 2 seasons

lection areas do not reflect the entire gradient, they reflect the environmental gradient contrasting conditions between the upstream and downstream areas, with differences in salinity, turbidity, among others (Fig. 2), and an important part of the species taxonomic differences along the gradient (i.e. species turnover, Fig. S1, Fig. 5). In the upstream areas of both estuaries, basal food sources and fish showed depleted carbon isotope values when compared to the downstream area, for both seasons. This trend was more pronounced in Mamanguape during the wet season. In addition, sources and consumers showed enriched nitrogen isotope values in upstream areas, in both estuaries and seasons (Fig. 5). For *Laguncularia racemosa*, a mangrove species found on all occasions,  $\delta^{15}\text{N}$  was generally higher for Paraiba compared to Mamanguape and also highest in the upstream oligohaline areas and for the dry season in both estuaries (Fig. 5).

## DISCUSSION

### What did we learn from the traditional approaches?

Both estuarine systems are marked by strong environmental changes along the spatial gradient, due to changes in salinity, pH, temperature, turbidity, as also discussed by Alves et al. (2016). These environmental variables were measured at the 2 sampling dates, and therefore are a static representation of the variability that may be found in each system. Still, the highest nutrient concentrations were clearly associated with Paraiba, as expected, since this estuary has a higher anthropogenic impact, in particular from the urban areas and intensive aquaculture. Salinity was higher in the downstream areas, as usual in estuarine areas. Yet the upstream areas of the Mamanguape were also characterised by high salinity during the



dry season, suggesting influence by oceanic waters further upstream in a season with reduced freshwater runoff. Mamanguape also had a lower number of river effluents than Paraiba, which might contribute to a lower freshwater runoff.

Tropical systems are known to have higher taxonomic diversity levels compared to other marine biogeographic realms (Mouillot et al. 2014, Pasquaud et al. 2015, Vasconcelos et al. 2015). Our systems had lower species richness when compared to other tropical estuarine fish communities (e.g. Vil  ger et al. 2012, Vilar et al. 2013, Vasconcelos et al. 2015), which could be related to the sampling technique elected to sample juveniles and sampling frequency. Still, Mamanguape estuary had generally lower species diversity, when we expected higher values, taking into account its environmental protection regime. In a regional context, the estuarine area and estuary mouth width have been known to influence fish diversity levels (Pasquaud et al. 2015, Vasconcelos et al. 2015). Paraiba and Mamanguape are markedly different in this respect, which might contribute to the higher species diversity in Paraiba: Paraiba is at least 4 times larger, its estuary mouth is wider and Mamanguape is partially dammed by a reef line. The reef line at the downstream areas of Mamanguape may be contributing to habitat complexity for a variety of fish (Xavier et al. 2012), but, at the same time, may be limiting the entrance of adventitious marine species or limiting the recruitment of fish from adjacent oceanic areas, as discussed in other studies (Nicolas et al. 2010, Vasconcelos et al. 2015). Other reasons for the generally lower fish richness in these 2 tropical systems as

compared to others might be that the different habitats were not considered in the study surveys or due to disturbance impacts, a hypothesis discussed in greater detail below. Sampling occurred in the main channel, which is mostly composed of coarse sands along the whole estuarine gradient (Alves et al. 2016). We did not consider vegetated areas (e.g. mangrove and seagrass areas) and adjacent mudflats, potentially contributing with high fish diversity as observed for other tropical areas (Blaber 2007, 2013).

### What did we learn from the functional approaches?

The incorporation of the functional approaches, such as use of species traits and carbon and nitrogen stable isotopes for food web analyses, allowed us to have a different perspective on the tropical estuaries studied and contributed to a better understanding of their functioning. We were able to depict or confirm patterns that would not have been possible to detect with the traditional approach. This included (1) gaining an understanding of the processes that shaped species coexistence patterns (e.g. importance of habitat and competition evaluated with trait and stable isotope analyses), (2) recognising the basal resources in the food web (evaluated with stable isotope analysis), (3) recognising anthropogenic impact sources in both systems (evaluated with stable isotope analysis), and (4) inferring the potential resilience of each system towards that disturbance (evaluated with trait analysis).

Fig. 5. Dual carbon and nitrogen stable isotope plots for basal food sources (black circles), macroinvertebrates (grey triangles), and fish (squares colour coded to distinguish estuaries and areas) collected in the (a) Mamanguape and (b) Paraiba estuaries, in the upstream and downstream areas, during dry (filled squares) and wet seasons (open squares). Basal food sources: *Bc*, *Bostrychia calliptera*; *Cc*, *Catenella caespitosa*; *Cl*, *Caloglossa leprieurii*; *Cop*, Copepoda; *Dic*, *Dictyota* sp.; *Ec*, *Eichhornia crassipes*; MPB, microphytobenthos; *Lr*, *Laguncularia racemosa*; POM, particulate organic matter; *St l*, *Schinus terebinthifolius* leaves; *St s*, *Schinus terebinthifolius* seeds; SOM, sedimentary organic matter; Zo, zoea *Brachyura*. Fish names in black: *Ade*, *Achirus declivis*; *Ali*, *Achirus lineatus*; *Aja*, *Anchoa januaria*; *Ama*, *Anchoa marinii*; *Asp*, *Anchoa spinifera*; *Acl*, *Anchovia clupeioides*; *Ale*, *Anchoviella lepidentostole*; *Afa*, *Astyanax fasciatus*; *Abr*, *Atherinella brasiliensis*; *Bso*, *Bathygobius soporator*; *Cl*, *Caranx latus*; *Cma*, *Citharichthys macrops*; *Csp*, *Citharichthys spilopterus*; *Cun*, *Centropomus undecimalis*; *Cbo*, *Ctenogobius boleosoma*; *Drh*, *Diapterus rhombeus*; *Esa*, *Elops saurus*; *Esm*, *Erotelis smaragdus*; *Ear*, *Eucinostomus argenteus*; *Eme*, *Eucinostomus melanopterus*; *Ebr*, *Eugerres brasilianus*; *Gst*, *Gobionellus stomatus*; *Goc*, *Gobionellus oceanicus*; *Hro*, *Hyporhamphus roberti*; *Hun*, *Hyporhamphus unifasciatus*; *Lgr*, *Lycengraulis grossidens*; *Mcu*, *Mugil curema*; *Mcv*, *Mugil curvidens*; *Osa*, *Oligoplites saurus*; *Oog*, *Opisthonema oglinum*; *Pvi*, *Poecilia vivipara*; *Rba*, *Rhinosardinia bahiensis*; *She*, *Sciadodes herzbergii*; *Sgr*, *Sphoeroides greeleyi*; *Ste*, *Sphoeroides testudineus*; *Sma*, *Strongylura marina*; *Sts*, *Symphurus tessellatus*; *Tpa*, *Trinectes paulistanus*; Invertebrate names in grey: Decapoda — *Alp*, *Alpheus* sp.; *Ano*, Anomura not identified (n id); *Api*, *Aratus pisonii*; *Cda*, *Callinectes danae*; *Cex*, *Callinectes exasperatus*; *Cma*, *Callinectes marginatus*; *Eli*, *Eurytium limosum*; *Pbr*, *Penaeus brasiliensis*; *Psc*, *Penaeus schmitti*; *Mac*, *Macrobrachium acanthurus*; *Mbo*, *Macrobrachium borelli*; *Psu*, *Penaeus subtilis*; *Uma*, *Uca maracoani*; *Uca*, *Uca* sp.; *Uco*, *Ucides cordatus*. Bivalvia — *Afl*, *Anomalocardia flexuosa*; *Cra*, *Crassostrea* sp.; *Mco*, *Macoma constricta*; *Msa*, *Mytilopsis sallei*. Gastropoda — *Nvi*, *Neritina virginea*; *Nze*, *Neritina zebra*; *Ppo*, *Phrontis polygonata*. Polychaeta — *Gly*, *Glycinde* sp.; *Gcr*, *Goniopsis cruentata*; *Lae*, *Laeonereis* sp.; *Ner*, Nereididae n id; *Pol*, Polychaeta n id

Habitat filtering and species biotic interactions have been broadly described as the main processes driving ecological communities (Cornwell et al. 2006): habitat filtering reduces the spread of ecological strategies, resulting in trait-convergence; biotic interactions maximise the differentiation between coexisting species, resulting in trait-divergence (De Bello et al. 2012). Our results from taxonomic and trait analyses were consistent with both hypotheses acting on different scales, which is a pattern to be expected for fish communities in estuarine systems (Sosa-López et al. 2007, Villéger et al. 2013). The strong environmental gradients along each estuary resulted in a high species turnover, meaning that species identity changed considerably along the estuarine gradient. However, although species changed, their functions were similar (low functional  $\beta$ -diversity), resulting in trait-convergence and suggesting high functional redundancy. Therefore, on a regional scale, habitat (or environment) selects particular characteristics that allow species to survive under such conditions, as has been previously pointed out (Mouillot et al. 2006, Dolbeth et al. 2013, Villéger et al. 2013), a pattern that we were only able to depict with the trait analyses. Nevertheless, this low functional  $\beta$ -diversity also means that the competition for resources might be considerably high, because species tend to use the system in a similar way. The  $^{13}\text{C}$  and  $^{15}\text{N}$  analyses supported this hypothesis, by detecting a narrow range of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among the fish species in the communities from upstream and downstream areas. This could mean that fish species shared a similar trophic level by feeding on similar prey items. As a consequence, on a local scale (i.e. each sampling site), competition for resources by the juvenile fish may dominate, translating into some trait differentiation within each community, allowing species to use different resources in that area (i.e. functional diversity RaoQ:  $\alpha > \beta$ ). These patterns were consistent throughout both estuaries and seasons.

As mentioned above, it has been suggested that the tropical systems examined in this study have a high functional redundancy, a characteristic that has been assumed for tropical systems in general, owing to their vast regional species pools, which are dominated by generalist species (Villéger et al. 2013, Mouillot et al. 2014). It is precisely this high functional redundancy that promotes resilience to environmental change, by ensuring against loss of ecosystem functioning after declines in species diversity (Villéger et al. 2013, Mouillot et al. 2014). Recent

studies have shown though that some species may contribute disproportionately to ecosystem functioning (Mouillot et al. 2013b), and for this reason high diversity and high functional redundancy can no longer be assumed to be attributes able to prevent ecosystem functioning impairment (Mouillot et al. 2014). In our case studies, we found some evidence that a few species could contribute with different functions depending on the season. Even so, when functional diversity is weighted by abundance, its values become similar for both estuaries and seasons, suggesting the maintenance of functions. However, functional redundancy was at least 1/3 lower for Mamanguape than for Paraiba, suggesting that Mamanguape could be more prone to human disturbance impacts.

Dual stable isotope analyses also enabled us to understand possible pathways in the fish food webs. The depleted  $\delta^{13}\text{C}$  values observed in basal sources and fish species from upstream areas were a clear indication of terrestrial/freshwater autochthonous material inputs into the water column food web, reflecting the dominant primary producers at each location. In these tropical systems, mangroves are considered to be the main primary producers (Baretta et al. 2010, Abrantes et al. 2015, Claudino et al. 2015). However, most mangrove production is not directly consumed but rather returned to the environment as detritus, thus playing a role in sustaining these ecosystems. In the present study, we found evidence that mangrove-derived nutrients were important food sources for the fish, particularly in the upstream areas, as was also observed by Claudino et al. (2015), and were consumed as detritus (Pessanha et al. 2015) or indirectly via consumption of invertebrates that feed upon mangrove detritus (Claudino et al. 2015).

### Anthropogenic impact

Despite the huge differences in the nutrient concentrations of both estuaries, the enriched  $\delta^{15}\text{N}$  of both primary producers and consumers from the upstream areas were a sign of anthropogenic impacts on both estuaries, regardless of their environmental protection framework. In our study,  $\delta^{15}\text{N}$  ranged from 10 to 20‰ in some primary producers and their consumers (invertebrates and juvenile fish), particularly in the upstream areas, suggesting anthropogenic sources of nitrogen (compared to the atmospheric deposition whose values are <6‰; Kendall 1998). In estuaries, different sources

of nitrogen may influence its isotope signature (e.g. catchment area, marine–freshwater interplay), which may hinder a clear relationship between isotopic signature and anthropogenic disturbance (Warry et al. 2016). Nonetheless, enriched values are usually associated with urban wastewater and intensive agriculture (either directly or after nutrient fraction by biogeochemical processes; Warry et al. 2016). In our case study, the enriched  $\delta^{15}\text{N}$  may have resulted from sewage discharges, sugar and alcohol-cane plantations, usually on the northeast Brazilian coast (Barletta et al. 2010), and from intensive shrimp aquaculture in the surrounding area. In addition,  $\delta^{15}\text{N}$  for *Laguncularia racemosa*, a typical mangrove species found in the upstream areas of NE tropical estuaries (Claudino et al. 2015), was  $11.8 \pm 0.5\text{‰}$  in the dry season, a value of 5 and 3‰ higher (in Mamanguape and Paraiba, respectively) than the one measured during the wet season, suggesting that the disturbance impact was higher during the dry season. The anthropogenic isotopic indicators were higher for the dry season, despite the fact that the highest nutrient discharges occurred during the wet season. Likewise, functional richness and redundancy increased in the wet season, suggesting that a decrease in the freshwater runoff during the dry season might cause greater disturbance on both systems, which might be related to an increase in the water residence time, i.e. the time needed for the water renovation in the system. For Mamanguape, this higher anthropogenic impact might be related to its particular hydro-morphological characteristics, as its downstream reef line probably results in a considerably longer water residence time of the nutrient-enriched waters. In line with the hypothesis, salinity was also high at the upstream areas of Mamanguape during the dry season, suggesting that water from terrestrial sources is not easily washed out from the estuarine area. In addition, mean depth is considerably lower at Mamanguape compared to Paraiba, particularly in the upstream areas. Therefore, even though Mamanguape has a lower nutrient load than Paraiba, damage to its ecological quality may be greater, due to its particular hydro-morphology. Overall, the conservation measures currently employed in Mamanguape thus seem insufficient to ensure a high environmental quality, and as previously mentioned, the fish community may be less resilient to disturbance owing to a lower functional redundancy as compared to the highly urbanised Paraiba.

## CONCLUSIONS

This study showed that different approaches to examining marine ecosystem functioning provide complementary information and may be used together to improve our knowledge about how ecosystems work, thus forming a basis for better conservation and management measures. Yet, as we mentioned at the outset, the time and resources spent on gaining this improved knowledge depends on the research question and the resources available to address it, so our effort in gaining this knowledge must be context dependent. In our study, we came to the conclusion that the environment (habitat filtering) was an important driver of species coexistence, which narrows functioning into similar ways of using the available resources in the system, translating into similar functional diversity for both estuaries. We were also able to detect anthropogenic impacts in the structure and functioning in both systems, despite environmental protection, which highlights the need for increased conservation and more effective management measures. Furthermore, functional redundancy was lower for the protected system than for the heavily impacted system, suggesting that the fish community might be less resilient in the former to future disturbance events.

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